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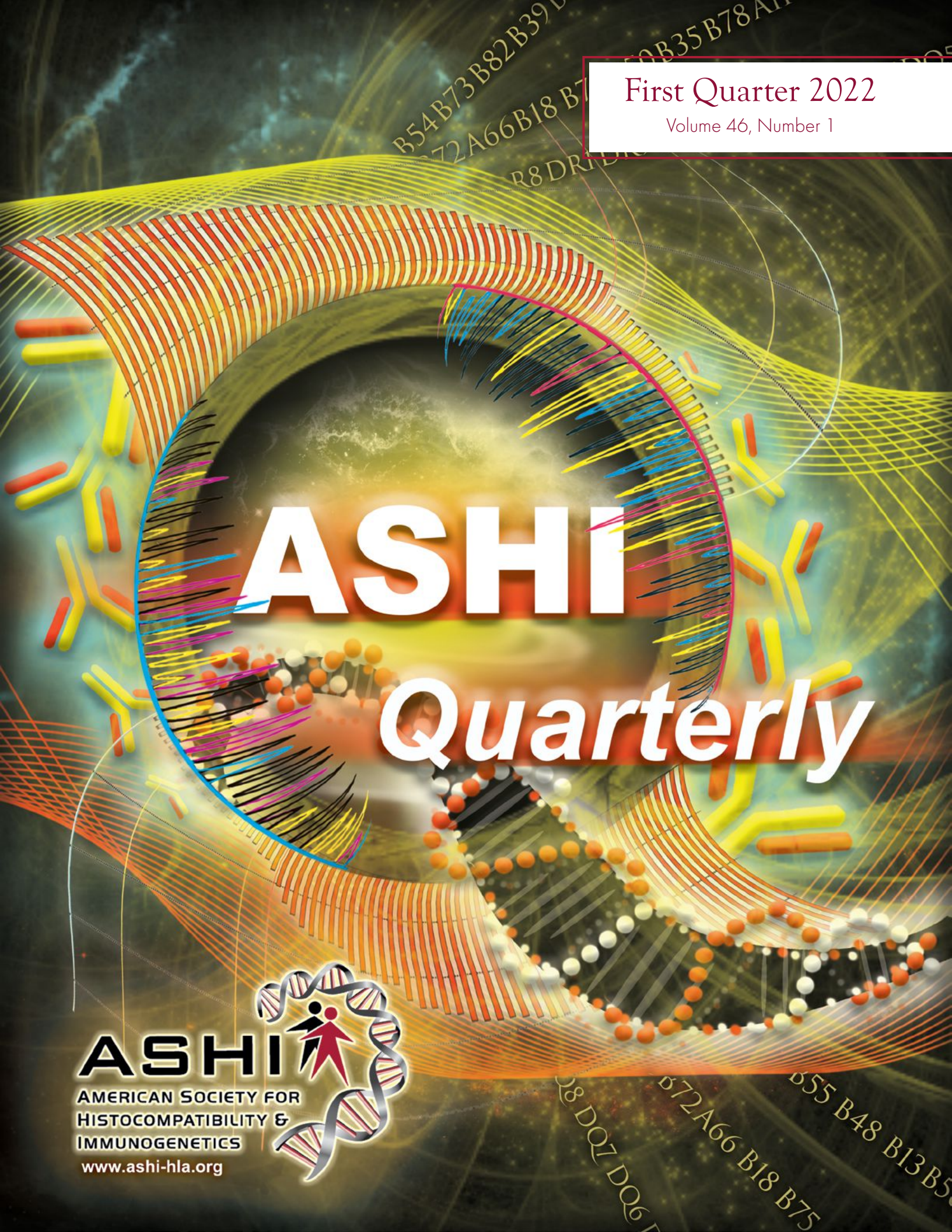

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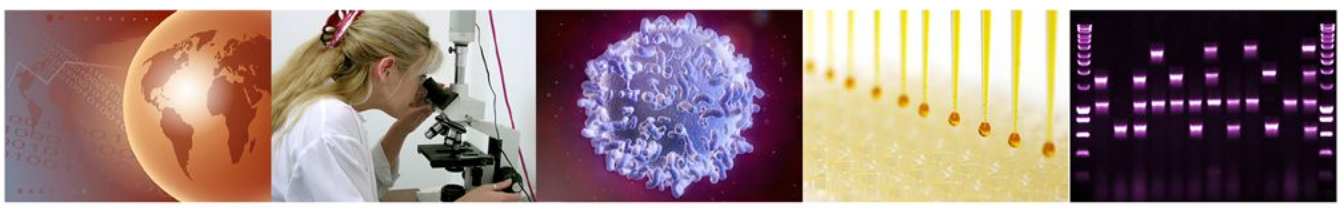
Program Director
***Sandra Rosen-Bronson***
E-mail: bronson@gunet.georgetown.edu

Program Administrator
***Andre Thalberg***
E-mail: andre.thalberg@georgetown.edu

ASHI
American Society for Histocompatibility & Immunogenetics

# Table of Contents

ASHI Quarterly

American Society for Histocompatibility and Immunogenetics

Volume 46, Number 1

## Features



## Departments



## Editor's Note

Individuals interested in submitting articles for *ASHI Quarterly* should observe the following requirements:

- All articles must be submitted via email in Microsoft Word format
- All articles must be double-spaced

Article submissions should be forwarded to:
Editor-In-Chief
Lee Ann Baxter-Lowe, PhD, F(ACHI)
Email: lbaxterlowe@chla.usc.edu

Copy deadlines for future issues:
Third Quarter 2022: July 1, 2022
Fourth Quarter 2022: October 1, 2022



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EDITORIAL

# From the Editor-In-Chief

Lee Ann Baxter-Lowe, *PhD, F(ACHI)*

As the incoming Editor-in-Chief, I welcome you to the first issue of the *ASHI Quarterly* in 2022. I would like to begin by thanking the people who make the *ASHI Quarterly* a reality. Each issue is the product of inspirational brain-storming and hard work of the Editorial Board, contributing authors who have volunteered to share their knowledge, the ASHI Publications Committee which ensures that content meets ASHI standards, ASHI's Board which supports the publication, and the *ASHI Quarterly* staff liaison Kathy Giovetsis, who keeps production of the publication on track. I am grateful that the Associate Editors from 2021 have agreed to remain on the Board in 2022: Dr. Sara Dionne, Ms. Anne Halpin, Dr. Sivadasan Kanangat, Dr. James Lan, and Dr. Chang Liu. I am pleased to announce that Dr. Nicholas Brown has agreed to fill my former position of Associate Editor for clinical science. Fortunately, past Editors-in-Chief, Dr. Robert Liwski and Dr. Manish Gandhi, have agreed to continue to serve as advisors. I am looking forward to working with this exceptional team in 2022.

The 2022 Editorial Board plans to continue featuring society activities, providing scientific articles which are relevant to ASHI members, and sharing inspirational insights from members and friends of ASHI. Society activities featured in this issue include:

- **Dr. Annette Jackson's President's Column** highlighting ASHI's 2022 Educational Workshops, the 48th Annual ASHI Meeting, and STIC Committee initiatives.
- The **PT Committee's** behind-the-scenes "View of the ASHI PT Program" including answers to FAQs.
- An **ARB Update** describing vaccination requirements for inspectors, a UNOS-ASHI taskforce that is performing a crosswalk of ASHI Standards and UNOS Policies, and key dates for 2022 inspection cycles.
- The **ACHI Certification Corner** which describes key differences between ACHI certification and ASHI membership and recognizes those who have recently passed the ACHI exam.
- A report from the **Director's Affairs Committee** which shares the results of their survey on NGS-based HLA typing.
- A **CEMC Update** which describes a new requirement for plain language summaries for abstracts submitted for the 2022 Annual ASHI Meeting. The update provides guidance for writing a plain language summary and 13 plain language summaries from the 2021 meeting which a panel of experts recognized for their high-quality research and clinical progress.

In **My Way to HLA**, Alix Raymond, a histocompatibility technologist working at Versiti-WI shares her enthusiasm for science, describes her experience during her first year of working in an HLA laboratory, and her plans to become certified as a CHT. In the article "**The need to "pull out all the stops" for a Kidney Transplant,**" Runying Tian describes the challenges of assessing donor compatibility for a highly sensitized patient. In this example, high resolution HLA typing and analysis of HLA epitopes played key roles in evaluating donor compatibility. This is an excellent illustration of how emerging technology and knowledge are advancing our field.

In 2021, the Q1 issue featured articles about SARS-CoV-2 (COVID-19), and this theme is continued in this first issue of 2022 because COVID-19 continues to play a major role in our personal and professional lives. The rapid expansion of research in this field is unprecedented with more than 223,000 articles appearing in a PubMed search for "COVID" including 599 involving HLA. This issue features several articles describing recent progress.

- **The Immunogenetics of COVID-19 Infections, Disease, and Immune Response to Vaccination.** Dr. Kanangat's review of the current literature provides insights into the roles of T cells, NK cells, and B cells in infections and diseases and illustrates the role of HLA diversity in these responses.
- **Antigen Presentation in SARS-CoV-2 Infection: The Role of Class I HLA and ERAP polymorphisms.** Dr. Saulle et al. describe current knowledge of host genetics including variation in HLA genes in the immune response to Coronaviruses. The authors note that results to date have often been contradictory, in part due to small sample size, heterogeneous study designs, and reliance on associations to identify factors related to diseases and their potential mechanisms.
- **Humoral Immune Mechanisms Involved in Protective and Pathological Immunity During COVID-19.** Dr. Widjaja et al. provide an excellent description of the pathophysiology of complex and often ambiguous manifestations of COVID-19, the pathogenesis of acute lung inflammation, and the dynamics of cytokine production during SARS-CoV-2 infection. The authors also describe B-cell responses, laboratory tests to monitor those responses, and antibody therapy.
- **COVID-19 and Lung Transplantation: From Donors to Recipients ~ Where Are We in 2022?** Dr. Deborah Jo Levine provides an up-to-date overview of COVID-19 and its impact in lung transplantation including donation of lungs, identification of lung transplant candidates who are at risk for developing COVID-19 ARDS, and evaluation organ recipients who are infected with the virus and their transplant outcomes.

In closing, I would like to thank those who have contributed to this issue including the authors of the articles and reports, the Editorial Board, the Publications Committee, Kathy Giovetsis, and *Human Immunology* Editor in Chief, Dr. Amy Hahn, for permitting the *ASHI Quarterly* to reprint articles from the journal. I hope that you enjoy this issue and welcome your suggestions for, and contributions to, future issues. Sharing ideas and expertise is key to continued success in our field.

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# President's Column

Annette M. Jackson, *PhD, F(ACHI)*

Dear ASHI community,

**Happy New Year!** The ASHI Board, Staff, and Committees are looking forward to an exciting and productive 2022. **Click here** to view ASHI committee activities from 2021. Additionally, if you missed it, my last column contained links to different communication venues showcasing ALL ASHI activities for the coming year.

The **ASHI Educational Workshops** (formerly ASHI Regional Education Workshops) have taken on a new name but are still packed with excellent educational opportunities. This year's **first** workshop will be held in person from June 23-25, 2022 in Fort Lauderdale, Florida. A **second** workshop will be held virtually on consecutive Fridays, July 22 and July 29. Both workshops will include a **Technologist Interactive Session on HLA Antibody Analysis** using csv files sent to you in advance and additional case studies presented by stellar ASHI Technologists. **ASHI Travel Awards** will be available, so keep watch for application deadlines.


The **ASHI 48th Annual Scientific Meeting** will be held in Las Vegas from October 24-28, 2022. The Monday **Pre-Meeting Symposium** will be organized by the **Society for Immune Polymorphism** and will highlight new data relating to MHC-associated disease, population genetics, KIR, and much more.

The **Annual Meeting Plenary and Workshop** sessions will cover a broad range of topics including xenotransplantation, cell-based therapeutics, clinical applications from the 18th International HLA Workshop, and best practices for HLA antibody testing and analysis.

I am pleased to recognize the 2022 **Science Technology Initiatives Committee (STIC)** promoting scientific endeavors within ASHI. The first **ASHI Innovation Award** under the category of **Outstanding Original Science** was awarded to **Dr. Eric Weimer** for his *Journal of Molecular Diagnostics* paper on nanopore whole-transcriptome sequencing to elucidate HLA genotyping and HLA allele expression. The second 2022 **ASHI Innovation Award** under the category of **Development of a publicly available informatics or analytical tool, software, web app, or other interpretative resource** was awarded to **Dr. Nicholas Brown** for his innovative work in creating R for HLA data: a suite of tools for managing and analyzing data from clinical HLA laboratory information systems.

Thank you to the entire ASHI staff and ASHI community with special thanks to the **Membership and Marketing Committees** for their work in expanding ASHI membership. **Click here** to see the great news about our ever-growing community. The Membership Committee also developed new and equitable membership categories that include Human Development Index (HDI) rankings to expand HLA educational opportunities worldwide.

ASHI is expanding and engaging clinical and research interests surrounding HLA. Stay tuned to hear more about how **ASHI can add value to your career and prepare you for the future of histocompatibility and immunogenetics**.

Sincerely,

Annette M. Jackson, PhD, F(ACHI)

ASHI President 2021-2022

EDITORIAL

# My Way to HLA

Alix Raymond
Histocompatibility Technologist

I have always had a great interest for science. Science classes were always my favorite classes in school, and I knew pretty early on that I wanted to continue down the science path in my future education. As soon as I took my first biology class in my sophomore year in high school, I was hooked. Constantly amazed by the complexity, coordination, and sophistication of the human body on a molecular level, I always wanted to learn and understand more. Flash forward to college and I was so excited about the number of interesting biology classes offered — many of which had very hands-on lab components to them. This is when I knew that I wanted to work in a lab in the future. I loved the testing process, problem solving, troubleshooting, analyzing and interpreting results, and ways to modify the assay to optimize the results. I thrived in the lab! I always felt comfortable and confident in what I was doing and always wanted a good understanding of the biology going on behind the assay: the cellular interactions, what substances were binding to which receptors, and the result of that interaction.

I left college with a semi-clear picture of what I wanted to do. I knew I wanted to work in a lab, and I knew I wanted to work in the medical field. In my job search during my last semester in school, I submitted my resume to Versiti-WI. I got a call back and set up an interview with another lab at the company that was hiring at the time. Unfortunately, that opportunity did not move forward. A few months later, after I had graduated, I saw that Versiti was hiring lab positions again, so I submitted my application again. I got a call back from HR to discuss opportunities in a few labs that were hiring — one of which was the HLA lab. While I didn't know too much about HLA at the time, I was excited about it because I had just finished an immunology course, which was one of my favorite and most interesting courses I took in college.

I started working in the HLA lab at Versiti in February 2020, right before the COVID-19 pandemic hit in full force. Due to COVID, my training on assays was delayed a couple of months, but I used that time to learn more about the histocompatibility field to better understand the biology behind HLA proteins themselves and the role that they play in solid organ transplantation. After we were able to resume training, I learned the assays for HLA typing, HLA antibody testing, and flow crossmatching quickly. Always having an inquisitive mind, I asked many questions to fully understand everything going on biology-wise in each assay. I very quickly understood the clinical significance of each of the assays and the incredible importance for the accuracy of patient care results.

Even after only working in an HLA lab for just over a year, I have come to realize just how much of a feat of medicine that successful organ transplantation is. There are so many factors involved in the testing leading up to transplant and the immune monitoring afterwards, not to mention the individuality of each person's immune system and the complexities at play there. It is, however, precisely that complexity that intrigues me the most and makes me want to learn more.

I am hoping to continue my career in HLA and organ transplantation and continue learning more and more about this amazing and complex system. However, I also realize that there is a whole area of histocompatibility that I have not yet really been exposed to bone marrow transplantation. Only having started in the HLA lab just over a year ago, my primary learning focus has been on solid organ transplantation, since that is the side of histocompatibility that I work in currently. However, now that I am starting to feel like I have a solid general grasp on the subject, I can branch out and become more familiar with the bone marrow side of HLA. Since I have just recently passed my one-year mark of working in a histocompatibility lab, my next goal is to get my CHT certification. I know that in preparing for this exam, I will be exposed to new information and have many things to learn, but I look forward to this challenge as it brings me closer to having a full understanding of the histocompatibility system. I am truly looking forward to the day that I can call myself an expert in HLA.

# Attention Laboratory Directors in Training!

The American College of Histocompatibility & Immunogenetics (ACHI) requires a robust training plan for new Laboratory Directors. ASHI Standards must be met and training time must be completed at an ASHI or EFI accredited laboratory. The process can sometimes take years, so the ACHI has streamlined the process and is here to help!

**DID YOU KNOW...**

It is important to get registered with the ACHI as soon as you start your training? Visit the links below to get started.

## Resources

We have gathered some exemplary DIT reviewed cases as a model for your own portfolio of cases

## Checking In

We check in with you & your mentor halfway through your training for a progress report and help you start to prepare your portfolio

## Examination

The ACHI exam is one option for required board certification

## Oral Interview

The oral interview is separate from the exam and required for ALL new Directors

**www.ashi-hla.org/ACHI**

**MWeeks@ashi-hla.org**

# The Immunogenetics of COVID-19 Infections, Disease, and Immune Response to Vaccination

Siva Kanangat, *PhD, F(ACHI)*

The COVID-19 pandemic is still persisting with the constant evolution of variants with many varying severities of disease manifestations. Tremendous efforts have been made to induce immunity through mRNA and DNA derived vaccines. Both appear to induce reasonable levels of protective immune responses to alleviate the severity of the disease with repeated immunization sustaining this protective immune response with newly acquired infections in already immunized individuals. This COVID-19 variant that crossed from animals to humans have been relentless in evading immune responses and evolving with tremendous mutations in its surface proteins (spike proteins that help them enter the host cells). A wide variation in severity of infections, in vaccinated and unvaccinated individuals have been noted world-wide. While prevention of viral infection is achieved by neutralizing antibodies that binds to viral spiks, clearance of virally infected cells is mediated by effective CD8+ T cells as well as NK cells. The adaptive antibody responses — i.e., virus-specific neutralizing IgG antibodies — also require cognate help from the virus-specific helper CD4+ T cells. The T cell responses depend on the specific peptides presented in the peptide binding groves of specific HLA alleles. While the HLA alleles by themselves can select peptides of high affinity to their peptide binding grove, several steps that can regulate the selection of the specific peptides that are presented, starting from proteasome processing of the viral proteins, its transportation, regulation of HLA expression etc. Therefore, it is reasonable to assume individual variations in elicitation of virus-specific T cell and antibody responses. With respect to the NK cell mediated antiviral activity HLA alleles play a crucial role in determining the functional status of the NK cells in terms of their ability to inhibit the NK cell function. As the reports emerge, it appears that COVID-19 infections, irrespective of the strains, cause a sort of immune dysregulation with a "cytokine storm" as the prominent manifestation and also in many cases development of or exacerbation of autoimmune diseases due to polyclonal activation of B cells. So, considering all these, it is conceivable that the immunogenetics as a whole drives the course of infection and disease and also the immune responses following vaccinations. Reports along these lines are coming in, although it will become essential to have studies with high statistical power to correlate all aspects of immunogenetics with COVID-19 infection/severity and responses to vaccinations.

## Association Between HLA Alleles and COVID-19 Infection/Disease

The initial reports on COVID-19 infection and severity seem to be dependent on certain HLA alleles/haplotypes — however, concrete proof is yet to emerge. Langton et al., reported on the potential correlation between HLA genotype and severity of COVID-19 infection.[1] These authors using NGS, analyzed the class I and class II classical HLA genes of 147 individuals of European descent experiencing varying degrees of clinical outcomes after proven COVID-19 infections. In all, 49 out of 147 patients with no significant co-morbidities were admitted to the hospital with severe respiratory disease. These patients were compared to 69 asymptomatic hospital workers who had evidence of COVID exposure based on antibody profile with respect to their HLA typing. The authors found significant difference in the allele frequency of HLA-DRB1*04:01 in the severe patient compared to the asymptomatic staff group (5.1% vs. 16.7%, P = .003 after adjustment for age and sex). They also observed a significantly lower frequency of the haplotype DQA1*01:01-DQB1*05:01-DRB1*01:01 in the asymptomatic group (P = .007). These authors claimed significant influence of DRB1*04:01 on the clinical severity of COVID-19. As the authors rightly pointed out, the complexity of the genetic architecture of the immune system across different geographies and ethnicities needs to be considered.

Castelli et al.,[2] studied 83 Brazilian couples where one individual was infected and symptomatic while the partner was asymptomatic and antibody negative for at least six months despite sharing everything during the infection. The whole-exome sequencing followed by a state-of-the-art method to call genotypes and haplotypes across the highly polymorphic major histocompatibility complex (MHC) region was done. The couples had comparable ages and genetic ancestry with the exception that women were overrepresented (65%) in the asymptomatic group. The authors observed an association between HLA-DRB1 alleles encoding Lys at residue 71 (mostly DRB1*03:01 and DRB1*04:01) and DOB*01:02 with symptomatic infections and *HLA-A* alleles encoding 144Q/151R with asymptomatic seronegative women. With respect to other genes of immune modulation, the authors observed variants in *MICA* and *MICB* associated with symptomatic infections in terms of higher expression of soluble MICA and low expression of MICB. The quantitative differences

in these molecules may modulate natural killer (NK) activity leading to susceptibility to COVID-19 by downregulating NK cell cytotoxic activity in infected individuals. This study highlights the importance of potential T and NK cell effects on virally invaded cells.

Another study by Littera R et al.,[3] examined clinical, genetic and immunogenetic factors, emphasizing HLA class I and II molecules, to evaluate their influence on susceptibility to COVID-19 infection and outcome. They had 619 healthy Sardinian controls and 182 COVID-19 patients, and 39 patients required hospital care and 143 were asymptomatic or pauci-symptomatic or with mild disease. The HLA allele and haplotype frequencies were recorded. These authors found that hospitalized patients had a higher frequency of autoimmune diseases and glucose-6-phosphate-dehydrogenase (G6PDH) deficiency. Interestingly, the extended haplotype HLA-A*02:05, B*58:01, C*07:01, DRB1*03:01 [OR 0.1 (95% CI 0–0.6), Pc = 0.015] was absent in all 182 patients, while the HLA-C*04:01 allele and the three-loci haplotype HLA-A*30:02, B*14:02, C*08:02 [OR 3.8 (95% CI 1.8–8.1), Pc = 0.025] were more frequently represented in patients compared to controls. Also, among in-patients and home care patients, the HLA-DRB1*08:01 allele was exclusively present in the hospitalized patients [OR > 2.5 (95% CI 2.7–220.6), Pc = 0.024]. This study in a defined population shows that the extended haplotype HLA-A*02:05, B*58:01, C*07:01, DRB1*03:01 may have a protective effect against SARS-CoV-2 infection in the Sardinian population.

## More Defined Role of Immunogenetics in COVID-19 Infection/Disease Severity

The research report by Eric de Sousa et al., described more focused role of HLA class I and class II presentation of COVID-19 and its variants (antigenic peptides) that could potentially determine the outcome of the infection/disease.[4] These authors concluded that the SARS-CoV-2 genome may change peptide binding to the most frequent MHC-class I and -II alleles in Africa, Asia, and Europe. They studied how a single mutation in the wildtype sequence of SARS-CoV-2 could influence the peptide binding of SARS-CoV-2 variants to MHC class II, but not to MHC class I alleles. It is possible that selective pressure from MHC class II alleles may select for viral variants and subsequently affect the quality and quantity of cellular immune responses against COVID-19. This is an extremely important finding that needs to be further explored in order to understand the immunogenetics of COVID-19 pathogenesis in the short-term and long-term basis.

Furthermore, getting into the mechanisms of HLA association and COVID-19 infections in relation to variabilities in severity, Copley et al., reported on the influence of HLA-Class II polymorphisms in cellular responses towards COVID-19 infections.[5]

It is known that the development of adaptive immunity after COVID-19 infection and vaccination depend on the recognition of viral peptides, presented on HLA class II molecules, by CD4+ T-cells. Virally infected cells of course can elicit class I response. These authors used extensive high-resolution HLA data on 25 human race/ethnic populations to investigate the role of HLA polymorphism on SARS-CoV-2 immunogenicity at the population and individual level. They emphasized the role of HLA on development of protective immunity after SARS-CoV-2 infection and after vaccination and a firm basis for further experimental studies in this field.

However, another study also showed the mechanistic aspects of HLA dependent severity of COVID-19. Desterke et al., reported the importance of HLA on the heterogeneous outcomes in lung disease due to COVID-19.[6] Desterke et al., studied the Transcriptome and single cell of COVID-19 lungs and integrated with deep learning analysis of MHC class I immunopeptidome against SARS-COV-2 proteome. This analysis indicated that activation of MHC class I antigen presentation in these tissues was correlated with the amount of COVID-19 RNA present. The authors also observed a positive correlation in these samples between the level of COVID-19 and the expression of a genomic cluster located in the 6p21.32 region and in the MHC-II cluster that could influence the immunoproteasome structure and functions. These authors concluded that HLA-dependent heterogeneity in macrophage immunoproteasome activation during lung COVID-19 disease could determine the effectiveness of the immune responses to various types of vaccines floating around the globe claiming effectiveness.

## The indirect effect of COVID-19 infection related to its pathogenicity.

Finally, the report by Vanderbeke L et al.,[7] based on epidemiological and clinical reports indicate that SARS-CoV-2 virulence depends on initiating an abnormal immune response, in addition to the virally induced direct cell damage. These authors performed cytokine and multiplex immune profiling in COVID-19 patients and showed hypercytokinemia in COVID-19 in critical versus mild-moderate COVID-19. The deep immune profiling demonstrated that this hypercytokine production was driven by macrophages and also resulted in reduced T cells. Furthermore, these authors noted that antigen presenting system was impaired in critical COVID-19 disease. In addition, neutrophils were associated with severity and tissue damage, further enhancing cytokine production. Mahdi illustrated that how COVID-19 infection leads to type III hypersensitivity reaction.[8]

Furthermore, we should not forget the influence of NK cells on viral infections as an innate immune response. There are NK cells and NKT cells and these are also highly regulated by various activating and inhibitory receptors. The immunogenetics of these important partners of innate immunity and the bridge between innate and adaptive immunity also needs to be considered in order to fully understand the immunoprotection and immunopathogenesis of COVID-19 infections/disease severity and the emergence of variants.

## Conclusions

The COVID-19 pandemic has gone on for probably longer than the experts had expected. Interestingly it appears that there are more variations in terms of disease severity with this viral infection compared to many of the other respiratory viral infections. The emerging facts that the pathogenicity is related to host immune response, especially hypercytokinemia rather than direct virally induced damage to the host cells, deserve further deeper investigations into all aspects of immunogenetics of this particular viral infection.

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# The Unexpected Hassles During a Kidney Transplant Crossmatch

By Runying Tian, *CHS(ACHI),* and Dongfeng Chen, *PhD, F(ACHI)*

The HLA lab serves as a gatekeeper to assess transplant risk by providing adequate resolution of HLA typing[1-3] and accurate unacceptable antigen profiles to make sure that organs are allocated to compatible recipients. Our daily lab work constantly challenges our knowledge and occasionally brings to light limitations of current assays and analysis strategies. A case of a highly sensitized potential kidney recipient is reported herein to demonstrate some of those challenges.

A 67-year-old African American female homozygous for DR11 and DQ7 was evaluated for a second deceased donor kidney transplant. HLA typing was performed using PCR-rSSOP and RT-PCR methods (One Lambda Inc). HLA antibody screen and specificity were done by FlowPRA (One Lambda Inc.) and Luminex single antigen bead (SAB) assays (One Lambda and Immucor). All identified HLA antibodies were listed as unacceptable antigens in UNet. Her prior transplant in 2003 was mismatched for DR1, DR15, and DR51, and she was highly sensitized with a 99% on class II Flow PRA at the time of the current offer.

A potential donor with a reported typing of DR11, 13, and DQ7, was tested in the flow cytometry crossmatch assay, which gave an unexpected T cell negative, B cell positive result. Initially, it appeared that there was no DSA present, but further investigation into DR11 (self) reactivity observed on the SAB led to the question, "Is the anti-DR11 reactivity real or background?"

The recipient did not have any known autoimmune disease, and recipient cells were not available for an auto-crossmatch at the time of the offer. The DR11 antigen bead reactivities on the SAB panel were DRB1*11:01 (MFI=2891) and DRB1*11:04 (MFI=2245) for One Lambda, DRB1*11:01/03/04 (average MFI=4070) for Immucor (Figure 1).

The patient's most likely typing was DRB1*11:02, based on the rSSOP and RT-PCR typing results. Eplet analysis of DR unacceptable antigens (DR1, DR103, DR8, DR9, DRB1*12:02, DR15, DR16, DR51, DR53) was performed in Fusion. The difference between DRB1*11:02 and DRB1*11:01/04 alleles is at position 67 (67I vs 67F [Figures 2 and 3]). DRB5*01:01, containing 67F, might be the immunizer since the previous donor was DR51 mismatched. This recognized eplet reactivity also explains why the DRB1*12:02 bead was positive in the single antigen assay, but DRB1*12:01 was negative (Figure 3).

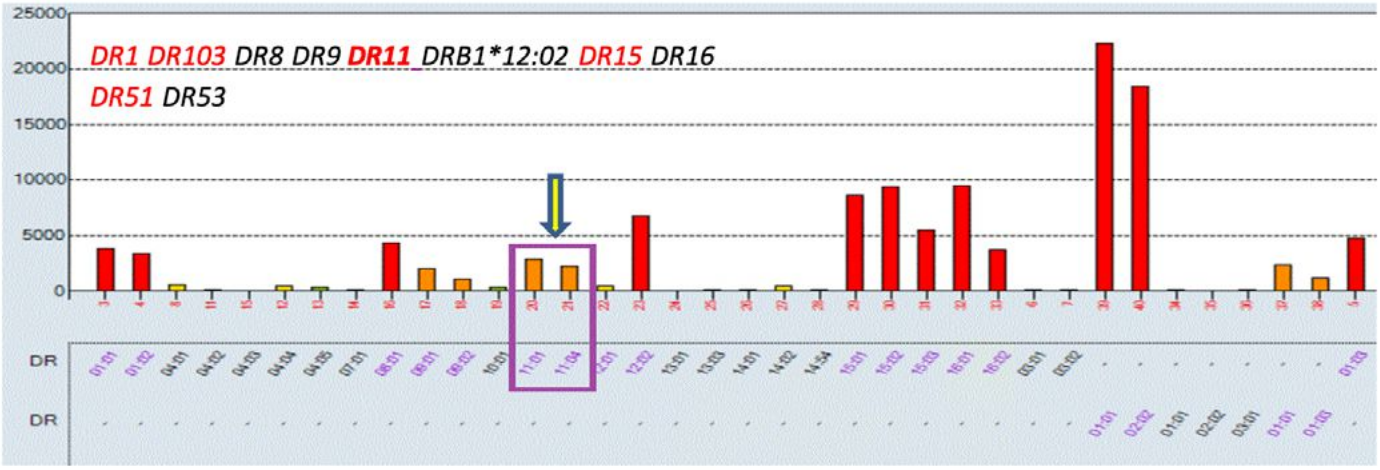
High resolution HLA typing by NGS (Immucor) showed DRB1*11:02 homozygosity for the recipient and DRB1*11:01, 13:03 for donor. The results indicated that the patient had allele specific antibodies reactive to the donor DR11 alleles but likely not to the self DRB1*11:02 allele. DRB1*11:02, which is less frequent in US population, is not represented on the SAB panels or One Lambda's supplemental SAB panel. DR11 was listed in UNOS for the recipient HLA typing because DRB1*11:02 was not represented in the UNOS list. DRB1*11:01/03/04 were listed as unacceptable antigens after the investigation.

This case exposes several limitations of current testing methodologies: 1) The lack of high-resolution typing of potential donors; 2) Incomplete HLA allele representation on SAB assays; 3) Limitations of UNet HLA typing entry choices.[4] When these problems are compounded, the unexpected hassles were experienced.

*Note: This work was done at the Clinical Transplantation Immunology Laboratory at Duke University Medical Center. Duke HLA lab is currently providing high resolution typing for all solid organ candidates and utilization of eplet-based HLA antibody analysis to be prepared for potential similar challenges in the future.*

FIGURE 1.

## Luminex Single Antigen Results (One Lambda on the top and Immucor at the bottom)




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6096 | 6212 | 11.00 | 4913 | 5007 | Positive | DRB1*08:02 | | | | | DR8 | |
| 5854 | 5951 | 6.41 | 4906 | 4987 | Positive | | DQA1*04:01 | DQB1*04:01 | | | | DQ4 |
| 5100 | 5232 | 6.32 | 4794 | 4918 | Positive | | DQA1*02:01 | DQB1*03:02 | | | | DQ8(3) |
| 7867 | 8042 | 9.69 | 4311 | 4407 | Positive | DRB1*11:04 | | | | | DR11(5) | |
| 7420 | 7559 | 9.19 | 4103 | 4180 | Positive | DRB1*11:01 | | | | | DR11(5) | |
| 3415 | 3520 | 8.41 | 3713 | 3827 | Positive | DRB4*01:01 | | | | | DR53 | |
| 4875 | 4919 | 8.03 | 3622 | 3655 | Positive | DRB1*08:01 | | | | | DR8 | |
| 5359 | 5440 | 7.96 | 3569 | 3623 | Positive | DRB1*11:03 | | | | | DR11(5) | |
| 2981 | 3033 | 7.75 | 3467 | 3528 | Positive | DRB3*01:01 | | | | | DR52 | |

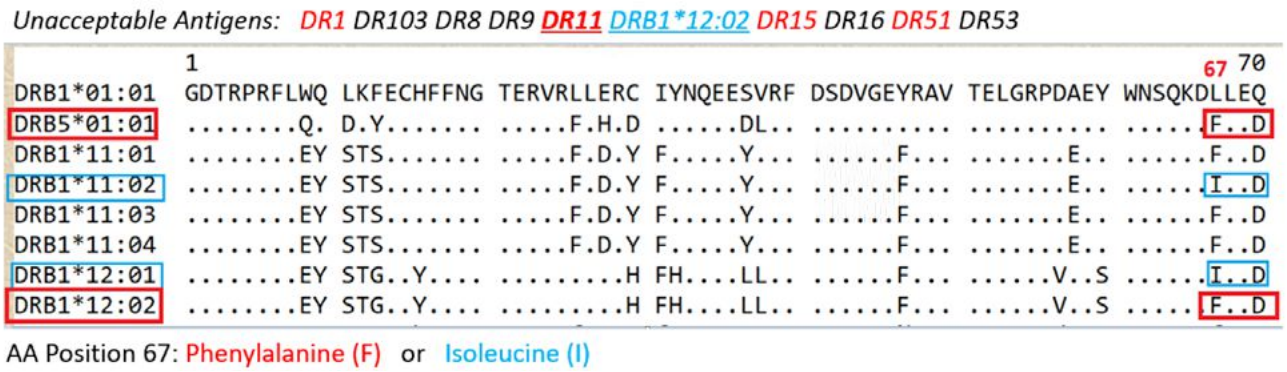
FIGURE 2.

## Amino Acid Alignment of All the Patient's DR Unacceptable Antigens

```
                1                                                                                                   67
DRB1*01:01  GDTRPRFLWQ LKFECHFFNG TERVRLLERC IYNQEESVRF DSDVGEYRAV TELGRPDAEY WNSQKDLLEQ RRAAVDTYCR
DRB1*01:02  .......... .......... .......... .......... .......... .......... .......... ..........
DRB1*01:03  .......... .......... .......... .......... .......... .......... ......I..D E.........
DRB1*08:01  ........EY STG..Y.... .....F.D.Y F.....Y... .......... ......S... ......F..D ...L......
DRB1*09:01  ...Q....K. D......... .....Y.H.G ......N... .......... ......V..S ......F..R ...E...V..
DRB1*09:02  ------...K. D......... .....Y.H.G ......N... .......... .......... ......F..R ...E...V..
DRB1*10:01  ........EE V......... .........R VH....YA.Y .......... .......... .........R ..........
DRB1*11:01  ........EY STS....... .....F.D.Y F.....Y... ......F... .......E.. .....F..D ..........
DRB1*11:02  ........EY STS....... .....F.D.Y F.....Y... ......F... .......E.. .....I..D E.........
DRB1*11:03  ........EY STS....... .....F.D.Y F.....Y... ......F... .......E.. .....F..D E.........
DRB1*11:04  ........EY STS....... .....F.D.Y F.....Y... ......F... .......E.. .....F..D ..........
DRB1*12:01  ........EY STG..Y.... .........H FH....LL.. ......F... ......V..S ......I..D ..........
DRB1*12:02  ........EY STG..Y.... .........H FH....LL.. ......F... ......V..S ......F..D ..........
DRB1*15:01  .......... P.R....... .....F.D.Y F......... ......F... .......... ......I... A.........
DRB1*15:02  .......... P.R....... .....F.D.Y F......... ......F... .......... ......I... A.........
DRB1*15:03  .......... P.R....... .....F.D.H F......... ......F... .......... ......I... A.........
DRB1*16:01  .......... P.R....... .....F.D.Y F......... .......... .......... ......F..D ..........
DRB1*16:02  .......... P.R....... .....F.D.Y F......... .......... .......... .........D ..........
DRB4*01:01  ...Q....E. A.C....L.. ....WN.I.Y ......YA.Y N..L...Q.. .......... .........R ...E......
DRB4*01:03  ...Q....E. A.C....L.. ....WN.I.Y ......YA.Y N..L...Q.. .......... .........R ...E......
DRB5*01:01  ........Q. D.Y....... .....F.H.D ......DL.. .......... .......... ......F..D ..........
DRB5*02:02  .....C..Q. D.Y....... .....F.H.G ......N... .......... .......... ......I... A.........
```

FIGURE 3.

Eplet Analysis of DR Unacceptable Antigens, Highlighting DR11 and DR12 Allele.

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Research article

# Antigen presentation in SARS-CoV-2 infection: the role of class I HLA and ERAP polymorphisms

Irma Saulle [a,b,*,1], Chiara Vicentini [a,1], Mario Clerici [b,c], Mara Biasin [a]

[a] Department of Biomedical and Clinical Sciences- L. Sacco, University of Milan, Italy
[b] Department of Pathophysiology and Transplantation, Milan, Italy
[c] SM Nascente Scientific Institute, IRCCS, Don C Gnocchi Foundation, Milan, Italy

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ABSTRACT

Given the highly polymorphic nature of Human Leukocyte Antigen (HLA) molecules, it is not surprising that they function as key regulators of the host immune response to almost all invading pathogens, including SARS-CoV-2, the etiological agent responsible for the recent COVID-19 pandemic. Several correlations have already been established between the expression of a specific HLA allele/haplotype and susceptibility/progression of SARS-CoV-2 infection and new ones are continuously emerging. Protective and harmful HLA variants have been described in both mild and severe forms of the disease, but considering the huge amount of existing variants, the data gathered in such a brief span of time are to some extent confusing and contradictory. The aim of this mini-review is to provide a snap-shot of the main findings so far collected on the HLA-SARS-CoV-2 interaction, so as to partially untangle this intricate yarn. As key factors in the generation of antigenic peptides to be presented by HLA molecules, ERAP1 and ERAP2 role in SARS-CoV-2 infection will be revised as well.


## 1. Introduction

Infectious diseases are still a significant challenge for public health worldwide, as they are responsible for millions of deaths, mainly, but not only, among older adults and immunosuppressed or chronically ill people. The virulence of the pathogen and the efficacy of the host immune response are the key factors conditioning the onset and progression of infectious diseases. In the context of adaptive immunity, a pivotal role in protection and recovery from infections is played by CD8+ T lymphocytes [1]. These cells detect antigenic peptides bound by major histocompatibility complex (MHC) class I molecules, known as the human leukocyte antigen (HLA) class I in humans, and work through different pathways in order to eradicate the pathogen and the infected cells.

Given the major role played by CD8+ T cells in host immunity, it is conceivable that plenty of studies have tried to decipher the consequences of a jam in CD8+ T cell activation in their response to pathogens. In this setting, since the very first report establishing a correlation between HLA-B27 and Ankylosing Spondylitis [2,3], the MHC has been recognized as the region of the genome that is associated with the highest number of human diseases [4]. For this same reason, allelic variants and altered MHC expression have been associated with disease severity following infection with several microbes. In parallel, aminopeptidases – in particular endoplasmic reticulum aminopeptidase 1 (ERAP1), ERAP2 and partly insulin-regulated aminopeptidase (IRAP) – have drawn the scientific attention as well. Indeed, they are responsible for antigenic peptide trimming within the ER, thus conditioning the antigen processing pathway [5] in both physiological and pathological contexts including those mediated by infectious agents, among which severe acute respiratory disease coronavirus 2 (SARS-CoV-2) [6].

*Abbreviations:* SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; MHC, major histocompatibility complex; HLA, between Human Leukocyte Antigen; ERAP, endoplasmic reticulum aminopeptidase; WHO, World Health Organization; COVID-19, coronavirus disease 2019; MERS-CoV, Middle East respiratory syndrome coronavirus; ACE2, angiotensin-converting enzyme-2 receptor; ARDS, acute respiratory distress syndrome; IRAP, insulin-regulated aminopeptidase; RDB, receptor-binding domain; TMPRSS2, type II transmembrane serine protease; TAP, transporter associated with antigen processing; GWAS, genome-wide association study; SNP, single nucleotide polymorphism, NMD, nonsense-mediated-decay; ERGIC, ER Golgi Intermediate Compartment; ER, endoplasmic reticulum; CTL, Cytotoxic T Lymphocytes.

* Corresponding author at: Department of Biomedical and Clinical Sciences- L. Sacco, University of Milan, Via G.B.Grassi 74, 20157 Milan, Italy.
*E-mail address:* irma.saulle@unimi.it (I. Saulle).
[1] These authors equally contributed to this work.

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0198-8859/

Since SARS-CoV-2 outbreak, the pathogen responsible for **CO**rona**VI**rus **D**isease **19** (COVID-19), the scientific community has tried to identify those factors controlling the susceptibility/outcome of the disease and playing a major role in determining the appearance of acute respiratory distress syndrome (ARDS), the life-threatening form of infection [7]. This virus belongs to the family of Coronaviruses, responsible for other two epidemics over the last twenty years: SARS-CoV (Severe Acute Respiratory Syndrome) in Asia in 2003 and MERS-CoV (Middle East Respiratory Syndrome Coronavirus) in Arabian Peninsula in 2012. As an obligate intracellular parasite, SARS-CoV-2 replicates inside the host cells exploiting nucleic acid and protein synthesis mechanisms to facilitate its spreading from one individual to another. The biology of the virus, its infectious and replicative cycle, as well as the human host factors directly or indirectly contributing to its maintenance/annihilation within cells have been exhaustively described elsewhere [8,9]. Throughout these phases, viral proteins can be unfolded, degraded and further processed by cytosolic and nuclear proteasomes inside host cells; the resulting peptides, 8–16 amino acid long, are then transported into the endoplasmic reticulum (ER) by transporter associated with antigen processing (TAP). Herein, they are further trimmed by ERAP1 and ERAP2 proteins in order to achieve the optimal length to be loaded onto the clefts of MHC class I molecules. In turn, MHC class I molecules, by loading intracellular compartment-derived antigens, provide a recapitulation of the events occurring inside the cell, thus allowing CD8+ T cells to monitor possible ongoing infections (Fig. 1).

Given the direct participation of ERAP proteins in the antigen presentation pathway of MHC class I, this manuscript primarily aims to review the data correlating antigen presentation by MHC class I molecules and COVID-19 susceptibility/severity so far reported in the scientific literature by in silico and genetic-association studies. Noteworthy, data concerning the role displayed by MHCII and non-classical HLA in SARS-CoV-2 infection will be reported as well. Although this topic has already been reviewed by other authors [10,11], due to the rising global attention on the current pandemic, researchers are rapidly accumulating an incredible amount of data and an updated revision of the literature is constantly necessary.

## 2. Antigen presentation by HLA class I in SARS-CoV-2 infection

HLA are highly polymorphic molecules that play a key role in individual genetic susceptibility to human diseases including those provoked by infectious agents [6,12]. For example, in HIV-1 infection different HLA – mainly HLA-A*29, HLA-B*27, HLA-B*35 and HLA-B*57 – were shown to be correlated with both susceptibility/progression of the disease [13]. Notably, as reviewed in early June 2020 in a work by Ovsyannikova and colleagues, a collection of data provides evaluations of the role of host genetics - including variation in HLA genes - in the immune response to Coronaviruses, among others SARS-CoV-2 [14]. In contrast to what had emerged in studies concerning SARS-CoV [15,16] and MERS-CoV [17,18], correlations between SARS-CoV-2 and HLA are still not entirely defined and results so far obtained are sometimes contradictory. Herein we report the so far collected data on this issue (Table 1).

### 2.1. Genetic associations on case-control and cohort studies

Because of the relatively recent occurrence of SARS-CoV-2, quite limited published studies have explored the relative frequencies of HLA alleles in case-control, cohort, and observational studies. The results so far obtained are not univocal and limited by relatively small sample size and by heterogeneity in study design. Moreover, in most cases the reported results are merely associative and the mechanism of action possibly displayed by different HLA alleles in SARS-CoV-2 antigen presentation remains elusive. Nonetheless, comparison with previous coronavirus infections and correlation with clinical symptoms may provide essential information prior to a specific mandatory multicenter study to be performed, in the attempt to identify biomarkers of susceptibility/progression of SARS-CoV-2 infection.

One of the first studies in the field documented HLA-A*24:02 to be correlated with SARS-CoV-2 susceptibility. This allele was found to be expressed in four out of five patients from Wuhan, during the first stages of the pandemic [19]. HLA-A*24:02 frequency in Chinese population is typically 17.2%, a value that is significantly lower than the observed frequency (80%) reported in the infected patients enrolled in the study. As it is conceivable, considering the extremely small sample size, the study lacks statistical power and requires validation in larger sample cohorts. However, soon after the publication of these results, Tomita *et al.* reported an association between HLA-A*02:01 and an increased risk for COVID-19. HLA-A*02:01, indeed, showed a relatively lower capacity to present SARS-CoV-2 antigens compared with other frequent HLA class I molecules, mainly HLA-A*11:01 or HLA-A*24:02. Therefore, the authors suggest that subjects carrying HLA-A*11:01 or HLA-A*24:02 genotypes may trigger a more efficient T cell-mediated antiviral responses to SARS-CoV-2 compared to HLA-A*02:01 [20]. A subsequent study conducted by Yung *et al.* determined a positive association between HLA-B*22 serotype with SARS-CoV-2 susceptibility in 190 Hong Kong Chinese patients [21].

In order to define the HLA haplotypes associated with susceptibility to COVID-19 disease, a group of Italian researchers – through a geographical epidemiological analysis – focused on describing the pattern of distribution of the two most common HLA haplotypes (HLA-A*01:01g-B*08:01g-C*07:01g-DRB1*03:01g and HLA-A*02:01g-B*18:01g-C*07:01g-DRB1*11:04g) in the Italian population [22]. They discovered that the huge incidence of infection and mortality rate in the northern regions of Italy correlates with high frequency of HLA-A*01:01g-B*08:01g-C*07:01g-DRB1*03:01 g haplotype, suggesting that such haplotype is a potential 'susceptibility' marker of the disease. Contrariwise, a lower incidence and mortality for COVID-19 were observed in the central-southern regions of the country, where higher frequency values of the HLA-A*02:01g-B*18:01g-C*07:01g-DRB1*11:04g were reported, allowing to speculate on a defensive mechanism towards SARS-CoV-2 infection elicited in subjects carrying this haplotype. Other possible explanations to such a differing mortality rate from north to south of Italy – e.g. climatic differences, migration and pollution – were considered by the authors. Nevertheless, none of these factors seemed to be that significant. Indeed, no substantial climatic differences distinguish the northern and southern regions, and atmospheric emissions of PM10, PM2.5 and $NO_2$ are actually higher in the southern areas. Finally, the uncontrolled north–south exoduses, which took place just before the beginning of the lockdown, did not result into the spreading of the infection in the southern Italian regions as expected.

Another study conducted by Novelli and colleagues [23], on a small sample of 99 Italian patients affected by a severe or extremely severe form of COVID-19, investigated the HLA allele frequency distribution, in order to identify variants possibly associated to a worst COVID-19 outcome. Despite the restricted sample size, the researchers found a strong correlation for *HLA B*27:07*, *DRB1*15:01*, *DQB1*06:02* alleles after comparing the results to a reference group of 1017 Italian individuals. Notably, these data are in line with those published by Kachuri *et al.* recently identifying DRB1 and DQB1 as key genetic factors controlling host susceptibility to viral infections [24]. Even more recently, HLA class I typing was performed within a pilot study on 45 Spanish patients with different COVID-19 symptoms severity [25]. The

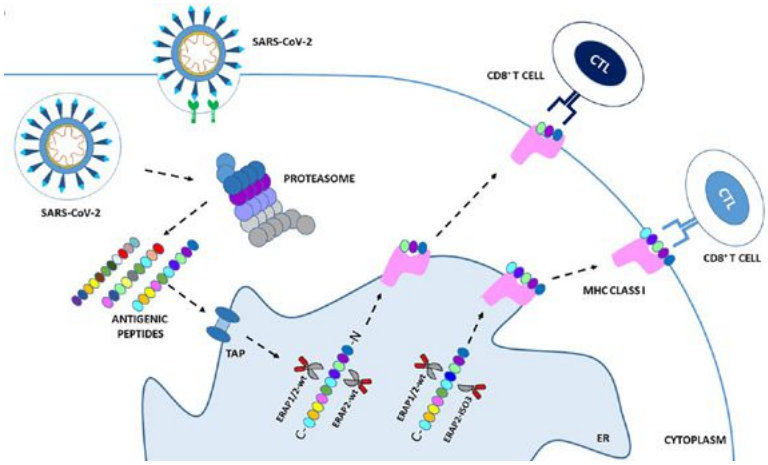


**Fig. 1.** Schematic overview of the MHC-I antigen processing and presentation pathway in SARS-CoV-2 infection. Following SARS-CoV-2 infection rs2248374-A ERAP2 expressing cells produce wild type ERAP2 (ERAP2-wt) which can homodimerize or heterodimerize with ERAP1-wt (ERAP2-wt + ERAP2-wt; ERAP1-wt + ERAP2-wt), in order to process viral antigens to be presented on cell surface for recognition by specific CD8+ cytotoxic T lymphocyte (CTL) clones. Rs2248374-G ERAP2 expressing cells may also produce an alternative spliced isoform: ERAP2-ISO3. This variant, unlike ERAP2-wt, lack the catalytic domain but can still heterodimerize with both ERAP2-wt and ERAP1-wt. As a result, these unconventional heterodimers (ISO3 + ERAP2-wt; ISO3 + ERAP1-wt) may process viral antigens differently from the canonical ones, generating an alternative antigenic repertoire. This in turn may activate other CTL clones possibly triggering a more or less protective immune system response. ER: Endoplasmic reticulum; TAP:

results obtained suggest that patients exhibiting a mild form of the disease presented HLA class I molecules characterized by a higher binding affinity to SARS-CoV-2 peptides and showed a higher percentage of heterozygous HLA molecules compared to patients exhibiting moderate and severe symptoms. In addition, the authors stressed the fact that theoretically protective alleles of HLA, such as HLA-B*15:03, were found in patients who died because of a severe evolution of the disease; while, on the other hand, alleles showing low affinity for the viral peptides, such as HLA-A*25:01, were present in patients with a moderate evolution of the disease. Their observation suggests that studying the affinity of the entire HLA genotype for SARS-CoV-2 may be more fruitful than focusing on the specific positive or negative roles of different HLA alleles.

Lorente *et al.* analysed a total of 3886 healthy controls and 72 COVID-19 patients (10 non-survivor and 62 survivor patients at 30 days) and showed that there was a higher frequency of HLA-A*32 alleles in healthy controls than in COVID-19 patients. conversely, HLA-B*39 and HLA-C*16 were more represented in COVID-19 patients compared to healthy controls. However, the correlation did not reach statistical significance after correction for multiple parameters [26].

Sakuraba *et al.*, by analyzing the frequency of HLA allele in 74 countries from the Allele Frequency Net Database and worldometer.info, in order to investigate the association between class I MHC, HLA-A, -B and -C, and the risk of death due to SARS-CoV-2 infection, found HLA-C*05 allele to be potentially correlated with mortality at a global level [27]. HLA-C molecules work as killer cell immunoglobulin-like receptor (KIR) ligands [28]. The latter are highly polymorphic receptors expressed on natural killer (NK) and T-cells membranes and are distinguished into activating or inhibitory ones, depending on the length of their cytoplasmatic tail [29]. KIRs indeed control the inhibition and activation of cell responses by recognizing polymorphic motifs on HLA I molecules (i.e. HLA-A*03, HLA-A*11, HLA-Bw4, HLA-C1, HLA-C2) expressed on target cells, thus playing a crucial role in regulating the innate immune defense against cancerous cells, adaptive immune responses as well as viral infections [30]. An example is represented by the interaction between KIR3DL1/S1 and HLA-Bw4 and different outcome of HIV-infection as reviewed in [31]. In Sakuraba work, the authors hypothesized that this HLA-KIR combination could lead to immune over-activation, subsequently causing negative selection. Indeed, populations with the highest mortalities (France, Italy and Spain) were demonstrated to present the greatest number of carriers for HLA-C*05 and its receptor *KIR2DS4fl*. Therefore, patients with a HLA-C*05 and *KIR2DS4fl* pair may be predisposed to develop an excessive cytokine response and suffer from hyper-cytokinemia, strongly associated to severe forms of the disease and COVID-19 mortality [27]. Incidentally, the central role of HLA-C in virus immune-escape has already been documented, as reported for example by Fredj and colleagues in a study showing an increase in human herpesviruses (HHV) risk of infection in KIR2DL2 and HLA-C1 positive multiple sclerosis (MS) patients [32]. HLA-C1 molecules may induce inhibitory signals in KIR2DL2 positive NK cells from these patients, thus creating an anergic environment with very low levels of IFNγ and consequent lack of NK cell activation and of innate protection to virus infection [33]. Such correlation was more recently associated with an increased susceptibility to HHV-6A infection in patients with a severe Alzheimer's Disease (AD) status [34].

As was to be expected, a more complete picture of the features of the immune response to SARS-CoV-2 was gained through large-scale genome-wide association studies (GWAS) and biological validation studies based on larger cohorts of patients. Ellinghaus *et al.* performed a GWAS on 1980 COVID-19 patients enrolled in Spain and Italy. The authors found an association between two regions in the human genome and the virus-induced respiratory failure [35]. One of them is located on chromosome 3 in an area which includes six genes, namely SLC6A20, LZTFL1, FYCO1, CXCR6, XCR1, CCR9. Some of them encode chemokine receptors, while SLC6A20 is translated into a protein with transportation functions, which has been demonstrated to interact with ACE2. The other identified locus is situated within the ABO blood groups locus on chromosome 9, assigning to blood type O a protective role against the disease and, on the other hand, linking group A to a more severe form of the disease. This finding is consistent with other studies reporting similar results [36–38]. However, any link between HLA

**Table 1**
HLA variants involved in susceptibility to SARS-CoV-2 infection and/or in disease severity.

| HLA variants | Associated to | Mechanism of action |
|---|---|---|
| *Genetic association on case-control and cohort studies* | | |
| HLA-A*24:02 | SARS-CoV-2 susceptibility | Unknown [19] |
| HLA-A*02:01 | Increased risk for severe COVID-19 outcome | Lower capacity to present SARS-CoV-2 antigens [20] |
| HLA-A*11:01, HLA-A*24:02 | Protection against COVID-19 | More efficient T cell-mediated antiviral responses to SARS-CoV-2 [20] |
| HLA-B*22 | Susceptibility marker for SARS-CoV-2 | Unknown [21] |
| HLA-A*01:01 g-B*08:01 g-C*07:01 g-DRB1*03:01 g | Susceptibility marker of SARS-CoV-2 infection and severe COVID-19 outcome | Unknown [22] |
| HLA-A*02:01 g-B*18:01 g-C*07:01 g-DRB1*11:04 g | Protection against SARS-CoV-2 infection | Unknown [22] |
| HLA-B*27:07, HLA-DRB1*15:01, HLA-DQB1*06:02 | Worst COVID-19 outcome | Unknown [23] |
| HLA-DRB1, HLA-DQB1 | Higher susceptibility to COVID-19 | Unknown [24] |
| HLA-B*15:03 | Severe evolution of COVID-19 | Unknown [25] |
| HLA-A*25:01 | Moderate evolution of COVID-19 | Unknown [25] |
| HLA-A*32 | Higher frequency in healthy controls | Unknown [26] |
| HLA-B*39, HLA-C*16 | More represented in COVID-19 patients | Unknown [26] |
| HLA-C*05 | Higher mortality | Unknown [27] |
| HLA-DRB1*08 | Higher risk of and death | Unknown [36] |
| HLA-E*0101 | High severity of COVID-19 | Lower NKG2C + NK cell response [66] |
| *Bioinformatic* in silico *epitope prediction studies* | | |
| HLA-B*15:03 | Protection against SARS-CoV-2 | High presentation of SARS-CoV-2 immunogenic epitopes [47] |
| HLA-B*46:01 | Severe symptoms | Reduced presentation of SARS-CoV-2 peptides [47] |
| HLA-A*02:02, HLA-A*11:01, HLA-B*40:01, HLA-B*35:01 | Lower risk for severe COVID-19 | High capacity to present SARS-CoV-2 antigens [49] |
| HLA-DRB1*01 | Fatality rate in hospitalized patients | Unknown [51] |
| HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*23:01, HLA-A*24:02, HLA-A*26:01, HLA-A*30:02, HLA-A*31:01, HLA-A*68:01, HLA-B*07:02, HLA-B*18:01, HLA-B*35:03, HLA-B*38:01, HLA-B*44:02, HLA-B*44:03, HLA-B*51:01, HLA-C*05:01, HLA-C*07:01, HLA-C*07:02, HLA-C*08:02, HLA-C*15:02, HLA-C*17:01 | Protection against COVID-19 | Strong binding to SARS-CoV-2 peptides [52] |

alleles and disease susceptibility or severity was found in Ellinghaus' study. As genetic hyper-polymorphism across MHC locus differentiates thousands of HLA alleles and the clinical manifestations may significantly differ between affected patients, it is possible that the sample size analyzed in this study was not large enough to identify any statistically significant association.

The role of MHC class II molecules, which intervene in antigen presentation to helper CD4+ T cells to facilitate the humoral immune response, has been investigated as well, in relation to SARS-CoV-2 infection. Some of the results obtained have already been reported in the above paragraphs [22,23], however the most encouraging findings seem to be related to HLA-DR expression levels, as its dowregulation – mainly in monocytes – is often associated with a dysregulated immune response, [39,40] even in SARS-CoV-2 infection as recently reviewed in [41].

In this perspective, Giamarellos-Bourboulis *et al.* [42] performed a well-designed study on 54 COVID-19 patients, who showed hyper-inflammatory reactions in the form of either macrophage activation syndrome (MAS) or immune dysregulation. The latter was characterized by lower expression of HLA-DR on CD14 + monocytes, secondary to monocyte hyperactivation, excessive release of interleukin-6 (IL-6), and severe lymphopenia. The leading hypothesis was that IL-6 was responsible for the reduced level of HLA-DR on CD14+ monocytes. The increase of circulating HLA-DR+ cells during the healing period of one patient with a moderately severe infection from SARS-CoV-2 further endorsed this hypothesis [43]. In conclusion, they identified a new feature of immune dysregulation in SARS-CoV-2 patients, which supports the rationale of clinical trials – which were ongoing at the time this work was published – based on the use of Anakinra, Sarilumab, Siltuximab, and Tocilizumab to hamper the production of inflammatory cytokines in these patients. In another study conducted by Amoroso and colleagues [36], they analyzed HLA-A, B, and DRB1 frequencies on a sample of 40 904 individuals (32294 transplant recipients and 8610 waitlisted patients) and found a higher frequency of HLA-DRB1*08 in COVID-19 patients and a significant correlation with an increased risk of death. Consistently, the peptide binding prediction analyses demonstrated that the DRB1*08 allele is unable to bind any viral peptide with high affinity. Although the obtained data needs further confirmation, the present study provides promising results, especially if the consistency with previous works is taken into account.

### 2.2. Bioinformatic in-silico epitope prediction studies

Because of the highly polymorphic nature of HLA molecules and the limited quantity of biological data gathered in roughly a year of pandemics, some authors focused on the use of predictive algorithms to find which HLA alleles are associated with viral peptide epitope recognition [44–46]. In particular, Nguyen *et al.* analyzed peptides from SARS-CoV-2 proteome across more than a hundred HLA I alleles with the aim of mapping susceptibility loci for COVID-19 [47]. Results of these analyses showed that HLA-B*15:03 is highly capable of presenting peptides from SARS-CoV-2, suggesting a possible protective role for this allele against SARS-CoV-2 infection. On the other hand, HLA-B*46:01 was predicted to bind to the fewest number of peptides from the virus, suggesting that immune response in carriers of this allele may be weaker, resulting in more severe symptoms. Consistently with that, HLA-B*46:01 has already been reported to be significantly associated with the severity of SARS-CoV infection in Asian populations [48].

A recent study by La Porta and colleagues compared the different binding affinities between coronavirus-derived peptides and a series of HLA class I molecules for SARS-CoV-2, SARS-CoV, and HCoV-OC43. The first two share 80% of the genome and are both responsible of the potential onset of severe symptoms following infection, while HCoV-OC43 is a coronavirus associated with mild respiratory symptoms [49]. Specifically, using two epitope prediction algorithms – both based on artificial neural networks – the authors evaluated binding affinities between SARS-CoV-2 peptides and 79 HLA class I molecules; results were compared with the ones

from analogous predictions made for SARS-CoV and HCoV-OC43. What emerged was a strong similarity in the binding patterns for SARS-CoV-2 and SARS-CoV. Importantly, HCoV-OC43 was characterized by peptides with a stronger HLA binding ability compared to peptides belonging to the other two viruses. The authors individuated two sets of haplotypes respectively correlated to weak and strong binding capacity towards SARS-CoV-2 peptides, the latter including HLA-A*02:02, HLA-A*11:01, HLA-B*40:01 and HLA-B*35:01. They then investigated the heterogeneous responses to SARS-CoV-2 infection in human populations by measuring the prevalence of the haplotypes in different human populations, finding that strongly binding haplotypes are more represented in Asian populations. Despite no clinical or immunological consequences related to this haplotypes were investigated in this work, these data could be relevant to study the diffusion of the disease across the world and could represent the basis to develop individualized tests in order to identify the immune susceptibility to COVID-19 among different populations. This study may be considered as another significant step towards the possibility to perform population screening and to predict individual severity scores of infection, in order to develop personalized therapeutic strategies against COVID-19.

A recent report described peptide-binding affinities between 438 HLA class I and class II proteins and the proteomes of seven pandemic viruses, including coronaviruses, influenza viruses and the immunodeficiency virus [50]. In this work HLA alleles were examined in relation to peptide-binding affinities and then grouped into four categories, namely strong, regular, weak or non-binding, based on the different kind of affinity they show towards various peptides. Notably the authors observed that the frequencies of the strongest and weakest HLA molecules are influenced by geographical location. Indeed, among native Americans the frequencies are higher for the strongest and lower for the weakest HLA binders, possibly as a consequence of previous selective pressure applied by historical infectious agents. However, results demonstrated that the majority of HLA proteins are not specific binders of SARS-CoV-2 peptides, as they bind viral peptides in an aspecific way.

Romero-Lòpez *et al.* performed a bioinformatic prediction of which epitopes of the SARS-CoV-2 spike protein are significantly immunogenic and could be presented by HLA Class I and II in different populations [51]. They also established an ecological correlation of HLA allele frequency with the predicted fatality rate in hospitalized patients of 28 states in Mexico. The only negative significant correlation observed was between the frequency of HLA-DRB1*01 and the fatality rate in hospitalized patients in Mexico. Remarkably, this correlation was weak, suggesting that other key factors, apart from HLA, could be involved in COVID-19 outcome and further experimental studies are needed to reinforce these results.

Using a different approach based on panHLA analysis, Campbell *et al.* were able to recognize 368,145 unique combinations of peptide-HLA complexes (pMHCs) with a strong binding affinity and an overlap between class I and II predicted pMHCs [44]. Though highly informative this kind of approach surely presents some limitations: 1) the analysis focused on pMHC complexes wizth predicted binding affinities of less than 500 nM, which could lead to underestimate the number of alleles correlated to the predicted antigenic peptides; 2) the research was restricted to 9-mers and 15-mers, which represent the length of most but not all reported HLA class I and class II binding peptides; 3) the data does not provide any measure of the quantity and timing of viral protein expression in host cells; and 4) the research of global population frequencies was conducted only on a restricted number of HLA alleles and countries. Overall, however, this valuable pan-HLA approach allowed identification of new possible interactions between HLA molecules and peptides, while leading to the recognition of other peptides from less prevalent HLA types. Besides, the overlap between class I and II predicted pMHCs makes conceivable that some epitopes may be presented to both CD4+ and CD8+ T lymphocytes.

De Moura *et al.* described 24 epitopes derived from the SARS-CoV-2 S protein that could interact with 17 different MHC-I alleles in the Brazilian population [52]. These epitopes can elicit an effective CD8+ T cells immune response and may be useful to develop strategic methods for vaccines against COVID-19. The immunoinformatic approach reveals that the protective MHC class I alleles include HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*11:01, HLA-A*23:01, HLA-A*24:02, HLAA*26:01, HLA-A*30:02, HLA-A*31:01, HLA-A*68:01, HLA-B*07:02, HLA-B*18:01, HLA-B*35:01, HLA-B*35:03, HLA-B*38:01, HLA-B*44:02, HLAB*44:03, HLA-B*51:01, HLA-C*05:01, HLA-C*07:01, HLA-C*07:02, HLA-C*08:02, HLA-C*15:02 and HLA-C*17:01.

Since the very first identification of allele specific motifs for T-cell antigens by HLA class I molecules, in 1991, epitope identification and characterization has been significantly simplified by in silico prediction strategies [53]. Indeed, many ongoing vaccines designs, targeting infectious pathogens, have been exploiting prediction algorithms, whose accuracy is progressively improving. Nevertheless, these tools present some limitations, mainly consisting in: differences between the real and the Protein Data Bank structure, accuracy of the methods for simulate proteasome cleavage, imperfections in the reproduction of molecular modelling, docking and dynamics and others, which have been recently revised in [54]. Such considerations should be taken into account to properly use the innovative programs for T-cell immunogen design for epitope-based therapies and future epidemiological investigations.

## 3. Antigen presentation by non-classical HLA in SARS-CoV-2 infection

HLA-G and HLA-E are both non classical molecules with tolerogenic and immunosuppressive properties which influence the onset of autoimmune and infectious diseases [55–57]. In particular, HLA-G was shown to be upregulated following HIV, HCMV and HCV infections leading to the suggestion that this could represent an immune evasion strategy ([58,59]. Actually, HLA-G can bind immune inhibitory receptors such as ILT2 and ILT4, thus preventing the generation of optimal immune responses and facilitating virus immune escape [60]. Based on these premises, HLA-G and HLA-E were considered by different authors as possible biomarkers to monitor SARS-CoV-2 infection, as exhaustively revised by Zidi [61].

In particular, Zhang and colleagues published a case report aimed to analyze the expressions of HLA-G and its receptors (ILT2, ILT4 and KIR2DL4) in peripheral immune cells of a patient critically infected with SARS-CoV-2, during the 23-day hospitalization [62]. In this study, HLA-G expression in peripheral immune cells was shown to follow a high–low–high pattern, which may reflect the three stages of infection, indicating that the status of SARS-CoV-2 infection may influence the regulation of HLA-G expression. However, questioning the real relevance of this observation, results showing that the expression of the HLA-G receptors, ILT4 and KIR2DL4, remained relatively stable during the disease were reported. As being a single case-report, the relevance of this findings should be confirmed by further independent studies.

Another correlation between SARS-CoV-2 and a non-classical HLA was investigated in a study by Bortolotti and colleagues with the aim of evaluating the effect of SARS-CoV-2 spike (SP1) protein expression in the control of NK cell activation [63]. Results showed

that SP1 expression provoked: 1) HLA-E induction, whose expression was stabilized by the interaction with a SP1-derived HLA-E-binding peptide; 2) increased levels of the inhibitory receptor NKG2A/CD94); and 3) NK cell-reduced degranulation. In view of deepen new aspects and therapeutic strategies against COVID-19, this study highlights the potential of targeting the S1 protein or using the anti-NKG2A monoclonal antibody – already in use against rheumatoid arthritis and some neoplastic diseases [64] – in an attempt to enhance the innate immune response at the early stage of SARS-CoV-2 infection [65].

Vietzen *et al.* performed an analysis on a total of 361 Austrian COVID-19 patients to assess the influence of host genetic variants on the severity of COVID-19. Considering the significant reduction of NK cells observed in patients with a severe clinical outcome [66], they focused on NKG2C – an activating NK cell receptor encoded by the KLRC2 gene – which binds to HLA-E on infected cells allowing NK cell activation. The study confirmed that NKG2C$^+$ NK are potent antiviral effector cells, even in SARS-CoV-2 infection. Consistently, the deletion of KLRC2 which naturally occurs, together with a higher degree of the heterozygous HLA-E*0101/0103 variant and the HLA-E*0101 allele were registered in hospitalized patients, especially those who required intensive care in comparison to patients with mild symptoms. Other *in vitro* studies had already highlighted a lower cell surface expression levels for HLA-E*0101/0101 than for HLA-E*0103/0103 [66], which likely results in decreased NKG2C$^+$ NK cell response, thus influencing the severity of COVID-19. These antiviral mechanisms driven by NK cell could play a critical role in gaining new insight about the protective immune responses against SARS-CoV-2 and further studies are needed to support and clarify these findings.

## 4. ERAP1 and ERAP2 role in SARS-CoV-2 infection

ERAP1 and ERAP2 are two aminopeptidases which share 50% homology. They belong to the M1 family of zinc-dependent aminopeptidases and play key roles in the activation of the human adaptive immune response [67]. All of them are characterized by the presence of four functional domains: domain I comprises 3 beta sheets and contains the antigenic peptide binding site; domain II constitutes the catalytic site; domain III acts as a hinge between domain II and IV, thus allowing conformational changes of the protein; domain IV forms an arc with domain II and determines the closed state of the protein [47,68].

Within the Endoplasmic Reticulum (ER), ERAP1 and ERAP2 cleave the N-terminus of precursor peptides, previously processed in the cytoplasm by the proteasome, so as to generate antigenic peptides of 8–9 amino acid residues, which perfectly accommodate within the binding groove of MHC I molecules.

These two proteins act in concert but their activity is not redundant as they maintain marked differences in their enzymatic specificity. ERAP1 preferentially cleaves peptides with lysine, leucine, asparagine and tyrosine residues [69] and shows a strong tendency to cut 9–16 amino acids peptides into pieces of 8–9 amino acids, the optimal length for loading onto MHC I molecules [70–73]. Contrariwise, ERAP2 presents a striking predilection for the positively charged arginine and lysine residues situated at the N-terminal and shows a greater efficiency toward shorter peptides, that ERAP1 processes poorly [74].

These aminopeptidases are, therefore, essential for creating the appropriate immunopeptidome, capable of activating a suitable immune response by CD8+ T cells. For this same reason, ERAPs polymorphisms altering their functionality and/or expression level have been demonstrated to influence the onset and progression of several diseases. Indeed, ERAP1 has been associated with Ankylosing spondylitis (AS), psoriasis and Behçet's disease (BD) in epistasis with the risk HLA-B*27[75] and -B*40:01 [76], -C*06:02, [77] and HLA-B*51 alleles [78], respectively. ERAP2 has been associated with AS, psoriasis and natural resistance to HIV-1 infection [79,80] as well, but only in this latter it seems to be in epistasis with HLA-B*57.

As expected, an altered functioning of these aminopeptidases, due to specific mutations, has evident consequences even on susceptibility/progression of infectious diseases included COVID-19 [6].

A work by Stamatakis *et al.* was pivotal in understanding how these enzymes within the ER process SARS-CoV-2 antigens and suggested the possibility of modulating ERAPs efficacy to improve and boost the effectiveness of antiviral responses [81]. In particular, they utilized a novel approach to investigate the trimming activity of ERAP1, ERAP2 and IRAP, focusing on S1 spike glycoprotein, known to be the largest antigen of the virus. The authors incubated a mixture of synthetic peptides derived from the sequence of the SARS-CoV-2 S1 spike glycoprotein with either ERAP1, ERAP2, or IRAP alone, or with a mixture of ERAP1 and ERAP2. All three aminopeptidases generated shorter peptides with sequences appropriate for binding onto HLA alleles, even if with different trimming specificities. ERAP1 was the most efficient in generating peptides 8-11aa long – the correct length for HLA binding – ERAP2 and the ERAP1/ERAP2 mixture followed, while IRAP was the less efficient. The conclusions of these elegant analyses confirmed the hypothesis that, as long as peptide trimming is mediated by ERAP1, HLA-B*15:03 is likely to present more SARS-CoV-2 epitopes than HLA-B*46:01; notably, ERAP1 is considered to have the leading trimming activity in the ER. The authors also discovered that only 7% of the SARS-CoV-2 antigenic peptides potentially presented by the above-mentioned HLA alleles were produced by either ERAP1, ERAP2 or IRAP. This finding reveals that, through their trimming activity, aminopeptidases can markedly filter and determine which antigens can be presented by MHC I molecules. Thus, ERAPs genes and their allelic variants, which encode for proteins that establish a bottleneck for the fitting of processed antigens into the binding pocket of MHC I molecules, represent a further variable which should be taken into account in the context of peptide prediction algorithms. In line with this, one of the main functions recently ascribed to ERAP1 is to limit the peptides available for MHC I [82]. Therefore, this innovative approach could be useful in optimizing bioinformatic predictions of potential MHC I epitopes.

In a subsequent study, Lu *et al.* correlated ERAP2 genotype to COVID-19 severity. The authors examined 193 deaths from 1412 infections in a group of 5871 UK Biobank SARS-CoV-2 positive patients and found rs150892504 variant in ERAP2 gene to be one – out of the 5 novel risk variants in 4 genes discovered – of the genetic risk factors associated with survival from infection in SARS-CoV-2-infected individuals [83].

Even more recently, our group conducted a study based on the consistent amount of data giving ERAP2 a pivotal role in viral infections [6]. The work was built on the 2018 results of Ye and colleagues showing the presence of two novel genetic variants – ERAP2/Iso3 and ERAP2/Iso4 – transcribed following influenza viral stimuli by monocyte-derived dendritic cells isolated from homozygous HapB-carrying individuals [84]. These latter carry the G allele for rs2248374, a single nucleotide polymorphism (SNP) previously demonstrated to prime only the transcription of a spliced ERAP2 variant (ERAP2/Iso2), further degraded by nonsense-mediated-decay (NMD) [85]. Conversely, the A allele for this SNP leads to the transcription of ERAP2/Iso1, the wild type (WT) form of the enzyme, which is thoroughly functioning. The two short isoforms diverge from each other by alternative splicing at a secondary splice site at exon 15, but while ERAP2/Iso4 harbors a premature termination codon leading to its NMD, ERAP2/Iso3 may contribute to shape the antigen repertoire. Indeed, although it loses the

catalytic domain, its capacity to dimerize is preserved. The interaction of ERAP2/Iso3 with the WT-forms of ERAPs could result into an alternative trimming efficacy of viral peptides, thus suggesting its indirect, yet crucial involvement in the anti-microbial response (Fig. 1). The results reported in the paper answered three main issues: first, they demonstrated that the newly characterized ERAP2/Iso3 mRNA expression is not flu-specific, as being also prompted by other pathogens including HIV, SARS-CoV-2, CMV and Bacteria (LPS); second, they proved that ERAP2/Iso3mRNA can be translated into a protein in response to microbial infections; third, they showed that ERAP2/Iso3 mRNA is expressed in a dose-dependent manner following viral infections [86]. Indeed, ERAP2/Iso3 expression was found to be directly proportional to the multiplicity of infection (MOI) of SARS-CoV-2 or HIV-1 used to *in vitro* infect cells, leading to the conclusion that ERAP2/Iso3 expression is strictly dependent on the viral dose of exposure. Finally, these results also showed that SARS-CoV-2 exposure provokes the expression of ERAP1 and ERAP2/Iso1 in a dose-dependent way, once again suggesting ERAPs participation in the handling of the anti-viral response during SARS-CoV-2 infection.

Besides their direct participation in the antigen presentation machinery ERAPs may be released in the extracellular milieu by immunocompetent cells triggered by inflammatory stimuli [87,88], possibly conditioning SARS-CoV-2 infection through at least two alternative mechanisms. Firstly, once released ERAPs can modulate innate immunity besides acquired one, by promoting inflammasome activation, monocyte differentiation and phagocytic activity of THP-1–derived macrophages [89,90], thus altering the immunological microenvironment in which the virus is growing. Secondly, ERAPs are two renin-angiotensin system (RAS) regulators, a pathway which is often altered in patients showing a severe form of COVID-19 [91]. In particular, ERAP1 cleaves angiotensin II into angiotensin (Ang) III and IV while ERAP2 cuts Ang III into Ang IV [92,93]. Thus, loss-of-function variants of these aminopeptidases impair Ang III and Ang IV production, contributing to the increase of circulating Ang II levels resulting in hypertension [94]. As following SARS-CoV-2 infection the ACE2 receptor is depleted from cellular surface causing an accumulation of Ang II [95], ERAP1 and ERAP2 dysfunctional status may accentuate the clinical manifestations of the disease, thus further worsening the effects of SARS-CoV-2 infection [96].

## 5. Conclusions

The aim of this brief review is to collect and present the results actually available on the role of antigen presentation in SARS-CoV-2 infection. At the time of writing this paper, the COVID-19 pandemic is continuing its course, with a total of 155 million cases and more than 3 million deaths to date registered [97]. The scientific community is relentlessly focusing on identifying the features of the immune response against the virus and the role of genetics in influencing susceptibility and degree of severity of the disease. More and more studies are deepening the mechanisms of T cell response to SARS-CoV-2, considering that – unlike vaccines based on the formation of antibodies against the surface spike glycoprotein – T cell vaccines have the capacity to generate immune responses against viral proteome in its entirety [98]. In addition, a recent study states that robust cellular immunity against SARS-CoV-2 is likely to be present within the great majority of adults at six months following asymptomatic and mild to moderate infection. This also lowers the level of concern that immune responses following natural infection may not be lasting, thus predisposing to recurrent infection [99]. Even if different vaccines are currently available and distributed worldwide, it is important to highlight how the clarification of the complex interactions between this devastating coronavirus and our immune system is a matter of strong need, to learn how to control the ill-fated progression of COVID-19.

## Author contributions

I.S. and C.V are responsible for investigation, and writing the original draft. M.C. and M.B conceived the project supervised the work and reviewed the manuscript.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review

# Humoral immune mechanisms involved in protective and pathological immunity during COVID-19

Gunawan Widjaja [a], Abduladheem Turki Jalil [b], Heshu Sulaiman Rahman [c,d], Walid Kamal Abdelbasset [e,f], Dmitry O. Bokov [g], Wanich Suksatan [h], Mahnaz Ghaebi [i], Faroogh Marofi [j], Jamshid Gholizadeh Navashenaq [k], Farhad Jadidi-Niaragh [l], Majid Ahmadi [m,*]

[a] Universitas Krisnadwipayana Indonesia, Jakarta, Indonesia
[b] Faculty of Biology and Ecology, Yanka Kupala State University of Grodno, Grodno, Belarus
[c] College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq
[d] Department of Medical Laboratory Sciences, Komar University of Science and Technology, Chaq-Chaq Qularaise, Sulaimaniyah, Iraq
[e] Department of Health and Rehabilitation Sciences, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Al Kharj, Saudi Arabia
[f] Department of Physical Therapy, Kasr Al-Aini Hospital, Cairo University, Giza, Egypt
[g] Sechenov First Moscow State Medical University, 8 Trubetskaya St., bldg. 2, Moscow 119991, Russian Federation
[h] Faculty of Nursing, HRH Princess Chulabhorn College of Medical Science, Chulabhorn Royal Academy, Bangkok 10210, Thailand
[i] Department of Immunology, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
[j] Department of Hematology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[k] Noncommunicable Diseases Research Center, Bam University of Medical Sciences, Bam, Iran
[l] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[m] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing COVID-19 is associated with excessive inflammation, as a main reason for severe condition and death. Increased inflammatory cytokines and humoral response to SARS-CoV-2 correlate with COVID-19 immunity and pathogenesis. Importantly, the levels of pro-inflammatory cytokines that increase profoundly in systemic circulation appear as part of the clinical pictures of two overlapping conditions, sepsis and the hemophagocytic syndromes. Both conditions can develop lethal inflammatory responses that lead to tissue damage, however, in many patients hemophagocytic lymphohistiocytosis (HLH) can be differentiated from sepsis. This is a key issue because the life-saving aggressive immunosuppressive treatment, required in the HLH therapy, is absent in sepsis guidelines. This paper aims to describe the pathophysiology and clinical relevance of these distinct entities in the course of COVID-19 that resemble sepsis and further highlights two effector arms of the humoral immune response (inflammatory cytokine and immunoglobulin production) during COVID-19 infection.


*Abbreviations:* SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, 2019 novel coronavirus disease; CoV, coronavirus; WHO, World Health Organization; ACE2, Angiotensin-converting enzyme 2; IFNγ, Interferon gamma; IL-1β, interleukin-1β; IP-10, interferon-inducible protein 10; MCP1, monocyte chemoattractant protein 1; ICU, intensive care unit; TNF-α, tumor necrosis factor alpha; G-CSF, Granulocyte colony-stimulating factor; MIP1a, inflammatory protein 1a; CRS, Cytokine release syndrome; CAR T cells, Chimeric antigen receptor T cells; scRNA-seq, Single-cell RNA sequencing; NFIL3, ETS2, nuclear factor regulated by IL-3; PHLDA2, Pleckstrin Homology Like Domain Family A Member 2; HLH, hemophagocytic lymphohistiocytosis; ARDS, acute respiratory distress syndrome; NK cell, Natural killer cell; AST, aspartate aminotransferase; PB, plasmablast; CCL2, chemokine C–C motif ligand 2; SOFA, Sequential/Sepsis-related Organ Failure Assessment; SHLHOS, sepsis-HLH overlap syndrome; IgM, Immunoglobulin M; ELISA, enzyme-linked immunoassay; MERS-CoV, Middle Eastern Respiratory Syndrome Coronavirus.

* Corresponding author.
*E-mail address:* ahmadi.m@tbzmed.ac.ir (M. Ahmadi).

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**Contents**



## 1. Introduction

The novel human coronavirus (CoV) designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first distinguished in infected patients with pneumonia in Wuhan, China, in December 2019. The respiratory illness derived from SARS-CoV-2 was termed by the World Health Organization (WHO) as the corona virus disease 2019 (COVID-19) that has become the most severe public health issue worldwide. Since the first reports of COVID-19 in Wuhan, there has been an exponential growth in the number of individuals diagnosed with COVID-19 all over the world. On March 11, 2020, the outbreak was declared a pandemic by WHO [1].

Several studies have now established that the COVID-19 is associated with excessive inflammation, as a main reason for severe condition and death in infected patients [2-4]. A key question for hospitalized patients with COVID-19, then, is how immune responses alter over time in the course of COVID-19. Complete and comprehensive clinical assessment focusing on the immunological characteristics is fundamental to the appropriate selection of treatment for the patient groups and for reliable analysis of experimental results [5]. A proper comprehension of viral immunopathogenesis may help with earlier management of the severe complications and clarify the best approach in managing this disease and better monitoring of the treatment response as well as its clinical course. These include both cellular immune responses, such as the induction of a high level of Th1 responses and cytotoxicity and humoral immune responses, mediated by increased antibody and cytokine levels [6]. Humoral responses have been associated with clinical outcome in patients with SARS-CoV-2 virus infection. Although the humoral immune responses induced by SARS-CoV-2 is rapid and is elicited by most infected individuals, its magnitude and time course kinetics correlates with COVID-19 disease severity [7]. This is the key issue in the management of the pandemic since the main target of current vaccine approaches is B cells that produce antibodies to target virus and infected cells. Biomedical data has also evidenced the association between COVID-19 clinical outcome and inflammatory cytokines. The level of pro-inflammatory cytokines that increase profoundly in systemic circulation appear as part of the clinical pictures of two distinct but overlapping conditions, where a robust inflammation is elicited affecting multiple organ damage, sepsis that usually presents with respiratory distress and multiple organ dysfunction and the hemophagocytic syndromes which are mostly caused by the activation of macrophages as a result of an infection [8]. The review may shed some lights on the understanding of the pathophysiology of the ambiguous and complex manifestations of COVID-19 and will additionally inform about the clinicopathogenesis of acute lung inflammation caused by COVID-19, in a natural host-pathogen interaction. Given the key role of antibodies in protective immunity and immune pathogenesis of viral diseases, we focus this review to specific issues relating to a serological correlate of protection from SARS-CoV-2 for COVID-19 vaccine evaluation and discuss how they are related to viral load in acute infection and SARS-CoV-2-induced clinical illness severity. We further highlight the importance of screening in seroprevalence studies of the infection, seroconversion rate and the complications with rapid therapeutic intervention with immunoglobulin treatment.

## 2. COVID-19 pathology frameworks

### *2.1. COVID-19 pathology is substantially associated with perturbations in immune system compartments*

It seems that COVID-19 illness drives two distinct but related pathologies triggered by the virus itself and by the host response, albeit in different levels of severity [9]. The early reports suggest that in the establishment phase of the infection the symptom expression is similar in immunocompetent and immunoquiescent states as in the elderly, or transplant recipients [10]. Owing to the concomitant use of anti-inflammatory therapy in heart transplantation, the COVID-19 disease tends to be milder in the second phase which is determined by the inflammatory host response [10,11]. Accumulating evidence indicates a plausible link between the host immune response and disease progression during 2019-nCov infection and this plays an important role in shaping SARS-

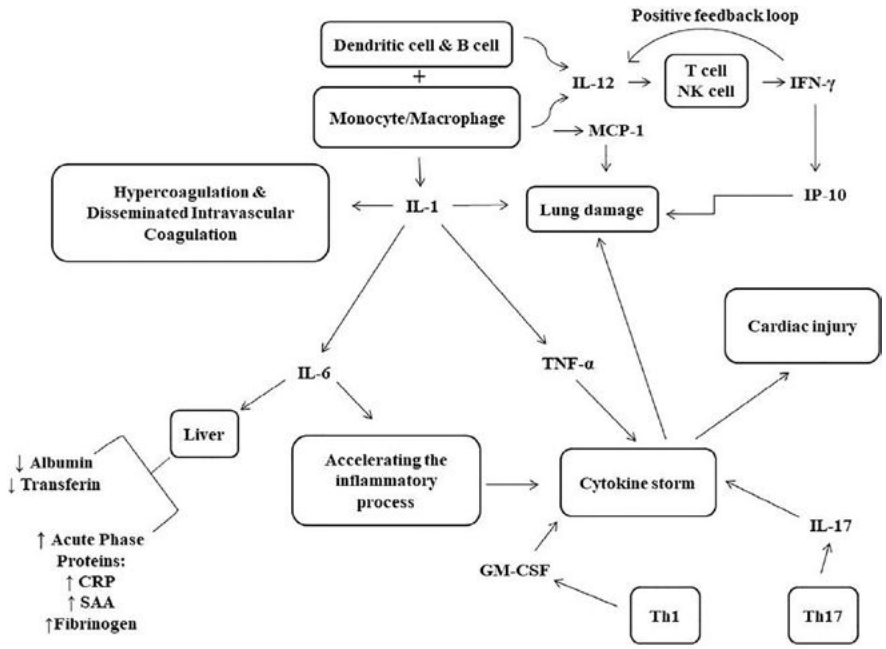


**Fig. 1.** Schematic diagram of the pathological effects of immune system cytokines in COVID-19. During the disease caused by SARS-CoV-2, activated monocytes and macrophages produce various cytokines such as IL-1, IL-6, and TNFα which can cause cytokine storm and multiorgan damage. IL-6 also can induce liver cells to synthesize acute phase proteins and is associated with low albumin and transferrin concentrations. In addition, IL-12, produced by monocyte/macrophage, dendritic cell and B cells, may induce NK and T cells to secrete IFN-γ which in turn stimulates IL-12 production in a positive feedback loop. SAA; serum amyloid A, CRP; C reactive protein, Th; helper T cell, IL-; interleukin-, TNF-α; tumor necrosis factor alpha, GM-CSF; granulocyte–macrophage colony-stimulating factor, MCP-1; monocyte chemoattractant protein1, IP-10; Interferon-Inducible Protein 10, IFNγ; interferon γ protein.

CoV-2 pathology. In this respect, at non-severe stages, development of COVID-19-induced immunity is thought to produce a classical two-phase immune profile that provides a protective response followed by pro-inflammatory damaging reactions at the severe stage [12]. Lung damage is a major source of morbidity and mortality limiting recovery in those severe patients and there are occasional serious complications associated with kidney failure or heart problems. It has been reported that severe COVID-19 disease is more likely in the elderly, who have weaker immune function. These population groups are considered to have an adverse outcome with regard to their general health status. This report establishes a role for good health in mounting a protective endogenous immune response that elicits specific antiviral immunity. When host-protective immune response is impaired, virus will propagate to a high extent causing massive destruction of the tissues with high expression of Angiotensin-converting enzyme 2 gene (ACE2) such as kidney and intestine. Impaired cells, as a result, contribute to innate immune activation leading to inflammation in the lungs [12]. Pulmonary inflammation is the most common feature of life-threatening respiratory disorders that afflict patients in the older age group/severe stages [13]. Although early adaptive immune response is needed to eradicate the virus in the early stages and likely contribute to the susceptibility of the host to infection but it may be even a causative factor in pulmonary pathologies. The first immune response emerges from innate immune cells including macrophages, neutrophils, and the NK cell activities initiated to try to eliminate the virus, which further activates the adaptive immune system [14]. The anti-viral adaptive immune response resides on cytotoxicity by CD8 + CTL, Th1 subset of CD4 + T cells, and antibody-secreting plasma cells [14]. Many of the evidenced severe COVID-19 cases demonstrate a large number of proinflammatory cytokines in serum [9,15]. Moreover, the existence of autoantibodies directed against a variety of proteins including cytokines, chemokines, and cell surface antigens in the serum of COVID-19 patients may contribute to the tissue damage by immune complex formation and activating complement [16]. Since an exacerbated immune response to the virus can aggravate a preexisting injury condition, being in a good overall health state may not be beneficial for those who have progressed to the severe stage of the disease. In the late phases, once severe lung damage occurs, treatment of virally driven hyperinflammation tailored to this demanding condition may be able to keep it from getting worse or stop it in order to reduce fatality rates. Yet identifying the immune mechanisms which determine the infection duration induced by the virus and discriminate between people with severe and non-severe (mild, moderate) COVID-19 infection has been the subject of debate.

### *2.2. The molecular dynamics of cytokine production during SARS-CoV-2 infection*

It would also be relevant to illuminate the molecular dynamics of cytokine response during the course of disease. Early studies have shown that 2019-nCoV infection induce increased concentrations of proinflammatory cytokines, including IFN-γ, IL-1β, IP-10, and MCP1 which is similar to features of infections caused by SARS-CoV [17] and MERS-CoV [18]. The role of these cytokines in pathophysiology of the disease is briefly explained as a diagram in Fig. 1. At the same time, SARS-CoV-2 may antagonize the antiviral interferon response of the host and thus evade innate immunity

[16]. Furthermore, similarities and differences of clinical features between severe and non-severe COVID-19 patients have been noted in regard to the kinetics of the immune response which is of major importance in the pathogenesis or progression of COVID-19 infection. In this regard, comparison between 2019-nCoV-infected patients admitted to the intensive care unit (ICU) and non-ICU patients has shown higher levels of specific cytokines (TNF-α, IL-2, IL-7, IL-10, MCP-1, G-CSF, MIP1A, and IP-10) in patients requiring ICU admission than did subgroups not requiring ICU admission [9] proposing that the cytokine storm strongly correlates with disease severity [19]. In the first critical COVID-19 case in Zhejiang Province, Zhang et al. [20] showed that elevated circulating levels of IL-6, IL-10 and IFN-γ decreased quickly while the levels of IL-4 and TNF-α increased when RT-PCR test for viral RNA returned negative. Since dynamics of the cytokine levels during SARS-CoV-2 infection appear to be related to disease severity, they may therefore serve as a potential biomarker for prognostic evaluation [21].

### *2.3. The primary source of the cytokine storm in response to SARS-CoV-2 infection*

Cytokine release syndrome (CRS) or cytokine storm is a complex hyperimmune response syndrome usually seen with T-cell activating therapeutics as in patients receiving CAR-T cell therapy which results in symptoms including fever, nausea, headache, and hypotension [22]. CRS can occur after a wide variety of infectious and non-infectious stimuli. Cytokine stimulation by infectious factors, or condition would exacerbate severity of the disease and an exaggerated cytokine response has been described as a driver of pathology in COVID-19 patients with advanced disease [23]. Since severe 2019-nCoV infection have been characterized with lymphocytopenia (indicating a state of immunosuppression), it is inferred that COVID-19-induced CRS may be the result of an overactive innate immune response mounted by other leukocytes [12]. Previous studies on SARS virus have shown the stronger host innate immune responses to viral infection in older animals inoculated with SARS-CoV compared to younger adults with a marked elevation in expression levels of inflammation related genes [24]. Regarding SARS-CoV-2, the innate immune response to the virus has been proposed to contribute to the development of acute respiratory distress syndrome (ARDS) due to the rapid onset of widespread inflammation in the lungs [13]. The pathological investigation of the lungs in fatal cases of COVID-19 reveals massive infiltration of alveolar macrophages with slight lymphocytic infiltration [25]. The composition of immune cells localized to the lung differs across patients ranging from mild to severe. In the severely injured lung, the predominant macrophage lineage is greatly inflammatory Fibronectin-like sequences within NC1+ (FCN1 + ) macrophages, a phenotype that associates with

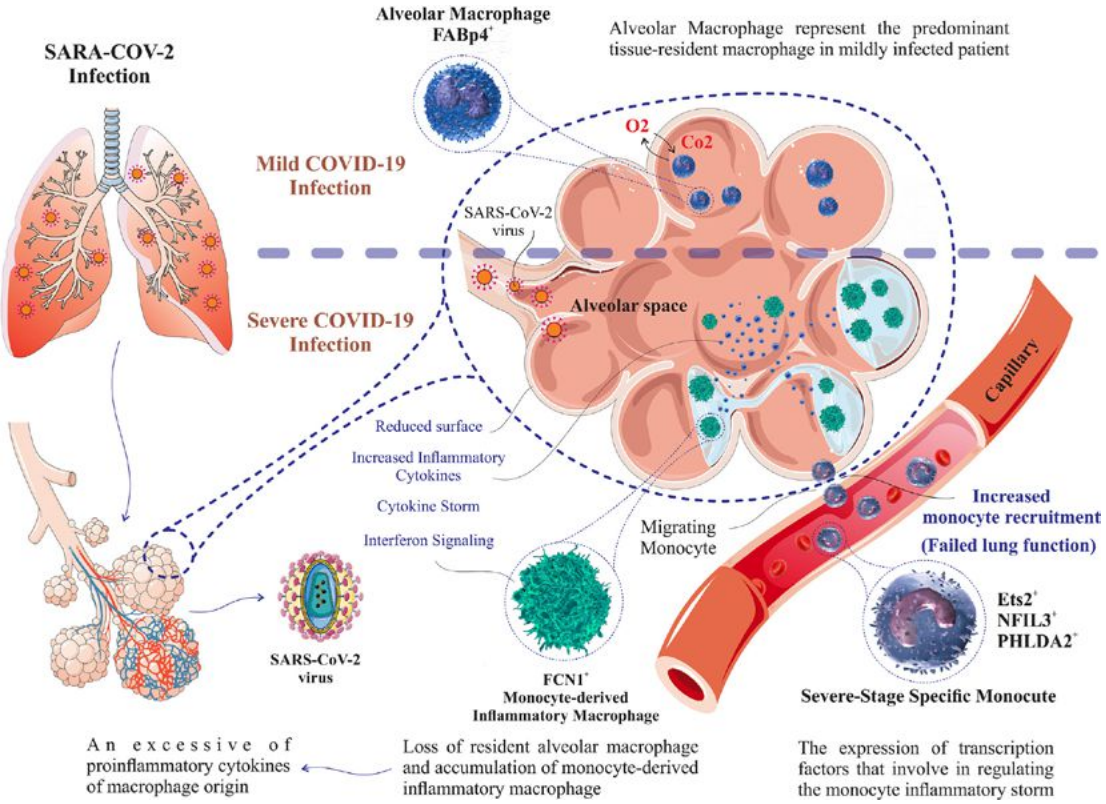


**Fig. 2.** Specific macrophage-monocyte lineage cells surrounding alveoli that cause local pulmonary inflammation after SARS-CoV-2 infection or COVID-19 disease. The composition of immune cells localized to the lung differs across patients ranging from mild to severe. The pathological investigation in mild cases of COVID-19 reveals massive infiltration of alveolar macrophages, while in the severely injured lung the predominant macrophage lineage is inflammatory FCN1 + macrophages, that associates with monocyte-derived macrophages. A unique monocyte subset called as "severe stage-specific monocyte" exists only in severe stage patients with COVID-19.

monocyte-derived macrophages [26], whereas, in both healthy subjects and mildly infected cases, the alveolar macrophages consisted the principle tissue-resident macrophages in the lungs. Indeed, in lung (bronchoalveolar lavage fluid) immune cell composition, FABP4+ (fatty acid-binding protein 4) alveolar macrophages with lipid metabolic functions replace the inflammatory monocyte-derived FCN1 + macrophages indicating a disturbed balance of lung macrophage subpopulations during the progression of severe COVID-19 [26]. Importantly, the depletion of alveolar macrophages, as effector cells for pulmonary cell-mediated immunity [27], in severely infected lungs is likely a leading cause of failed lung function. These data show that there is relationship between disease severity during the COVID-19 infection and the loss of resident alveolar macrophages accompanied by the accumulation of monocyte-derived inflammatory macrophages. Inflammatory macrophages having interferon signaling and monocyte-recruiting chemokine programs can lead to a macrophage excess and this may drive severe lethal pneumonia in SARS-CoV-2 infected individuals [28]. Profiling the peripheral immune cells in COVID-19 demonstrated a unique monocyte subset called as "severe stage-specific monocyte" which existed only in severe patients. Single-cell RNA sequencing (scRNA-seq) analysis of peripheral blood mononuclear cells suggest that distinct properties of these cells are dictated by a gene regulatory network consisted of ETS2, NFIL3 and PHLDA2 transcription factors that involved in regulating the monocyte inflammatory storm [29]. Taken together, the data convincingly propose that an excessive secretion of proinflammatory cytokines of macrophage origin is responsible for immunologically mediated adverse effects in SARS-CoV-2 infected patients. The critical issue is how to recognize and intervene early in those patients at increased risk of developing this complication. Fig. 2 depicts the local immune mechanisms and mediators of pulmonary hyperinflammation and impaired gas exchange in the lungs in patients with mild and severe COVID-19 illness.

### *2.4. Immunopathogenesis of SARS-CoV-2-induced disease: A potential infection-associated hemophagocytic lymphohistiocytosis or viral sepsis?*

Current data supports that hyper immune reaction, leading to cytokine storm in COVID-19, which is clinically specified by lymphopenia, pathological damage, respiratory failure, shock, and organ failure, is at least partially accounted for these poor outcomes. Two prominent immune dysregulation syndromes implicated as common causes of hyper inflammation associated with tissue injury include HLH and toxic shock syndrome/sepsis and thus it is crucial to think about it when facing a patient with fever, cytopenia, hepatosplenomegaly, and other systemic manifestations. Identification and characterization of the sepsis and HLH overlapping syndromes might be an obstacle to deal with in this setting, because both disorders cause a similar presentation. To explore better clinical care for critically COVID-19 ill patients with pneumonia, this section aimed to describe the clinical and laboratory manifestations of these patients to accurately define the immunopathogenesis derived from the systemic cytokine storm. To date, the underlying cause of hyperinflammation in patients with COVID-19 has remained elusive. Hemophagocytic lymphohistiocytosis (HLH; hemophagocytic syndrome), is known as a potentially fatal hyperinflammatory status that describes the phenomenon of activated macrophages which phagocytose hematopoietic cells such as leukocytes, platelets, erythrocytes, and their precursor cells in the bone marrow, lymph nodes, or liver, leading to the clinical symptoms. It is of two types - primary HLH (familial HLH) and secondary HLH (acquired HLH). The later occurs following strong immunologic activation such that occurs with systemic infection (virus, bacteria and protozoa), neoplasms, and autoimmune disease [30]. The clinical feature of the syndrome is mainly determined by prolonged fever, splenomegaly, and hemophagocytosis in the bone marrow and the major laboratory hallmarks include hyperferritinemia, hypertriglyceridemia, cytopenias, hypofibrinogenemia, decreased or absent activity of NK cells, and elevated sCD25 [31]. For SARS-CoV-1, HLH have been shown related to adverse clinical outcomes in a subset of fatal infections [32-35]. Analysis of laboratory results in a large cohort of inpatients with COVID-19 showed that such abnormalities as anemia, thrombocytopenia, elevated ferritin and ALT are significantly more frequent in non-survivors compared to survivors [36]. There is a small case-series report in literature describing hypertriglyceridemia, high fever, and hyperferritinemia, which are helpful in combination with distincting HLH from non-HLH COVID-19 patients with ARDS [37]. Progression to ARDS , that is, the upregulation of pro-inflammatory cytokines and chemokines, in several severe COVID-19 patients is very similar to the pattern found in macrophage activation syndrome or secondary hemophagocytic lymphohistiocytosis (sHLH), a clinical condition presenting as a cytokine storm syndrome associated with multi-organ system dysfunction [38]. Laboratory and clinical features of a severe COVID-19 patient often resemble that of HLH including fever, cytopenias, and pulmonary involvement [39,40]. A HLH-like cytokine profile involving enhanced the cytokine production, including IL-1β, IL-2, IL-6, IL-7, IL-8, TNF-α and chemokines such as CXCL10 and CCL2 predominate in the majority of severe COVID-19 infections [41,42]. Cytokine storm with features akin to HLH, however, is associated with profound immunosuppression which is evident with pronounced lymphopenia, and decreased natural killer cell function [42,43]. HLH is often diagnosed using clinical, laboratory, and histologic features [44]. Pathologic detection of hemophagocytosis plays an essential role in the diagnosis of HLH. Post-mortem findings in a series of 4 cases with laboratory-confirmed COVID-19 have documented histologic evidence of hemophagocytosis [37].

Sepsis as a distinct medical entity represents a state of uncontrolled inflammatory response [45]. Although bacterial infection has been the predominant cause of sepsis syndrome, viral infections can also elicit sepsis. This association has previously been described where it was shown that nearly 40% of community-acquired pneumonia adults had sepsis on account of viral infection [46]. Similarly, sepsis might be directly resulted from SARS-CoV-2 infection. A univariate and multivariate analysis for the risk factors of in-hospital death using retrospective data on 191 patients with COVID-19 detected the developed sepsis and no bacterial pathogens in more than half of patients [36]. According to the International Consensus definitions for sepsis and septic shock (Sepsis-3), the assessment of Sequential/Sepsis-related Organ Failure Assessment (SOFA) score is suggested as a measure of sepsis-associated organ dysfunction [47]. In addition to severe lung injury, many late phase COVID-19 patients satisfy several of the criteria required for sepsis diagnosis including cold extremities and weak peripheral pulses, severe metabolic acidosis, impaired liver [48] and kidney function [49] indicating possible recognition of sepsis in these patients [50].

Documentation of the mechanism of hyperimmune host reactions triggered by the virus that results in hypercytokinemia is found to be complicated because human COVID-19 disease has been associated with severe clinical manifestations in the form of sepsis and the overlapping disorder, HLH-like illness, as well. It seems possible that the inflammatory response elicited by SARS-CoV-2 virus may trigger a hyper-inflammatory disease course identified by HLH syndrome in at least a subset of patients. Experimental evidence in support of this concept has been given in a cohort of 16 fatal H1N1 adult patients where 81% exhibited HLH

histologically and 36% were identified to carry heterozygous mutations in genes associated with familial HLH [51]. Intriguingly, others have reported that 14% of the patients who develop HLH in adulthood harbor hypomorphic mutations in familial HLH–causing genes and these mutations might have an assisting role in developing the late-onset HLH when challenged by viral infection or other stresses [52]. These data may explain that both genetic and immunologic diagnostic testing may be beneficial in forecasting which individuals are at highest risk of cytokine storm and that HLH-directed treatment can reduce mortality associated with HLH in a subset of COVID-19 patients. There are also characteristics of sepsis with cytokine storm that might argue against HLH as the major cause for increased mortality in this pandemic setting. In spite of significant similarity of HLH to in terms of clinical manifestations and pathophysiologic characteristics, it can be discriminated from sepsis in many patients. Since the aggressive immunosuppressive regimen required to treat HLH is absent in sepsis guidelines, differential diagnosis is critically essential between these two conditions [53]. However, the majority of physicians consider sepsis as a leading cause of critical illness for understanding of severe COVID-19 pathogenesis [54] mostly due to the fact that severe COVID-19 presents with hypercytokinemia [9,55]. It is now evident that severe COVID-19 can cause sHLH [37,56]. Finally, however final conclusions cannot be made, we propose that sepsis-HLH overlap syndrome (SHLHOS) which represents a severe form of sepsis or a subgroup of septic patients who are suffering from dysregulated immune hyperactivity where infection triggers macrophage activation, might explain a significant fraction of critically ill COVID-19 patients with no clear dividing line between sepsis and HLH [57]. Identifying these patients might allow us to select those who would benefit most from immunomodulation.

## 3. Humoral outlines in SARS-CoV-2 infection

### 3.1. B cell responses

Although the development of lymphopenia is mainly related to the decrease in absolute T cell counts, contribution of B lymphocytes in this setting in COVID-19 pneumonia remains controversial. There are a significant number of studies that indicate the absolute numbers of B cells were within normal range in most patients during the course of COVID-19 disease [43,58]. Other reports suggest decrease in B cells in COVID-19 patients and that severe cases have a diminished level than mild cases [43,59]. In contrast, in a comparison between severe, recovery and healthy stages, a distinct difference has been observed between the groups; while the absolute number of total lymphocytes was decreased in COVID-19 patients, the proportion of B lymphocytes was found to be higher in most patients, more profoundly in severe cases [60]. In addition, plasma B cells, the antibody-secreting cells, were found enriched at severe and recovery stages versus healthy controls indicating that humoral immunity is crucial to fight off viral infection [29]. However, it remains a matter of debate whether antibody-dependent enhancement play roles in disease exacerbation [61,62]. Such a scenario has been considered especially based on the findings that COVID-19 ICU patients who had evidence of SARS-CoV2-specific antibodies were not protected yet, and may even be at increased risk for adverse outcome [63]. Meanwhile, in another study, it is suggested that B-cell response might be nonessential based on the observation that the two patients with X-linked agammaglobulinemia who were exposed to SARS-CoV-2 and developed pneumonia could recover from the COVID-19 disease [64] implying that the production of antibody is probably involving in disease progression. It also may reflect that normal T cell response may be sufficient in the immune response against SARS-CoV-2 infection.

The failure to document a definitive pattern of B cell kinetics in SARS-CoV-2 infection may be attributed to the analysis of the whole B cell population but not considering subpopulations. A deep profiling study of B cell populations has revealed several alterations in the distribution of B cell subsets in patients with COVID-19 [65]. Within the CD19 + B cells, plasmablast (PB) frequencies (CD19 + CD27 + CD38 + ) were often robustly increased, representing > 30% of circulating B cells in some cases, whereas IgD + CD27- naïve B cell counts were not. However, robust plasmablast populations were only observed in two third of cases, with the remaining patients presenting PB at similar frequencies to recovered cases and healthy subjects [65]. Conversely, class-switched (IgD – CD27 + ) and not-class-switched (IgD + CD27 + ) memory B cell subsets were decreased in COVID-19 patients compared to recovered patients and healthy controls. In following up patients longitudinally for temporal pattern of change in lymphocyte subpopulations, the prior study also found that COVID-19 patients maintain a stable frequency of PB cells at day 0 and day 7 of hospitalization, however there were significant changes in memory B cell subsets [65]. Findings from another study, using single-cell sequencing, found that naïve B cells expressing CD19, CD20 (MS4A1), TCL1A, IL4R, IGHD, and IGHM decreased significantly in the course of COVID-19 recovery stage [66], which contrasts with the previously mentioned report, using high dimensional cytometry, in patients who present with COVID-19 infection [65]. Overall, there is considerable inter-patient heterogeneity for circulating B cell responses, although it appears that both the proportion and number of B cells are not frequently decreased in both severe and non-severe patients [60]. Considering the dynamic acute immune response to SARS CoV-2 [67], a possible reason for the observed heterogeneity may rely in different sampling time points and different sample sizes in the discussed studies.

### 3.2. Antibody response dynamics in association with clinical manifestations

After SARS-CoV-2 virus exposure, adults are usually capable to mount strong, weak or no antibody response to SARS-CoV-2 nucleocapsid protein. Also, the magnitude of antibody responses produced in COVID-19-infected adults negatively correlates with clinical immunity [68]. It has been observed that the earlier response, and higher antibody titer is associated with disease severity, indicating that strong responders for IgM and IgG among patients with COVID-19 may be actually those with severe disease [69,70]. A high antibody titers, therefore, is suggested to be an independent risk factor for a worse clinical prognosis in COVID-19 [70]. The potential contribution of antibody response to viral clearance must also be considered, as patients with COVID-19 who were poor IgG responders followed with higher viral clearance rate than that of strong responders [68] which resemble SARS-CoV [62] and MERS-CoV [71] infections. Alternatively, a short duration of viral shedding has been reported to occur in patients with positive anti-SARS-CoV-2 IgM results compared to those with the absence of anti-SARS-CoV-2 IgM antibodies [72]. It has been documented that both anti-SARS-CoV-2 IgM and IgG are produced during COVID-19 infection but their contribution to viral clearance remains to be elucidated. Antibodies specific for the viral spike protein, which facilitate the infection of human immune cells independent of ACE2 receptor, comprise an important fraction of antibodies elicited by SARS-CoV-2 infection [73]. The basic idea and theoretical concern of antibody-dependent enhancement (ADE) with SARS-CoV-2 coronavirus is based primarily on experimental findings and limited clinical evidence [74]. These data indicate a

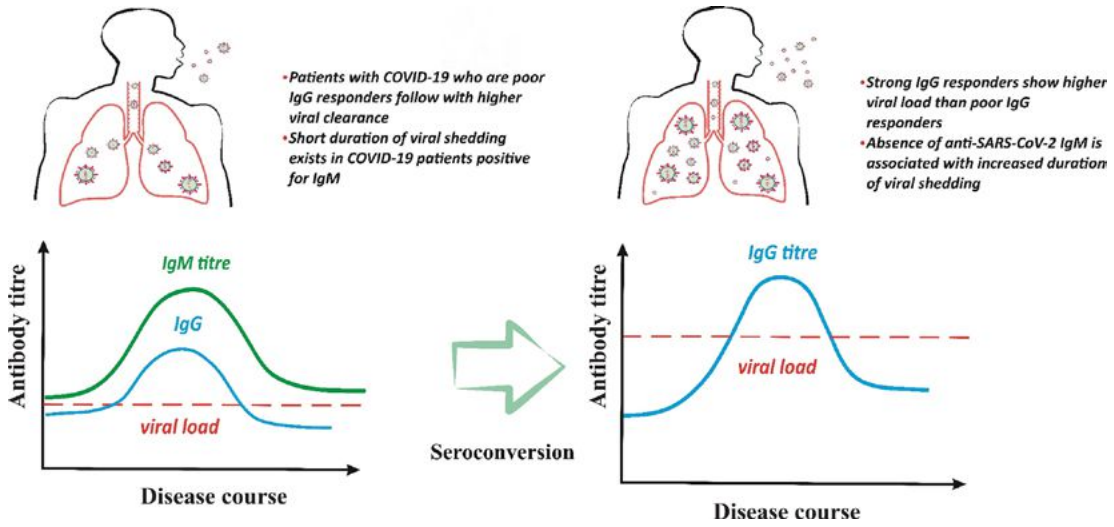


**Fig. 3.** Antibody response associates with viral load and shedding of patients with COVID-19 infection. In the early infection the specific antibody responses against SARS-CoV-2 is mainly the IgM antibody response that is correlated to higher viral clearance whereas following seroconversion or in the individuals who produce IgG earlier than IgM, the higher viral load and longer duration of viral shedding has been detected.

novel cell entry mechanism into immune cells known as antibody-mediated infection. Further evidence support the predominant role of Fcγ receptor (FcγR) in ADE of SARS-CoV-2. The immune cells expressing FcγR for IgG may be infected by IgG-FcγR interactions mediated by anti-SARS-CoV-2 spike protein IgG antibodies which are found in high levels in severe COVID-19 patients [75]. These findings may explain the reason of functional dichotomy between IgG and IgM in the pathogenesis of SARS-CoV-2. Prolonged virus shedding even after seroconversion has been demonstrated in an individual case report [76]. Fig. 3 illustrates the possible association between the specific antibody response in early infection which is mainly of IgM type and after seroconversion to IgG anti-SARS-CoV-2 antibodies with the viral load and shedding in COVID-19 patients.

The causal link between humoral response and critical illness is still poorly understood. Reasonable hypotheses can be made based on knowledge from MERS-CoV and SARS-CoV which indicate the possibility of antibody-dependent disease enhancement effects [62,77]. Assessment of IgM and IgG antibody responses in patients who underwent seroconversion, show that IgG and IgM titers were raised in the severe group compared to non-severe group. The serological courses of COVID-19 infection in 285 patients suggest that all had detectable antiviral IgM or IgG within 19 days after symptom onset and the median day of seroconversion for both IgG and IgM was day 13 [78]. Moreover, sequential analysis revealed three models of seroconversion including IgM seroconversion earlier than that of IgG, IgM seroconverted later than or synchronously with IgG [78]. These results are in great contradiction with the assumed principles of sequential serum antibody response to the pathogens switching from an early IgM response to a later IgG response [79], and suggest that the total antibody is more sensitive and rises faster than IgM and IgG for detecting SARS-CoV-2 infection [70]. As a mucosal pathogen, SARS-CoV-2 virus infects individuals mainly through the mucosal routes and it would thus be expected to induce secretory IgA (sIgA). One major effector molecule of mucosal anti-viral immunity is sIgA [80]. IgA-mediated protection prevents pathogens from binding and invading the host cells. A role for antibody-dependent cellular cytotoxicity (ADCC) has also been proposed as a mechanism of effector immune responses mediated by sIgA [81]. In particular, sIgA is able to drive activating signals, leading to cytokine release [82]. A recent study evaluating the pattern of humoral immune response to SARS-CoV-2 showed that remarkably higher level of IgA and IgG were found in severe patients compared to non-severe patients [83]. The positive association between the level of SARS-CoV-2-specific IgA and the disease severity has been established in COVID-19 patients [83]. However, we cannot draw final conclusions, there is overall agreement in that the great majority of confirmed COVID19 patients seroconvert and antibody response vary with different clinical manifestations and disease severity [72].

### *3.3. Serological assays provide a means for sero-diagnosis, sero-epidemiology and evidence of naturally acquired or vaccine induced immunity*

Although molecular diagnostic tests developed rapidly in the early phase of the pandemic, serologic assays are still somewhat limited. The role of adaptive immunity in the natural history of SARS-CoV-2 is particularly important. Adaptive immunity is expected to rise within one week from infection [84]. The use of serological assay as an indirect marker of infection is still debated in terms of its diagnostic values in SARS-CoV-2 infection [85]. Recently, interim guidance for laboratory testing are provided by the World Health Organisation (WHO) showing the strategic use of diagnostic testing in areas with different transmission/circulations of the COVID-19 outbreak [86]. That indicates where the COVID-19 virus is widely spread serological testing over time is recommended to support diagnosis. In areas with no established SARS-CoV-2 virus circulation, it is required to pay attention a case laboratory-confirmed by detecting the unique sequences of virus RNA by molecular testing such as real-time reverse-transcription polymerase chain reaction (rRT-PCR) for at least two different targets on the SARS-CoV-2 genome [86]. The reliability of RT-PCR depends on many factors, including the sample types (throat or nasopharyngeal swabs, sputum, blood, etc.), the quality of the sample (either during collection or shipment), and the quality and consistency of the PCR assays [87,88]. Characterization of serological profiles also may provide support for the diagnosis of either rein-

fection or relapse in cases. Serological tests have low sensitivity in acute phase of the disease because of a 5- to 7-day delay in the IgM antibodies produced after exposure and thus give the correct diagnosis in certain phases of the disease. The serological testing represents the main examination in tracking the infection and identification of humoral immune response in vaccinated individuals. It also provides more accurate information regarding epidemiological aspects of the disease related to any previous exposure to SARS-CoV-2 in populations. As an example, the seroprevalence rate of COVID-19 in Wuhan was estimated 3.2%−3.9% in March 2020 and a ~4.1% estimated seroprevalence rate has been recorded in California in April 2020 [16]. Another important aspect to consider is its use in those with mild symptoms or asymptomatic patients as an option for screening of populations including healthcare workers. Currently, there is a substantial group of people asymptomatically infected or very mild cases of COVID-19 infection who mask a population's true rate of infection [89]. This supports the view that screening is currently the strongest tool available in the fight against COVID-19 infection. A recent review of the literature describes the details of the various serological tests used for COVID-19 investigations including rapid antibody tests, and immunoenzymatic serological tests like indirect enzyme-linked immunosorbent assay (ELISA) [90]. Several ELISAs have been developed for the identification of individuals exposed to the virus and for the quantitation of IgG and IgM [90]. The diagnostic potential of the SARS-CoV-2 antibodies is an ongoing debate and further studies are needed to determine the best time to use them for disease assessment. Analysis of the humoral response of 140 cases diagnosed as confirmed (n = 82) and probable COVID-19 (n = 58) cases has shown that the early IgM and IgA antibodies increased both between days 8–14 but were not sustained between days 15–21 of infection or thereafter, whereas the IgG antibody titers increased on days 8–14 and tended to rise until days 15–21 peaking on day 21 [91]. That means that the lack of detection sensitivity at early time-points has limited this approach in early stage infection where the ELISA titer is virtually undetectable at days 0–7 [91]. Generally, however, such serological assays is not employed to make diagnosis of acute infections, they help support some relevant applications [92]. Up to date, serological testing for clinical diagnostic purposes is mostly requested in hospitalized patients when despite a strong clinical suspicion, RNA testing remains negative, or for patients whose samples are collected after the acute phase of the infection, as well as in patients who have low viral loads and await decision to end isolation in clinical practice [93]. One study testing ELISAs using the main immunogenic coronavirus proteins demonstrated that among the spike protein antigens tested, receptor binding domain (RBD), and the N protein antigen were more sensitive than S1 subunit of S protein, while S1 subunit specific IgG ELISA was more specific in detecting SARS-CoV-2 antibodies [94]. The specificity of serological testing for SARS-CoV-2 is of critical importance because cross reactions may occur due to the presence of antibodies against other circulating coronaviruses in the community.

Furthermore, serological tools should be considered to identify potential highly reactive human donors for generation of convalescent plasma/serum therapeutics [92]. Titration of neutralizing antibodies is effective prior to use convalescent plasma therapy. Neutralizing antibodies arise during the course of infection in some infected hosts to enable virus clearance and confer protection in an uninfected host that exposed to the virus [95]. For many viral infections, it is widely accepted that neutralizing antibodies are a main correlate of protection [96-98]. As an instance, testing for neutralizing antibodies has been an established gold standard for assessing individual protection from polioviruses [99]. In addition, the induction of neutralizing antibody is a crucial criterion of vaccine efficacy studies and can be used in the evaluation of population immunity [100]. Antibodies against SARS-CoV-2 S protein are likely most important to block binding of SARS-CoV-2 virus to the receptor [101]. Monoclonal antibodies against a series of immunodominant regions on the viral proteins—for example, the spike glycoprotein are serotype-specific, while other potential epitopes are not. Two immunodominant linear B-cell epitopes (S14P5 and S21P2) present on SARS-CoV-2 spike glycoprotein have been shown to be associated with a robust immune response; antibodies recognizing these two epitopes could result in a significant inhibition of virus infection, as demonstrated by using sera of convalescent COVID-19 patients and pseudotyped lentivirus assay [102]. While the optimal dose and time point for screening potential plasma donors needs further investigation, it is to be noted that a neutralizing response has been detected for SARS-CoV-2 in a case from day 9 onwards [103].

## 4. Passive immunization - antibody therapies in Covid-19

### *4.1. Antibody therapy: Possible benefits and limiting drawbacks*

The great demand for the discovery of primary care-based therapeutic methods that combine the high specificity and accelerated development to control a serious viral outbreak often arise at times when vaccine and antivirals are not available. Therefore, it is urgent to consider rapid therapeutic interventions in order to enable emergency recovery from the severe condition of SARS-CoV-2 and its related consequences [104]. In view of the prior promising experience in treating other viral infections such as influenza, SARS, and MERS, great interest has been emphasized that passive immunotherapy and prophylaxis of SARS-CoV-2 infection would become possible by the potential utilization of antibodies [105]. Antibody therapy for infections includes plasma and monoclonal antibody therapies. To improve the emergency condition, passive immunotherapy in the form of convalescent sera represented a promising option where no other treatment was available. Immunotherapy by transferring the convalescent sera to infected patients may be capable of neutralizing the virus and prevent further infection. Early administration of convalescent plasma can be considered, although with some caution, for immunocompromised patients with suspected COVID-19 infection, a situation in which prolonged shedding of virus occurs frequently [106]. Treatment with passive antibody therapy can possibly reduce the viral load of infected patients and reduce the risk of subsequent mortality [107-111]. However, the challenges associated with availability of sufficient donors, viral kinetics, the influence of neutralizing antibodies on SARS-CoV-2 infection progression and underlying virus–host interactions are still under discussion. Also the challenges in developing these types of antibody-based treatments include the difficulties encountered with/in the viral safety of immunoglobulins preparations, the purity, and specificity. These factors have elicited the renew interest in applying antibody-based treatments to combat the COVID-19 virus. Chicken egg yolk antibodies (IgY), the main immunoglobulin present in avian blood (IgY), have proven useful for many biomedical applications [112]. IgY application as a non-invasive procedure has been successfully tested in human health. Specific anti-viral IgY monoclonal antibodies against SARS CoV-2 offers chances for rapid diagnosis and immunotherapy against COVID-19 [113]. It is more suitable than mammalian serum immunoglobulins because it does not react with components of the human immune system [114].

Monoclonal antibodies are specific therapeutic molecules capable of serving as highly effective treatment candidates protective against particular disease [115,116]. Accordingly, monoclonal antibodies against proteins present on the viral membrane or the receptor proteins located in the host cell surface can be used to

**Table 1**
Recruited clinical trials of immune-based treatments in COVID-19[a] patients.

| Intervention | Phase | Type of Intervention | Registration Code |
|---|---|---|---|
| Plasma Therapy | Phase 1 | Convalescent Plasma | NCT04333355, NCT04345679, NCT04340050, NCT04412486, NCT04397757, NCT04353206, NCT04388527, NCT04355897, NCT04377672 |
| | Phase 1/2 | Convalescent Plasma | NCT04344535, NCT04438694, NCT04366245, NCT04356482 |
| | Phase 2 | Convalescent Plasma | NCT04343755, NCT04345991, NCT04347681, NCT04323800, NCT04332380, NCT04343261, NCT04346446, NCT04354831, NCT04415086, NCT04405310, NCT04389710, NCT04442191, NCT04392414, NCT04434131, NCT04421404, NCT04390503, NCT04429854, NCT04375098, NCT04373460, NCT04403477, NCT04364737, NCT04357106, NCT04392232, NCT04385199, NCT04393727, NCT04359810, NCT04358783, NCT04323800 |
| | Phase 2/3 | Convalescent Plasma | NCT04342182, NCT04332835, NCT04385043, NCT04374526, NCT04384588 |
| | Phase 3 | Convalescent Plasma | NCT04348656, NCT04345289, NCT04381858, NCT04362176, NCT04425915, NCT04361253, NCT04376034 |
| | NA[b] | Convalescent Plasma | NCT04321421, NCT04344015, NCT04408209, NCT04327349, NCT04346589, NCT04338360, NCT04348877, NCT04389944, NCT04397523, NCT04352751, NCT04383535, NCT04356534 |
| IVIG | Phase 2 | Intravenous Ig[c] | NCT04403269 |
| | Phase 2/3 | Intravenous Ig | NCT04261426 |
| | Phase 3 | Intravenous Ig | NCT04350580, NCT04381858 |
| Monoclonal Abs | Phase 1/2 | Meplazumab (Anti-CD147) | NCT04275245 |
| | Phase 2 | Tocilizumab | NCT04317092, NCT04331795, NCT04332094, NCT04346355, NCT04335071, NCT04339712, NCT04335305, NCT04315480, NCT04377659, NCT04433910, NCT04363853, NCT04370834 |
| | | Clazakizumab | NCT04343989, NCT04348500, NCT04363502 |
| | | Leronlimab | NCT04347239, NCT04343651 |
| | | Sarilumab | NCT04321993, NCT04357808 |
| | | Gimsilumab | NCT04351243 |
| | | Canakinumab | NCT04365153 |
| | | Pembrolizumab | NCT04335305 |
| | | Bevacizumab | NCT04344782 |
| | | Siltuximab | NCT04329650 |
| | | Nivolumab | NCT04343144 |
| | | Eculizumab | NCT04346797 |
| | | Pamrevlumab | NCT04432298 |
| | | Mavrilimumab | NCT04337216, NCT04399980 |
| | Phase 2/3 | Sarilumab | NCT04315298, NCT04341870 |
| | | Emapalumab | NCT04324021 |
| | | Bevacizumab | NCT04275414 |
| | Phase 3 | Tocilizumab | NCT04345445, NCT04330638, NCT04320615, NCT04412772, NCT04334382, NCT04372186, NCT04409262, NCT04356937, NCT04403685 |
| | | Siltuximab | NCT04330638 |
| | | Olokizumab | NCT04380519 |
| | | Canakinumab | NCT04362813 |
| | | Lenzilumab | NCT04351152 |
| | | Ravulizumab | NCT04369469 |
| | | Sarilumab | NCT04345289, NCT04327388 |
| | Phase 4 | Tocilizumab | NCT04377750 |
| | | Ravulizumab | NCT04390464 |
| | NA | Tocilizumab | NCT04310228, NCT04310228, NCT04306705 |
| | | Bevacizumab | NCT04305106 |
| | | IC14 | NCT04346277 |
| | | Canakinumab | NCT04348448 |
| Checkpoint inhibitors | Phase 2 | PD-1[d] blocking antibody | NCT04268537 |
| JAK[e] inhibitors | Phase 1/2 | Ruxolitinib | NCT04334044 |
| | Phase 2 | Ruxolitinib | NCT04338958, NCT04403243 |
| | | Tofacitinib | NCT04332042 |
| | | Baricitinib | NCT04321993, NCT04373044 |
| | Phase 2/3 | Ruxolitinib | NCT04348071 |
| | | Baricitinib | NCT04340232, NCT04358614 |
| | Phase 3 | Baricitinib | NCT04320277, NCT04345289, NCT04401579, NCT04421027, |
| | | Ruxolitinib | NCT04377620, NCT04362137 |
| | Phase 4 | Baricitinib | NCT04390464 |
| | NA | Ruxolitinib | NCT04337359 |
| | | Ruxolitinib | NCT04331665 |

(*continued on next page*)

**Table 1** (*continued*)

| Intervention | Phase | Type of Intervention | Registration Code |
|---|---|---|---|
| Interferon-based immunotherapy | Phase 1 | Recombinant interferon-α1b | NCT04293887 |
| | Phase 2 | Interferon-β1b | NCT04350281 |
| | | Interferon-λ1α | NCT04331899, NCT04354259 |
| | Phase 3 | Recombinant interferon-α1b | NCT04320238 |
| | | Interferon-β1a | NCT04315948, NCT04315948, NCT04324463 |
| | Phase 4 | Interferon atomization | NCT04254874 |
| | | Interferon nebulization | NCT04291729 |
| | | Interferon-β1a | NCT04350671 |
| | | Interferon-β1a | NCT04350684 |
| | | Interferon-β1a/β1b | NCT04343768 |
| | NA | Interferon-α2b Spray | NCT04273763 |
| | | Alfa interferon | NCT04251871 |
| Interleukin-7 (IL-7) | Phase 2 | CYT107 | NCT04379076, NCT04407689 |

*Abbreviations*: [a]Coronavirus disease; [b]not applicable; [c]immunoglobulin; [d]Programmed cell death protein-1; [e]Janus kinase.

restrain virus binding and can thus be useful in methods of treating or preventing viral infection. This can be achieved by using either an overall strategy of anti-SARS-CoV-2 neutralizing monoclonal antibodies, or anti-ACE2 monoclonal antibodies. The S protein in the viral membrane is the main mediator of virus entry into the target cells and plays a major role in determining host cell specificity of the virus. Two functional subunits consisting of the S1 subunit- the receptor interaction site- with RBD domain and S2 subunit responsible for fusion to host cell have been identified in the S protein. Multiple human neutralizing monoclonal antibodies against SARS-CoV-2 virus have been recognized that include 47D11 which has been shown to target the S1 RBD of SARS-CoV and SARS-CoV-2 spike proteins [117], and the B38, and H4 monoclonal antibodies that are capable of binding to SARS-CoV-2 RBD, but not to SARS-CoV RBD [118]. Of note, the identification of SARS-CoV-2 reactive antibodies has suggested both novel diagnostics and potentially better therapeutic tools for patients. Table 1 lists the clinical trials of immunotherapeutic approaches that provide passive humoral immunity against the COVID-19 disease registered in the ClinicalTrials.gov web site.

## 5. Conclusion

Documentation of the mechanism of hyperimmune host reactions triggered by the virus that results in hypercytokinemia is found to be complicated because human COVID-19 disease has been associated with severe clinical manifestations in the form of sepsis and the overlapping disorder, HLH-like illness, as well. As defined on pathology data, it seems possible that the robust inflammatory response elicited by SARS-CoV-2 virus may trigger a hyper-inflammatory disease course identified by HLH syndrome, in at least a subset of patients. There are also characteristics of sepsis with cytokine storm that might argue against HLH as the major cause for increased mortality in this pandemic setting. Since the aggressive immunosuppressive regimen required to treat HLH is absent in sepsis guidelines, differential diagnosis is critically essential between these two conditions [53]. However, the majority of physicians consider sepsis as a leading cause of critical illness for understanding of severe COVID-19 pathogenesis [54] mostly due to the fact that severe COVID-19 presents with hypercytokinemia [9,55], it is now evident that severe COVID-19 can cause sHLH [37,56].

There is overall agreement in that the great majority of confirmed COVID19 patients seroconvert and antibody response vary with different clinical manifestations and disease severity [72]. Finally, there is considerable inter-patient heterogeneity for circulating B cell responses, although it appears that both the proportion and number of B cells are not frequently decreased in both severe and non-severe patients [60]. Considering the dynamic acute immune response to SARS CoV-2 [67], a possible reason for the observed heterogeneity may rely in different sampling time points and different sample sizes in the discussed studies.

## 6. Availability of data and materials

Not applicable.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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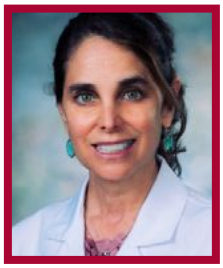
# COVID-19 and Lung Transplantation: From Donors to Recipients — Where Are We in 2022?

Deborah Jo Levine *MD, FCCP*



Coronavirus disease 2019 (COVID-2019) is due to the novel and highly infectious RNA coronavirus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Since the first human cases were described in December 2019, the disease has spread rapidly throughout the world and was declared a pandemic by the World Health Organization (WHO) on March 12, 2020,[2] impacting global healthcare systems and resulting in an immense volume of morbidity and mortality.

As of February 2022, there have been over 386,500,000 cases of COVID-19 diagnosed and over 5,700,00 deaths reported globally, with over 900,000 of these deaths in the United States alone.[2,3] The true prevalence of the disease has been suggested to be significantly underestimated, with seroprevalence studies suggesting a difference by a factor of up to 10x.[4] As healthcare systems became overwhelmed, public health measures and policies aimed their focus on limiting spread of the virus and maximizing available resources. The entire medical community, concentrated their attention and efforts on the acute clinical care of patients because of the sharp increase of infections.

Solid organ transplantation (SOT) was no different, and was not spared as the pandemic has profoundly affected every aspect of the field. Comprehensively, in the United States, the pandemic affected policy discussions and concerns on a center level as well as nationally in regards to the practice of transplantation. The primary concerns of the transplant centers initially were focused on acute care of candidates and recipients and shortages of resources (blood, staff, hospital facilities). This initially led to decreased transplant activity and increased morbidity and mortality in waiting list candidates and transplant recipients. In addition to the universal issues related to the pandemic, there have been many challenges specific to transplantation including rapidly changing trends in donor organ availability and procurement processes, modifications in candidate evaluation and waiting list additions, and candidate and recipient infections.

These issues along with others have particularly and uniquely impacted lung transplantation over any other solid organ transplant (SOT). This impact is primarily driven by the predominant pulmonary involvement of the virus, as well as its mode of transmission via the respiratory tract. Three significant areas of concern directly related to the field of lung transplantation are: (a) lung donation, (b) recognizing candidates for lung transplant who develop COVID-19 ARDS, and (c) evaluation and outcomes of recipients infected with the virus. Lung donation became a primary concern, not only in terms of concern of donor to recipient transmission, but also in terms of transmission to the transplant and procurement teams internationally. In terms of candidate selection, lung transplant teams are now considering which patients affected by COVID-19 ARDS or pulmonary fibrosis could be candidates for lung transplantation. Patients who have undergone lung transplant are a unique risk of post-viral complications that make them at a much higher risk for morbidity and mortality than other SOT recipients. We will be discussing these three topics in this review.

## Lung Donation and COVID-19

Lung transplantation is an established therapy for many patients with end-stage pulmonary disease. Advancements in surgical techniques, post-operative care and immunosuppression therapy have led to improved outcomes after transplantation. However, in the United States, in 2021 there were only 2524 lung transplants performed and there was a 10-15% mortality rate of those on the waiting list.[5] The major limitation to performing more transplants, in the US and globally, has been the general scarcity of lung donors in comparison to multi-organ donors. In the US, in 2021, there were 24,402 multi-organ deceased donors,[5] of those, only 2628 of those donors were lung donors.[5]

Already challenged by the general paucity of lung donors and high waitlist mortality, the field of lung transplantation suffered even more in the early part of the pandemic as there was a substantial decrease in the number of organ donations, leading to increased numbers of waitlist deaths. Initially, the processes of identifying and evaluating donors, sending them to the organ procurement organization (OPO) and on to procurement and transplantation was challenging as there was a decreased availability of OPO and transplant staffing, facilities and ICU beds.

As OPOs, transplant centers, transplant societies, and regulatory agencies gained knowledge and experience over the course of the pandemic, protocols were developed on all sides to increase the processes of organ donation and transplantation. In the *non-lung* organs (kidney, liver, heart and pancreas), centers explored first the use of donors with previous history of COVID but with a negative test, and then, carefully assessed the use of certain donors who tested positive with a remote history of infection and felt to be no longer infected. Multiple reports have been published of the utilization of *non-lung* organs from these donors for selected recipients with no reports of donor-derived transmissions.[6]

However, while these *non-pulmonary* transplants have been performed from COVID-19 positive donors, given the

uncertainties pertaining to lung damage and risk of transmission, significant concerns remain regarding transplanting lungs from these donors. In particular, since the respiratory tract carries the highest viral burden of SARS-CoV-2, lung donation is considered to have highest risk for transmission. As SARS-CoV-2 infection has the potential to cause pneumonia, diffuse alveolar damage, alveolar hemorrhage, and death in the recipient, at this time, these donors are not recommended for procurement for lung transplantation.

In fact, to date, UNOS reports three reported cases of unexpected donor derived SARS-CoV-2 transmissions occurring in lung recipients. Of note, in each of these cases, the donor had a negative COVID- 19 nasopharyngeal (NP) swab at the time of organ procurement, but was later found to have a positive SARS-CoV-2 result on bronchoalveolar lavage (BAL) (a lower respiratory tract sample)[7,8]

This issue was explored in more detail by the UNOS committee Disease Transmission Advisory Committee (DTAC) and they found from May 2021 to September 2021, that there was a total of 20 donors who were NP swab negative but BAL positive. In response to these findings, the OPTN mandated, effective May 2021, that all potential *lung* donors undergo testing of a lower respiratory tract specimen (i.e., tracheal aspirate, bronchoscopic washing, or bronchoalveolar lavage [BAL]) for SARS-CoV-2 by nucleic acid test (NAT) in addition to the normal screening (i.e., symptoms, history, exposures, upper respiratory tract sampling and any imaging). The test results need to be available prior to transplantation, thus allowing lung transplant programs time to evaluate the risk of donor-derived infections for potential recipients.[9,10] This mandate is based on evidence from prior studies in favor of greater sensitivity of the lower respiratory tract samples as compared to NP samples.

In conclusion, the use of non-lung organs from COVID- positive donors may present a viable pathway to transplant for selected patients who would benefit from an expanded donor pool, however, at this time the utilization of lungs from donors with a positive test is not recommended, given the risk of development of ARDS or pulmonary fibrosis after COVID-19 and the known association between respiratory viral infection and chronic lung allograft dysfunction (CLAD) in lung transplant recipients.

## Lung Transplant for COVID-19 ARDS and Pulmonary Fibrosis

Although there are multiple single center and international cohort studies which support transplant as a life-saving therapy in selected patients with severe COVID-19 associated ARDS, further experience is needed to identify those patients who would benefit from it.

As, lung transplant centers began seeing an increased number of referrals for these possible candidates, there were still so many unknowns on candidacy. In October 2020, Cypel and Keshavjee created and published recommendations[11] on how best to assess lung transplantation candidates in this difficult condition and to address clinical practice questions that transplant programs were facing. These recommendations included transplant consideration for: patients < 65 years of age, patients with single-organ dysfunction, at least 4-6 weeks after clinical signs of respiratory failure, those with radiological evidence of irreversible disease, those patients who are awake and able to discuss transplantation, those patients able to participate in physical rehabilitation, those patients who already meet the typical criteria for transplant and those patients who have a recent negative SARS-CoV-2 PCR result. The center should have substantial experience with high-risk transplantation and have access to a broad donor pool and low waiting list mortality.[11]

Beyond COVID-19 associated ARDS, there is a subset of patients who may improve clinically to the point where they may be discharged from the hospital, but still remain on oxygen as they have developed develop chronic pulmonary fibrosis from severe COVID-19. These patients may also benefit for lung transplantation long term.[12] Virus-induced lung injury, immune response, and attempts at healing are central to the process of leading to fibrosis. Further studies are needed to identify predictors of pulmonary fibrosis and understand which patients are most likely to progress to irreversible lung damage and might benefit from early lung transplantation.

## The Impact of COVID-19 in Lung Transplant Recipients

The coronavirus disease 2019 (COVID-19) pandemic has markedly impacted the field of pulmonary disease and lung transplantation. Lung transplant recipients, however, are uniquely affected and vulnerable to the effects of infection with SARS-CoV-2 when compared to patients with either chronic lung disease or recipients of other solid organ transplants.

Respiratory viruses, such as influenza, respiratory syncytial virus, parainfluenza, adenovirus, human metapneumovirus, rhinovirus and other coronaviruses, are pathogens that predominantly affect the respiratory tract. In lung transplant recipients, these viruses significantly impact the lung allograft as they have direct exposure enabling them to cause direct injury.[13]

Based on prior studies, acute lung injury and acute allograft rejection after a respiratory viral infection can occur in 5-55% of recipients. Patients are also susceptible to post-viral secondary bacterial infections. The most concerning issue, however, is that respiratory viruses are known risk factors for chronic lung allograft dysfunction (CLAD).[13] CLAD remains the major cause of graft dysfunction, failure and death after one year post lung transplantation. Following a respiratory virus, studies have reported a 20-30% progression to CLAD within one year post infection.[14]

The data regarding COVID-19 outcomes in lung transplantation are limited, as most large SOT cohorts contained only small numbers of lung recipients. The best strategy for therapy, as well as long term outcomes in this patient population is still lacking in data. Little is known about the impact of COVID-19 on pre-existing chronic lung allograft dysfunction (CLAD).

In terms of hospitalization and survival, international experience from multiple small single and multi-center lung transplant studies have been recently published. Morbidity and mortality rates were variable between the studies in 129 hospitalized patients.[15-21]

Collectively, hospitalization was required in 80-100% of COVID-19 infected lung transplant recipients with 10-42% requiring ICU admission [WASH U]. Respiratory support was required in 62-84% with 10-54% requiring mechanical ventilation. Severe disease occurred in 14-62%, and mortality ranged between 10-39%.[15-21] Treatment for COVID-19 was highly variable in the recipients among these studies. Overall, these series demonstrate worse outcomes in lung recipients compared to the general population. Since the number of lung transplant recipients is small worldwide, it will be important for all lung centers to follow and report long term outcomes of their patients, so better protocols and treatment regimens can be derived from a larger experience.[22]

## Conclusion

The COVID-19 pandemic has had a profound impact on the field of lung transplantation. Our current understanding of the SARS-CoV-2 virology and COVID-19 disease in the lung transplant population has grown significantly over the last two years as we have benefitted from the international collaboration and intense efforts of transplant physicians and scientists. As our experience continues to evolve, the transplant community will continue to navigate the best way to proceed to allow for lung transplant to be performed safely and optimally, donors to be evaluated efficiently and for those patients already transplanted, to make sure they have the most optimal preventative and therapeutic options available.

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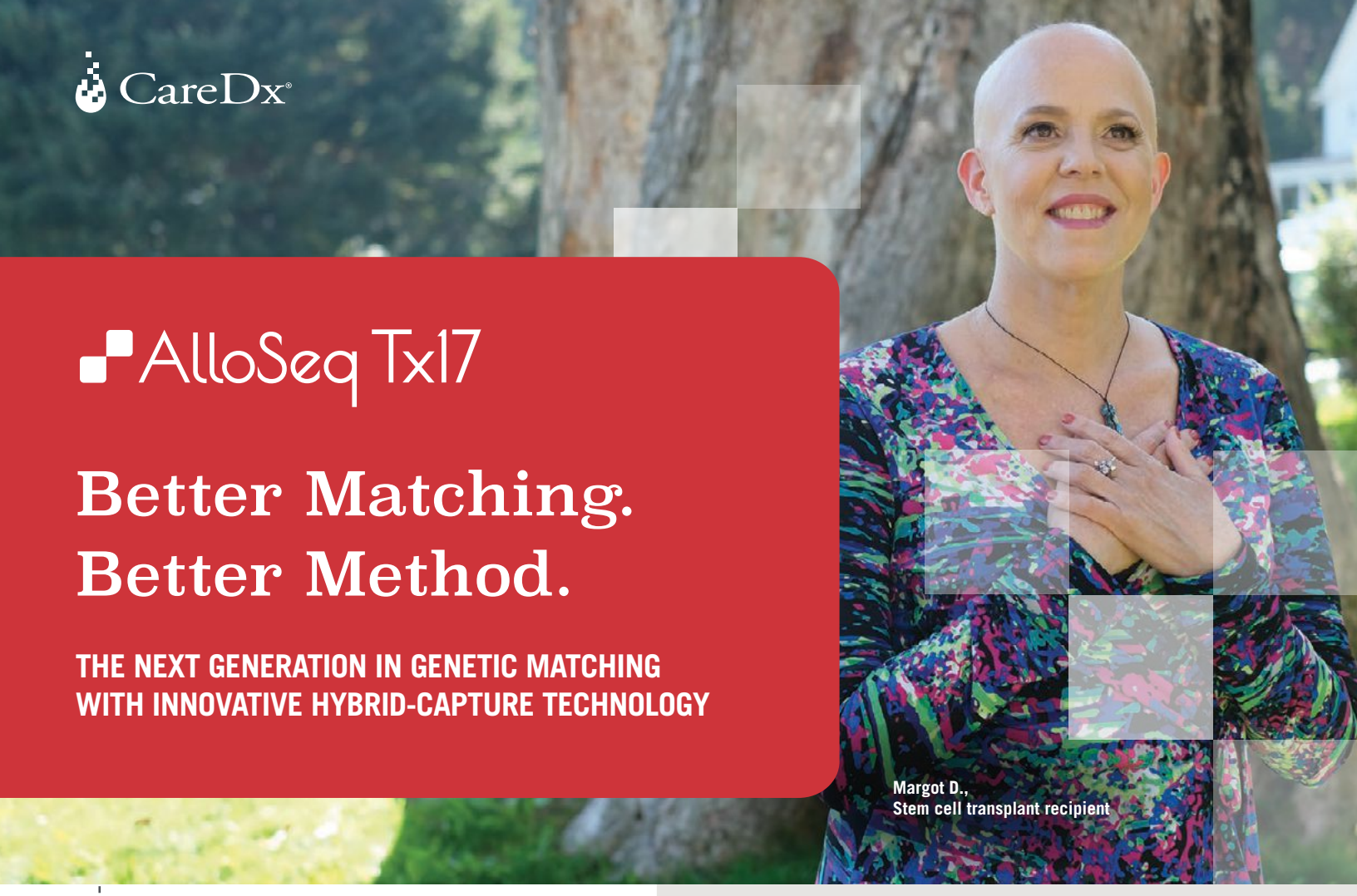


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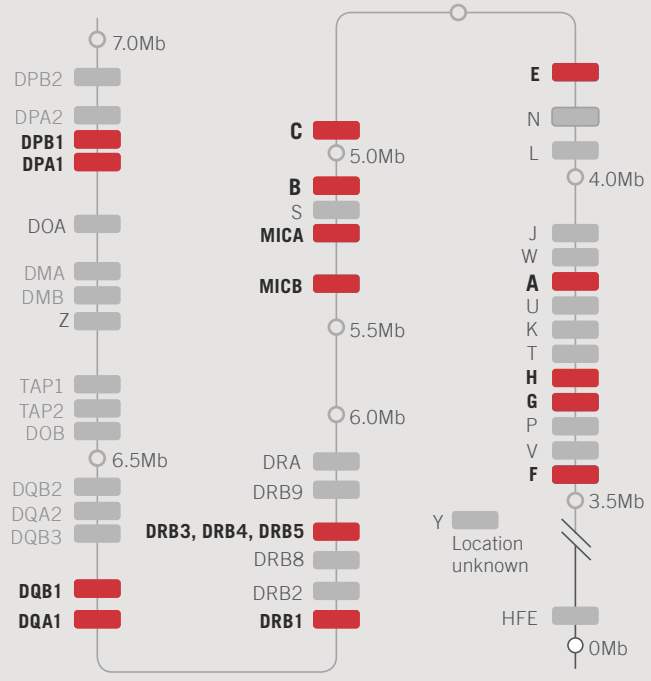


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# *ASHI Quarterly* Continuing Education Quiz

**Quiz Instructions:** The multiple-choice quiz below is provided as an alternative method of earning continuing education (CE) credit (CEC) hours. Read each article then select the ONE best answer to each question. The questions are based solely on the content of the article. Answer all of the questions and send the quiz answer sheet and fee (if applicable) to the ASHI Executive Office before the processing date listed on the answer sheet.

To be eligible for 3.0 credit/contact hours or 0.45 CECs, ALL questions must be answered, a passing score of 70% must be obtained and the answer sheet must be submitted with fee (if applicable) before the deadline. Quizzes may not be retaken, nor can ASHI staff respond to questions. Allow six weeks for paper processing following the submission deadline to receive return notification of your completion of the CE process. The CEC will be dated when it is submitted for grading. That date will determine the CE cycle year.

**Quiz may be taken online at ashi-u.com.**

## Saulle et al., Human Immunology 82 (2021) 551-560.

1. Loading of SARS-CoV-2-derived peptides onto HLA class I molecules allows which of the following cells to monitor possible ongoing infection?
   A. CD4+ T cells
   B. CD8+ T cells
   C. CD20+ B cells
   D. CD56+ NK cells

2. Which type of study was used to examine the relationship between SARS-CoV-2 infection and HLA?
   A. Cohort study
   B. Case-control study
   C. In silico epitope prediction study
   D. All of the above

3. True or False: The correlations between SARS-CoV-2 infection and HLA are still not entirely defined and results obtained so far are sometimes contradictory.
   A. True
   B. False

4. The physiological role of ERAP1 and ERAP2 aminopeptidases is:
   A. Degrading viral proteins in the cytosol
   B. Transporting peptides into endoplasmic reticulum (ER)
   C. Trimming peptides for loading onto class I HLA in the ER
   D. Degrading peptides in the ER

5. ERAP2 genotypes have been associated with which of the following conditions in the literature:
   A. Ankylosing spondylitis
   B. Psoriasis
   C. Resistance to HIV-1 infection
   D. Severe SARS-CoV-2 infection
   E. All of the above

## Humoral Immune Mechanisms Involved in Protective and Pathological Immunity During COVID-19

6. The first innate immune response against SARS-CoV-2 involve all of the following cells except:
   A. Macrophages
   B. Neutrophils
   C. Cytotoxic T lymphocytes
   D. NK cells

7. Which of the following is NOT a typical hallmark of the cytokine storm associated with SARS-CoV-2 infection?
   A. Cytokine storm is predictive of benign patient outcomes
   B. Clinical manifestations of fever, nausea, headache, and low blood pressure
   C. Increased concentrations of proinflammatory cytokines such as INF-g, IL-1, MCP-1
   D. Levels of circulating cytokines correlate with disease severity

8. Which of the following statements regarding humoral response against SARS-CoV-2 infection is FALSE?
   A. Plasma cells are enriched at severe and recovery stages
   B. Patients with X-linked agammaglobulinemia are not able to fight off and recover from Covid-19 infection
   C. There is considerable inter-patient heterogeneity for circulating B cell responses
   D. Unlike T cell responses, the number of B cells are generally not decreased in symptomatic Covid-19 patients

9. Humoral immunity against SARS-CoV-2 can involve the following model(s) of seroconversion:
   A. IgM seroconversion earlier than IgG
   B. IgM seroconversion synchronously with IgG
   C. IgM seroconversion later than IgG
   D. All of the above

10. Which of the following statements is TRUE regarding serologic testing for SARS-CoV-2?
   A. Serologic assays are sensitive for acute infection
   B. There is no role for serologic tests to be used clinically
   C. Early IgM and IgA antibodies are sustained well past the first month of infection
   D. The specificity of serologic test for SARS-CoV-2 is critical given the possibility of cross reactions with other community coronaviruses

## COVID-19 and Lung Transplantation: From Donors to Recipients — Where are we in 2022?

11. What was the approximate number of COVID-19 deaths reported globally as of February 2022?
   A. 3,865,000,000
   B. 386,500,000
   C. 5,700,000
   D. 900,000

12. True or false, COVID-19 has a unique impact on lung transplantation, compared to other solid organs, because of a predominant pulmonary involvement by the virus and its transmission through the respiratory tract.
   A. True
   B. False

13. True or false, in all three cases of COVID-19 transmission from donor to lung transplant recipient, the donor had a negative COVID-19 nasopharyngeal (NP) swab but was later found to have a positive COVID-19 bronchoalveolar lavage test.
   A. True
   B. False

14. OPTN mandates that all potential lung donors undergo the following:
   A. COVID-19 nasal swab rapid antigen test
   B. COVID-19 nucleic acid testing on a donor blood sample
   C. COVID-19 nucleic acid testing (NAT) of a lower respiratory tract specimen (such as tracheal aspirate, bronchoscopic washing, or bronchoalveolar lavage)
   D. All of the above

15. What percentage of lung transplant recipients infected with COVID-19 require hospitalization?
   A. 10-39%
   B. 10-54%
   C. 10-62%
   D. 80-100%

# Answer Sheet

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| | | | | | |
|---|---|---|---|---|---|
| 1 | A | B | C | D | |
| 2 | A | B | C | D | |
| 3 | A | B | | | |
| 4 | A | B | C | D | |
| 5 | A | B | C | D | E |
| 6 | A | B | C | D | |
| 7 | A | B | C | D | |
| 8 | A | B | C | D | |
| 9 | A | B | C | D | |
| 10 | A | B | | | |
| 11 | A | B | | | |
| 12 | A | B | | | |
| 13 | A | B | | | |
| 14 | A | B | C | D | |
| 15 | A | B | C | D | |

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**Answers to the 4th Quarter 2021 *ASHI Quarterly* Quiz:**

1. C; 2. D; 3. B; 4. E; 5. A; 6. D; 7. A; 8. B; 9. D; 10. A; 11. B; 12. C; 13. B; 14. D; 15. A

# Directors' Affairs Committee — NGS Survey report

Jennifer J. Schiller, *PhD, F(ACHI)*

Chee Loong Saw *PhD, HCLD, A(ACHI)*

Ahmed Mostafa, *MD, PhD, F(ACHI)*

Next-generation sequencing (NGS) technology has become a more and more common practice in HLA laboratories. Given its complex setup and steep learning curve, the Committee is aware that the method has not been fully adopted by the community for certain reasons. Those who have implemented the technology clearly see the advantages of it as a better tool to achieve higher accuracy and efficiency for genotyping work. While the experience of each laboratory is different, primarily due to the choice of technology and the time of their embarkation, the Committee understood that the collective experience of the laboratories, if put together, could be a good source of information for those who have not yet implemented NGS in the lab.

The ASHI Directors Affairs Committee thus conducted a survey aimed to gather information around and about member laboratory's experience in using NGS in November 2021. Fifteen questions were posed to HLA laboratories who have already or are in the middle of implementing NGS while five questions were posed to HLA laboratories who have not yet implemented NGS.

The survey was sent to 218 laboratory directors. The committee received a 20% participation rate. The demographics of laboratories that participated in the survey are represented in Table 1.

Among the labs that participated in the survey, 35 out of 44 and 41 out of 44 labs have identified themselves as labs performing HLA genotyping for stem cell transplantation and solid organ transplantation, respectively. Nine have identified themselves as OPO laboratories, while three identify as stem cell donor and cord blood registries. Four labs are from blood banks; five labs run immunology-testing, and four said they are also a molecular genetics laboratory while doing HLA genotyping work. Among all, seven labs identified themselves as research labs (Figure 1). Readers are reminded that these are service areas reported by individual labs where they could report having more than one service area.

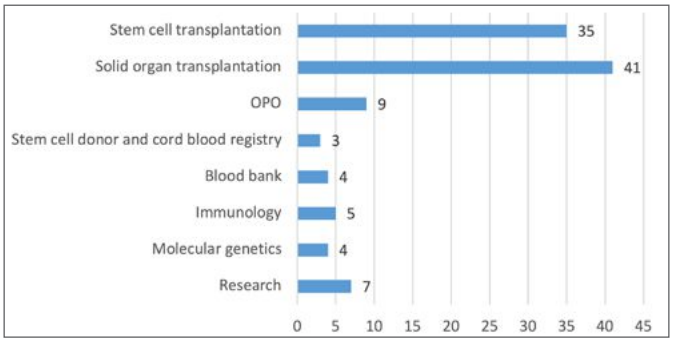
TABLE 1.

## Survey Participants

| | |
|---|---|
| Canada | 5 |
| New England (NH, VT, ME, MA, RI, CT) | 1 |
| Mid-Atlantic (NJ, NY, PA) | 1 |
| South Atlantic (DE, MD, DC, WV, VA, NC, SC, GA, FL, PR) | 7 |
| East North Central (WI, IL, IN, MI, OH) | 6 |
| East South Central (KY, TN, MS, AL) | 3 |
| West North Central (ND, SD, NE, KS, MN, IA, MO) | 1 |
| West South Central (TX, OK, AR, LA) | 4 |
| Mountain (MT, ID, WY, NV, UT, CO, AZ, NM) | 2 |
| Pacific (WA, OR, CA, AK, HI) | 7 |
| International Labs (Australia, Italy, UAE, Libya, Saudi Arabia) | 7 |
| TOTAL | 44 |

FIGURE 1.

## Laboratories' Service Areas

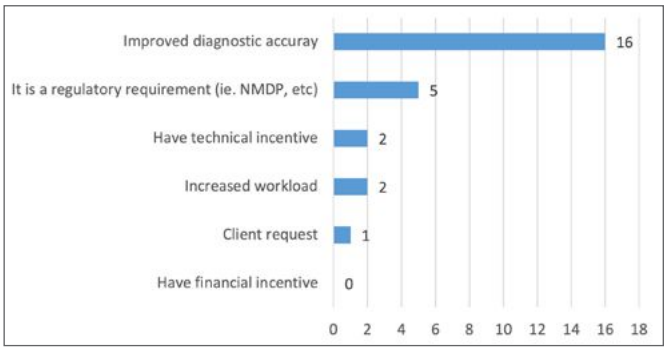



We categorize all participants into two groups: Group A are labs that have implemented, are in the middle or at the beginning of implementing, or have temporarily put their implementation on hold but have a timeline to implement NGS. Group B are labs that have thought about NGS or have tried to implement it but have put it on hold without a timeline to implement it, or have no current plan to implement NGS. There were 36 in Group A and 8 in Group B.

Asked what were the most important incentives and motivations for the labs who have implemented NGS (Group A), the clear message we found from those labs was that many of them wanted better diagnostic accuracy for their clients. The other important motivation for labs to implement NGS was that it is a regulatory requirement (i.e. NMDP) to employ sequencing-based technology for their work. In Figure 2, 30 responses are displayed and four irrelevant responses are left out.

FIGURE 2.

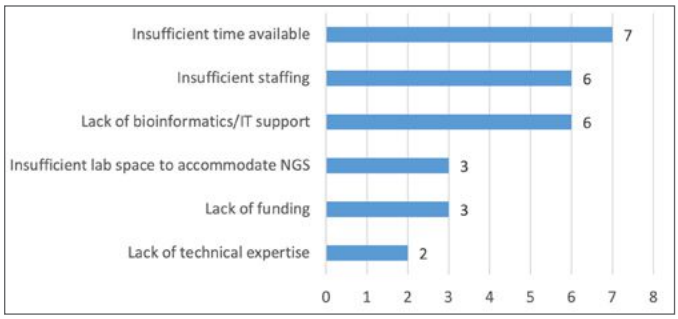
## Motivation to Implement NGS




Labs have also noted their major hurdles during the process of implementation. Among them are lack of bioinformatics/IT support (6 out of 30 labs), lack of technical expertise (2 out of 30 labs), and insufficient time available to the labs (7 out of 30 labs). The issue of lack of funding and lack of space to accommodate NGS were problems for a minority of labs (3 out of 30 labs) though not entirely irrelevant. In Figure 3 where 30 responses are displayed with three irrelevant responses left out.

FIGURE 3.

## Perceived most difficult aspect of the implementation

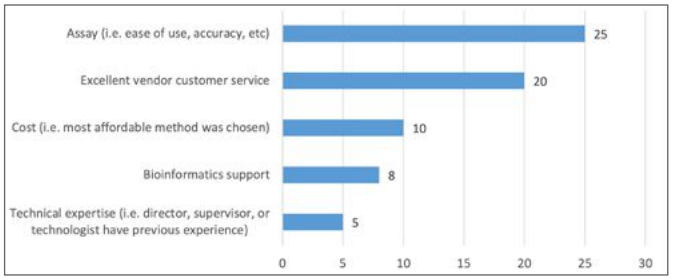



The Committee asked the HLA labs how much time is required for directors and technologists to plan for the implementation. These included, but not limited to, the following: reviewing technologies, learning, working on the bench, setting up LIMS, writing SOP, etc. Most laboratories estimated that time in the range of either 500-1000 hours or 1000-2000 hours are needed for a typical lab to set up NGS from scratch.

The Committee was also interested to find out among the few options known to labs, what have been the most important factors to labs when it comes to the decision on which NGS vendor to choose. Ranked by popularity, as many as 25 labs appreciate the ease of use of a particular NGS assay to be their reason for choosing the vendor (Figure 4). Twenty labs also agree that excellent vendor customer service is the next important reason why they would choose one NGS vendor over another. Affordability of NGS assay has become the third most important influencing factor to labs when they decide on which vendor to choose. Bioinformatics support the labs receive can be from the LIMS department, sequencer service company, or NGS assay vendor themselves. They were combined in our analysis and treated as one category of bioinformatics which the labs need to consistently deal with. To some laboratories, it does not appear that bioinformatics support was the major reason in a lab's decision process. We thought bioinformatics probably was a steep learning curve, but it did not amount to a problem too frequent or too severe that has hindered labs from using a particular bioinformatics solution or opted to use another. We reckon that similar to other LIMS solutions, NGS bioinformatics solutions (i.e. NGS analysis software) do come as a one-stop solution, and the installation is not necessarily handled by lab personnel. Instead, the LIMS department will be there to assist the lab in the installation and validation of the NGS analysis software. Once the hurdle is overcome, lab personnel rarely have to deal with the installation again. Labs do need to stay on top of the IMGT updates and the software updates. Speaking on the committee's own reflection in this exercise since all committee members' own HLA Labs have implemented NGS, we think it does take additional communication between the director, the supervisor, and the LIMS department to get the work off the ground. It also might take some extra time for labs who have not had any sequencing background to make the leap of moving from SSP/SSO-based methods, skipping the Sanger-based sequencing technology, and going straight to the NGS. Nevertheless, we agree that once this is done, labs would be rewarded for the effort they have made as they learn so much throughout the entire process.

FIGURE 4.

## Influencing Factors for Choice of Vendor/Method



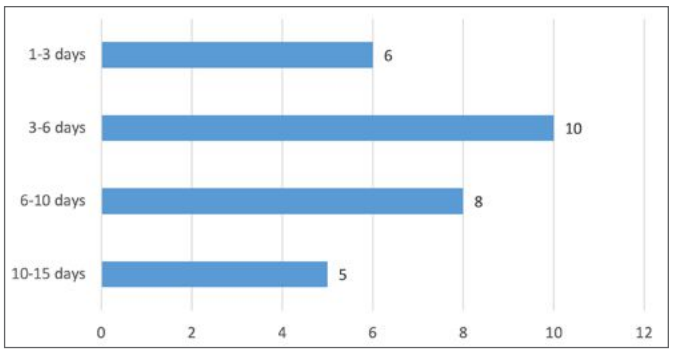

Most of the participants (25 over 30) said they have seen at least two or more NGS vendor demonstrations to understand the various properties and characteristics of different assays available to them. They also wanted to justify that what they choose to implement is really the optimal choice for their labs. Therefore seeing multiple vendor demonstrations has allowed many labs to do some comparison between the various vendors before committing themselves to one final decision.

That leads to the next question as to how many NGS methods had labs validated. We understand that seeing more demonstrations for oneself does not always mean that one had the resources to validate all of them. Most labs have seen more than one demonstration, but 18 labs only validated one method, nine labs validated two methods while three labs validated three or more methods. We did not ask the reason why labs validated multiple methods, understanding that labs who validated two methods did that just to have the second method as a backup method. This is similar to a common practice in the past where many labs validated SSP and/or SSO methods as a secondary method to their primary method.

Our survey found that most labs (19) have a wet bench procedure that goes up to 24 hours before loading the library into the sequencer. Four labs stated that they have a shorter than 24-hour process to prepare their library. Six labs have 1-3 days TAT that includes result reporting. Ten labs have 3-6 days, eight labs have 6-10 days, while five labs will send out the report in 10-15 days after the receipt of the samples (Figure 5).

FIGURE 5.

## NGS Turnaround Time




As expected, 84% (26 out of 30) of the labs reported using Illumina as their sequencer while 13% (four) said they use Ion Torrent. Among the Illumina platform users, 85% of labs use MiSeq, 19% of labs use MiniSeq, and 12% use ISeq.

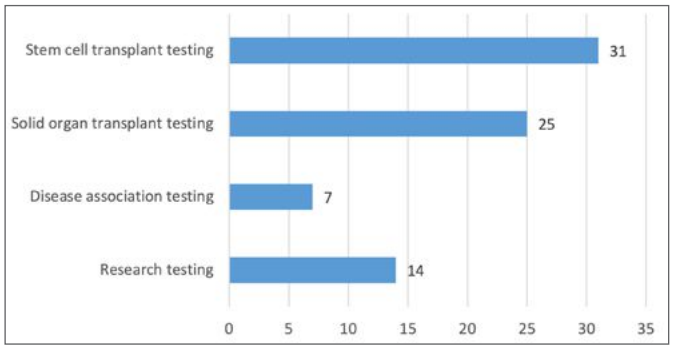
Two thirds of the labs do not have a liquid handler while seven labs mentioned they have access to liquid handlers that process their pre-, as well as post-PCR steps. Two labs said they only have access to liquid handle for their pre-PCR steps while two other labs said they only have access to liquid handle to do the post-PCR steps.

Qubit is undoubtedly the mainstay in labs that perform NGS genotyping: 96.7% of labs have one. One in 10 labs practice the PippinPrep library selection method. Two in 10 labs have access to a fluorescence reader for DNA quantification. Two labs use Bioanalyzer.

Of all labs that have implemented NGS, 31 said that they utilize NGS in testing for stem cell transplantation, an area of service that is clearly requiring labs to be up to date with the technologies and the regulatory requirements. The majority (80%) of the labs that have implemented NGS also utilize the method for solid organ transplantation testing. 45% (14) of the labs responded saying they use NGS for their research projects while just above 20% (7) of the labs would use NGS for disease association testing.

FIGURE 6.

## NGS Utilization




The Survey tried to capture the landscape of the labs that did not implement NGS (Group B). There were eight labs in total in Group B. Among these labs, 6 of 8 said they plan to implement NGS in the next 1-2 years while one lab said it would wait for another 3-6 years and one lab said it does not have a plan to validate NGS. Similarly, labs who have not validated NGS agree that NGS would improve diagnostic accuracy (70%) as the most important incentive for them in the future. A couple of labs think they would be more motivated to implement NGS if they had the technical expertise and if their client requested it. These labs currently use SSO (3) and RT-PCR (5) to reach allele-level or allele-group typing for their day-to-day operation.

The Committee extended the question further to help elucidate the hurdles that have hampered labs' intention to implement

NGS. A mixture of responses is what we found. Four labs out of eight think that NGS, at the current state, is too complex to handle, therefore they would wait for a more mature technology to be marketed. Four labs out of eight think they lack the technical expertise and experience as they have not done any prior sequencing-based method. One lab said their clients are not interested in NGS while two responses said there was a lack of approval from their administration and/or funding. One lab holds that being a solid organ only lab they do not see the need to implement NGS, see Table 2.

TABLE 2.
Major Hurdles Preventing Labs from Implementing NGS

| ARE THEY ACTUAL HURDLES? | IN RESPONDENTS' VIEW, THEY ARE |
|---|---|
| High test complexity | Seen as major hurdle |
| Lack of technical expertise | Seen as major hurdle |
| Lack of approval from administration | Secondary hurdle |
| Lack of funding | Secondary hurdle |
| Client not interested | Secondary hurdle |
| Lack of IT | Not significant |
| Insufficient space | Not significant |

## Summary

We hope that the Survey has provided a current understanding of the use of NGS in the HLA community. The Committee, whose labs have implemented the technology, agrees that NGS is certainly a valuable tool that can achieve more accurate diagnostic results for our patients and donors.[1-3] The benefits of NGS are undisputable and multifold. We hope our readers could review these data for their individual needs. For the vast majority of labs that have not yet implemented NGS, this survey could serve as a reference in the aspects if your lab is planning for one. The estimated range of hours, the perceived hurdles, the choice of sequencing platforms, and what TAT is expected are among the resources one should be looking into and be prepared to invest in. There certainly are hurdles that each individual lab has to overcome when comes to the validation work. Again, we reiterate that once this is done, labs would be rewarded for the effort they made because they will learn so much throughout the entire process. In the end, it is in us to fulfill our goal to advance the science and technology for patient testing with a better diagnostic tool.

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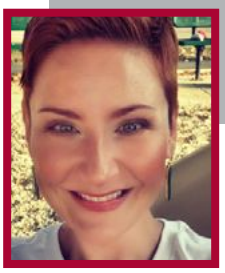
3. Smith AG, Pereira S, Jaramillo A et al. Comparison of sequence-specific oligonucleotide probe vs next generation sequencing of HLA-A, B, C, DRB1, DRB3/B4/B5, DQA1, DQB1, DPA1, and DPB1 typing: Toward single-pass high-resolution HLA typing in support of solid organ and hematopoietic cell transplant programs. *HLA.* 2019 Sep;94(3):296-306. doi: 10.1111/tan.13619

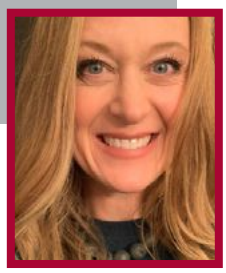
# Communication, Engagement, and Marketing Committee (CEMC) Update

## *Celebrating Abstract Success from the ASHI 2021 Annual Meeting*



Kelley Hitchman

Carey Killion



Anne Halpin

A highlight for attendees and participants at every ASHI meeting is the opportunity for clinicians and scientists to submit abstract summaries of ongoing and cutting-edge investigative projects. These abstract project summary submissions are scored by a large pool of experts from the ASHI community. The highest scoring and most innovative research projects are selected to be presented in short oral presentation format at the meeting, but not every project can earn this opportunity. The remaining high-quality project submissions are invited to be presented in poster format at the annual meeting. All of the work is valuable, and it is an honor for the investigators selected to share their results.

For the first time in ASHI history, the 2021 meeting included an option for those submitting abstracts to also describe their projects as a "plain language" summary fit for public consumption. These "plain language" summaries were meant to clear out the jargon and confusion allowing a broad public audience to connect with the excellent clinical and scientific research going on in the ASHI community. The ASHI community stepped up and the vast majority of submissions included a "plain language" summary of their ongoing work. The ASHI community was clearly excited to describe their ongoing work in a way that would be accessible to the patients, donors, and caregivers who would most benefit from these projects.

Of the "plain language" summaries submitted, several stood out for their quality of science and their translation to "plain language". Those 13 project summaries are presented below and represent a selection of high-quality research and clinical progress from the ASHI community in "plain language". These summaries were submitted to an esteemed panel of patient, donor, and caregiver partners for review and the scoring was based on 1) lack of jargon, 2) conciseness of summary, 3) potential for impact to the professional field, and 4) potential for patient impacts. ASHI is extremely grateful for the time and expertise of all who gave their time to review these project summaries. This expert review panel included:

- Amy Silverstein
  - Author, Lawyer, two-time heart transplant recipient and CEMC committee member
- Mary Libby
  - HLA Laboratory Technologist at The Cleveland Clinic, caregiver to a two-time kidney transplant recipient and CEMC committee member
- Keith Libby
  - Two-time kidney transplant recipient
- Jim Gleason
  - Heart transplant recipient and President of the Transplant Recipients International Organization (TRIO)
- Bethany Snipes
  - Clinical Laboratory Science student at UT Health San Antonio
- Johanna Henz
  - Volunteer, bone marrow recipient and lung transplant recipient

Of these 13 outstanding projects and "plain language" summaries, one stood out for the patient, donor and caregiver partner review panel. The inaugural "plain language" summary award was given to Dr. Anna Greenshields and her collaborators for their project, "Optimization and diversification of the LabScreen COVID PLUS assay". Reviewer Amy Silverstein commented that this work was an "excellent summary" which clearly "explains the what, why and how in helpful dimensions. After reading just once, I feel that I have a good grasp on the abstract"; Jim Gleason noted that the summary was "nicely done. Easy for this layman (with lots of medical experience and reading) to understand the first time through"; And Johanna Henz praised the abstract summary as "very good! Easy to understand, perfect length and timely subject matter. Especially with new variants, something like this has tremendous potential impact: results that much faster is incredible". Dr. Greenshields was awarded $250 for her winning "plain language" summary.

This effort to present ASHI research and innovation in a manner that will be useful for the broader public is only just beginning. Thanks to the enthusiastic participation in this effort in 2021, the "plain language" summary will be a required component for all abstract submissions and oral presentations given at the 2022 Annual ASHI meeting. The ASHI community is proud to have begun the journey of engaging with our patient, donor, and caregiver partners to enhance the inclusion of the benefactors of the research and the broad public.

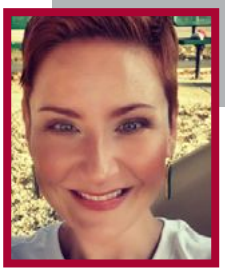
| | |
|---|---|
| **The impact of imlifidase on donor-specific antibodies (DSAs) in serum samples from patients diagnosed with antibody-mediated rejection (ABMR)**<br>Angela Maldonado | Donor-specific antibodies (DSAs) can cause kidney transplants to be rejected and possibly fail in a process called antibody mediated rejection (ABMR). Imlifidase is a medication which can interfere with the activity of DSAs. The impact of imlifidase on DSAs has the potential to offer kidney transplant patients diagnosed with ABMR, a treatment option in cases of ABMR where other agents may be less suitable at preventing damage to the kidney transplant. |
| **A WEB-BASED CPRA CALCULATOR BASED ON A COMPREHENSIVE HIGH RESOLUTION HLA GENOTYPE PANEL OF US STEM CELL DONOR**<br>Loren Gragert | Patients on the waiting list to receive an organ will often have antibodies in their blood that cause donors with certain tissue types to be incompatible. Our improved calculator will help the organ allocation system make more accurate adjustments to ensure that patients have equal access to organ transplantation, regardless of how many antibodies they have. |
| **Novel bead-based assay assesses the impact of imlifidase on ABO IgG antibodies**<br>Anne Halpin | ABO blood groups of donors and recipients are usually matched when we transplant organs. This is because humans naturally make ABO antibodies to ABO antigens we do not have. But sometimes it makes sense to transplant patients with organs from ABO mismatched donors to provide more opportunities for transplant. There are two kinds of antibodies: 'Y' shaped proteins called IgG and groups of 5 'Y' shaped proteins called IgM. Both IgG and IgM ABO antibodies are important in transplantation but the methods we currently use to measure the levels of both kinds of ABO antibodies don't always work well. We designed a new test to measure ABO antibodies and we tested it in many healthy people. We also tested it in the presence of a drug that removes IgG antibodies. This new assay shows us that people have a wide range of ABO antibodies and that this new test is good at detecting when antibodies are present and when they have been successfully removed. |
| **VISUALIZING HLA HAPLOTYPE DIVERSITY USING ALLUVIAL PLOTS**<br>Marian Dribus | For patients who need organ transplants, genetic markers can show if donors and recipients are good matches. We created a computer program to visualize how often certain combinations of genetic markers are found in the population. This makes it easier to identify which genetic markers are the most important for organ matching and disease risk. |
| **EDITING OF THE HLA GENE: A NOVEL THERAPY FOR AUTOIMMUNITY**<br>Christina Roark | This study is designed to show that we can perform "plastic surgery" on the immune system by selectively editing the HLA gene as potential therapy for autoimmunity. Specific tools have been developed to edit the HLA gene at a precise location and change a single amino acid in the HLA molecule. This change disrupts peptide binding and presentation to autoreactive T cells which turns off the immune response and attenuates disease. |
| **Individualized genetic makeup that controls natural killer cell function influences the efficacy of Isatuximab immunotherapy in patients with multiple myeloma**<br>Raja Rajalingam | Natural killer (NK) cells are a type of white blood cell that defends us from cancer. Genes in our body regulate NK cell response. These immune genes differ by the person in their structure and function, and such variation makes some individuals more susceptible to cancer. Herein, we discovered specific genes that influence NK cell antitumor activity and correlate with clinical outcomes in patients with multiple myeloma cancer treated with monoclonal antibodies. These findings offer biomarkers to identify, via precision medicine approach, cancer patients more likely to benefit from monoclonal antibody immunotherapy versus those who appear resistant. |
| **HMGB1-TLR4 and HMGB1-TLR9 Axes Differentially Polarize Macrophages to Distinct Phenotypes that Contribute to Alloimmunity Following Ischemia-Reperfusion Injury in Human Orthotopic Liver Transplantation**<br>Allyson Terry | Sometimes during a liver transplant, the donated liver experiences too much damage when it is surgically removed, transported, and then transplanted into the recipient. This damage at the time of transplant can influence how the immune system responds to the new liver after the transplant surgery. We studied how a protein released during the transplant surgery called disulfide HMGB1 can affect macrophages – the "middlemen" of the immune system – to ultimately interact with T and B cells – the immune cells responsible for long-term immune responses. We found that disulfide HMGB1 makes macrophages more likely to contribute to an inflamed environment in the liver, to activate T cells, and to engage with antibodies that are produced by B cells, all of which can be detrimental to the donated liver. We could block these functions with a treatment that prevented disulfide HMGB1 from initially activating the macrophages. This study shows how future treatments aimed at preventing disulfide HMGB1 from binding to macrophages can help decrease the immune response to donated livers and improve the health of liver transplant patients. |

| | |
|---|---|
| **GENE EDITING OF DRB1*04:01 AT POSITION 71 BLOCKS COLLAGEN SENSITIZATION AND AVOIDS ALLORECOGNITION**<br>Vibha Jha | Rheumatoid arthritis (RA) is a chronic disease where the immune system mistakenly attacks joints causing swelling, severe pain and in some cases, permanent damage. The HLA molecule, such as DRB1*04:01, is involved in activating immune cells that are responsible for these misguided attacks. We have shown that gene editing of DRB1*04:01 is safe and prevents activation of harmful immune cells in a mouse model which can then be translated to human patients to halt RA disease. |
| **OPTIMIZATION AND DIVERSIFICATION OF THE LABSCREEN COVID PLUS ASSAY**<br>Anna Greenshields<br>**WINNER OF THE BEST REAL LANGUAGE ABSTRACT AWARD** | It is important to understand how the body can protect itself from SARS-CoV-2, the virus that causes COVID-19. Tools that let us identify antibodies that target this virus are useful for monitoring the body's response to vaccination and for the development of new vaccines. Recently, One Lambda released a new test that allows researchers to identify SARS-CoV-2 specific antibodies in human blood. In our work, we describe a procedure that allows scientists to get antibody results 70% faster than with the original method. We also describe how the test can be expanded to look at different antibody types and functions |
| **Unique Molecular Identifier Enhanced HLA Typing and Transcript Quantitation using Nanopore Technology**<br>Eric Weimer | HLA matching is critical for optimal transplant outcomes. However, determining the specific HLA of person can be time consuming and takes longer than allowed for deceased donor kidney organ donation. To improve this, we've developed an assay that not only determines the specific HLA an individual possesses but also the specific amount of that HLA. We believe this will lead to improved matching for organs and better long-term organ survival. |
| **Visualization of Linkage Disequilibrium Patterns of Frequent Mismatched Amino Acid Positions Across HLA Class II Loci: Implications for Kidney Transplant Outcomes Studies**<br>Grace Wager | If the amino acids on an organ recipient's immune surveillance molecules do not match those of the donor organ it is known to increase the risk of graft failure. The current system for assessing the risk of mismatching only considers certain areas of the immune surveillance molecules and gives all their possible amino acid mismatch positions equal risk. We have found that the amount of risk varies with each amino acid mismatch position; therefore, weighted risk should be utilized in any organ allocation matching assessment, along with other variables not currently considered which are described in our abstract |
| **Precision medicine in transplantation: epitope identity or minimal mismatch at Human Leukocyte Antigen DQ or DR reduces the probability of donor-specific antibody and provides a practical and feasible strategy for optimizing compatibility**<br>Jenny Tran | More than half of transplanted kidneys are lost prematurely. The most important cause is rejection in which antibodies are formed to proteins called the human leukocyte antigens (HLA) on the kidney. The data we present here show that patients who are well-matched for special targets on these molecules (known as epitopes) have reduced risk of developing these antibodies and we propose a new strategy for epitope matching to reduce the risk of graft loss and prolong the life of the graft |
| **STEM CELL DONOR-DERIVED CPRA BETTER PREDICTS TRANSPLANT ACCESS DISPARITIES THAN CURRENT CPRA**<br>Kelsi Lindblad | CPRA is used to measure how difficult it is for a transplant candidate to find a compatible donor. We tested a new way of calculating CPRA and found that it is better at telling how many donors a candidate will be compatible with. Using this new CPRA would help order the transplant waiting list more fairly, with a better chance that the candidates who have the hardest time finding compatible donors will be at the front of the line when one of the few donors that work for them becomes available. |

A complete list of all the abstracts, the complete author lists, and their institutions as presented at the 2021 ASHI 47th Annual Meeting can be found on the Human Immunology website: https://www.sciencedirect.com/journal/human-immunology/vol/82/suppl/S

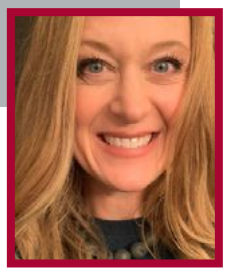
# Communication, Engagement, and Marketing Committee (CEMC) Update

## *Planning Your Lay Abstract for ASHI 2022!*



Kelley Hitchman

Carey Killion



Anne Halpin

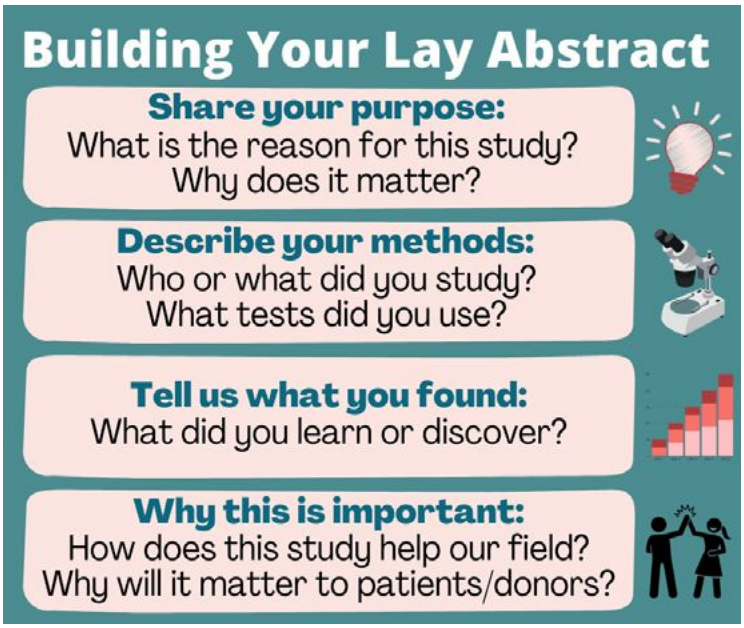
In this issue of the Quarterly we shared with you some of the best abstract lay/plain language (real speak) summaries from the ASHI 2021 meeting. It's already time to work on your abstract for the 48th Annual ASHI meeting which will be held from October 23-28, 2022 at the Bally's Las Vegas. Don't ask us how the year has flown by. We are as baffled as you are.

*Because of the fantastic response in submitting lay abstracts in 2021, these plain language summaries will be a mandatory requirement for all submitted abstracts for the 2022 ASHI annual meeting.*

Here are some tips for creating this submission:

- Avoid acronyms and jargon type language
- Have a friend or family member who doesn't work in histocompatibility or immunogenetics read your summary and provide feedback on its clarity (we recommend teenagers as they are very, very happy to critique things)
- Don't try to "convert" your science speak abstract; start with a fresh perspective and think how you would explain this to a patient, donor, or caregiver of a patient
- Focus on the key points as shown in the figure below

There is a growing push in many scientific communities to include lay summaries with published work and there are many great resources online to aide in crafting clear and concise plain language summaries. Thank you for helping ASHI to translate the work we do! Being able to translate our science, discoveries, and innovation to the world only expands our ability to better serve our patients, donors, and families.




**NO JARGON ALLOWED!**
**1-2 sentences for each section**

# PT Update

*A Behind-the-Scenes View of the ASHI PT Program*

Mary Philogene, *PhD, F(ACHI)*
ASHI PT Program Director

Cheryl Hartman, *PT Program Manager*

On behalf of the PT Committee, here is an update on ASHI's Proficiency Testing Program.

In 2021, a webinar was provided by the ASHI PT Program Manager entitled, "Behind the Scenes of ASHI PT: An Insider's Perspective on Access, Ordering, & FAQs" which focused on administrative information. The webinar was designed to benefit subscribers of the ASHI PT Program and to provide clarification on frequently asked questions. Information provided in the webinar is being presented in this article in a Question and Answer format, with some additional information, for those who may have missed the webinar. A recording of the original webinar is available in ASHI University.

## Q: What types of surveys does the ASHI PT Program offer?

**A:** Surveys offered by the ASHI PT Program include the HLA Typing (HT) survey, the Antibody Crossmatch/Identification (AC) survey, and the Engraftment Monitoring (EMO) survey. ASHI also provides educational offerings: Anti-Angiotensin II Type 1 Receptor (AT1RAb), Virtual Crossmatch (VXM), and Complement Component 1 q (C1q).

## Who oversees the ASHI PT Program?

The PT Committee oversees the ASHI PT Program. Members of the committee hold a variety of roles. The PT Executive Committee (PT EC) are members with the most experience and thus have the most responsibility. Each of the three PT EC co-chairs is responsible for reviewing one of the 3 surveys provided by the ASHI PT Program: HT (HLA Typing, HLA-B27 Detection), EMO (Engraftment Monitoring), and AC (Antibody Screening/Identification and Crossmatching) surveys. The newest members of the PT committee serve as reviewers of data entered by participating laboratories. Reviewers also provide support to the co-chairs by reviewing or drafting portions of each survey report.

## Who is the point of contact if I have a question about my PT shipment?

Because the vendor that ships blood to participants is not contracted to answer questions or emails directly from laboratories, the PT Program Manager must be the point of contact if a laboratory has a question about their shipment or their tracking information. The PT Program Manager is the ASHI staff member that provides administrative support to the PT Committee and acts as a liaison between the various components of the ASHI PT Program. The PT Program Manager serves as liaison between subscribing laboratories and the vendor that procures and ships blood, and as liaison between laboratories and committee members.

It is also the PT Program Manager's responsibility to coordinate discussions between vendors and the PT Committee. The PT Committee is always assessing ways to improve the process of getting blood shipped to laboratories and that may include discussing alternative options with the vendors in the hopes of reducing processing and shipping times.

## How many countries subscribe to the ASHI PT Program?

Currently, twenty-six countries, including Australia, Qatar, Pakistan, China, and New Zealand (among others) subscribe to the ASHI PT Program.

## Is ASHI a CAP-accredited PT Provider?

A: ASHI is accepted by the College of American Pathologists (CAP) as an alternative proficiency testing program provider for the HLA Typing, HLA-B27 Detection, Engraftment Monitoring, and Antibody Screening/Identification and Crossmatching surveys. If your laboratory is accredited by CAP, ASHI is able to report your ASHI PT Survey Performance Reports directly to CAP. For your convenience, this information is posted on the ASHI PT page of the ASHI website.

## Where can I find the PT shipping schedule?

When a participant logs into the ASHI website (www.ashi-hla.org), they can access PT information by selecting the Lab Programs tab, then selecting Proficiency Testing. The PT page contains information pertinent to the ASHI PT Program including the current shipping schedule indicating when the surveys will be shipped, the PT brochure detailing the contents of each survey, typing information for current and prior surveys, Virtual Crossmatch (VXM) articles, the Attestation Statement (PDF), and the PT Operations Manual which includes grading information and requirements pertaining to each shipment.

The PT page of the ASHI website also contains the link for the ASHI PT Lab Center, the portal which allows subscribing laboratories to enter PT results, access survey instructions for entering results, and view their laboratory's grades and reports.

Access typing information and detailed ASHI PT Program information, go to the PT info page.

Access the portal for entering PT results and viewing reports, go to the ASHI PT Lab Center.

## Who should I contact if I have a question about a grade received on a report?

PT-related questions should be sent directly to the PT Program Manager, Cheryl Hartman, at chartman@ashi-hla.org. Questions and concerns are then forwarded to the PT Executive Committee who reviews and provides a response once the issue has been discussed. This process is not immediate since each of the phases – review, discussion, and final decision - involves committee members located in different labs and in different time zones.

## What is the best way to submit PT-related questions to the ASHI PT Program Manager?

The quickest and most direct way to submit your PT-related questions is by emailing the PT Program Manager directly at chartman@ashi-hla.org. Sending emails through the general info box (info@ashi-hla.org) and through the contact form in the ASHI PT Lab Center is also possible, but is not as immediate as sending to the Program Manager's email.

## Tip: Screenshots help resolve issues more quickly.

Even when submitting basic questions regarding login and access issues, it is helpful to include a screenshot so the issue can be pinpointed more quickly.

## If I encounter an issue with the ASHI PT Lab Center, need help entering PT results or deciphering the survey instructions posted in the ASHI PT Lab Center, who should I contact?

All of these questions should be submitted to the ASHI PT Program Manager at chartman@ashi-hla.org. Labs with questions about PT result entry should be aware of the following:

### 1. Timing is key

The earlier a question is submitted prior to the submission deadline, the more likely you are to receive an appropriate and timely response. Result entry questions submitted during the last week of the deadline, especially during the last two days of the deadline, may be delayed due to a number of labs that choose to submit results during the last few days leading up to the deadline. Additionally, if the issue involves a software glitch, the IT team, which oversees many different platforms at once, may need to get involved which may take additional time to resolve. To avoid these types of issues and delays, laboratories are encouraged to submit results prior to the last week of the deadline.

Deliverables, deadlines, and projects required for the three different surveys offered by ASHI – HT, AC, and EMO surveys - often overlap so PT EC members must contend with a variety of questions and requests at any given time. Therefore, it is always recommended that laboratories submit their PT results a week before the deadline so there is adequate time to resolve any software issue or unexpected glitches that may arise.

### Tip: If necessary, re-submission of results is possible prior to the deadline

Laboratories can submit and re-submit as many times as necessary prior to the submission deadline. If a laboratory submits PT results early and discovers an error after submitting the results, participants can edit their submission and resubmit their PT results as long as it is prior to the submission deadline. Only the most recent version of the submitted results will be reviewed and graded since the last submission overwrites earlier submitted results.

### 2. Providing examples is very helpful

Sending screenshots showing an error message or the resulting page after encountering an issue is very helpful. Or, if a portion of the survey instructions must be clarified, including a screenshot of the specific paragraph and section is also helpful in speeding up the process.

### 3. Survey instructions posted in the ASHI PT Lab Center should be reviewed first

Detailed instructions for entering PT results are provided for each survey and are updated prior to every survey. If a participant has a question about result entry, the instructions should be consulted prior to contacting the PT Program Manager since the PT Executive Committee spends a considerable amount of time providing specific data entry information for each survey.

### 4. Data entry in the ASHI PT Lab Center should be reviewed to avoid errors

Laboratories are strongly encouraged to carefully review data entry for typos prior to submitting PT results. While it is understood that the software in the ASHI PT Lab Center is different from information systems used in HLA laboratories, PT result submissions that include data entry errors will result in a Discrepant grade and cannot be contested. Survey instructions posted in the ASHI PT Lab Center should be fully reviewed and followed during the data entry process to avoid Discrepant grades.

### 5. Data interpretation questions are not within the scope of the PT EC

It is not the role of the PT Executive Committee to recommend how laboratories should interpret the data. PT surveys are graded based on consensus among all participants. Though the PT EC may help participants interpret how the ASHI PT Lab Center portal operates and provide information on how data should be entered via the portal, the committee cannot recommend how to interpret the data.

The information contained in this article is part of the information provided in the webinar recording located in ASHI University. Additional information on the recording includes how the PT Executive Committee is structured, the difference between personal ASHI accounts and a MAIN LAB ACCOUNT, a live demo of the ASHI PT Lab Center, and a live Q&A session at the end of the webinar. If you have any questions regarding the ASHI PT Program, please feel free to contact the ASHI PT Program Manager directly at chartman@ashi-hla.org.

# ACHI Update

Shalini Pereira,
*PhD, F(ACHI),*
*ACHI President*

Melissa Weeks,
*ACHI Staff*

## Certification Corner

### ACHI Update for the ASHI Quarterly

When ACHI launched in October of 2020, questions from the community about the transition from the former ABHI to the new ACHI were anticipated. People have been familiar with ABHI for a couple decades as ASHI's sister organization, so adopting the new language is bound to take time. The ACHI's Board of Directors has committed to outreach and transparency on this topic in order to help with the transition.

One of the areas of confusion that has been noticed over the past year is concerning ACHI Certification and ASHI Membership, and the idea that the two are interchangeable. Quite a few ASHI members are also ACHI certified CHT, CHS, or Directors and vice versa. To clarify, ACHI and ASHI are independent entities governed by their own Presidents, Executive Committees, Boards of Directors, and have their own staff and organizational structures.

The goal of ACHI is to provide examinations for individuals at the predoctoral and postdoctoral levelsto ascertain fulfillment of published educational and competency standards, to issue certificates of competency to successful applicants (accrediting individuals) that meet the defined educational and competency requirements, and to maintain a registry of certified individuals.

ASHI is devoted to the scientific, technical, and administrative aspects of histocompatibility testing, the accreditation of histocompatibility and immunogenetics Laboratories, is a facilitator of proficiency testing programs and the authority and leading educational resource in immunogenetics and histocompatibility.

Both certification by ACHI and membership in ASHI require annual "maintenance." A breakdown of the two entities and their specialized areas are summarized in the table below:

ACHI offers the CHA, CHT, and CHS exams 4 times a year: in **March, June, September** and **December**. ACHI outsources all exam activities – including the application – to a company called **PSI**. ACHI provides a handbook detailing eligibility requirements for each level of certification offered, application instructions, a list of required documents, a detailed content outline for each certification, and application forms. The completed application forms need to be submitted to PSI and an exam appointment scheduled at one of their computer-based testing (CBT) sites. PSI is a large company and the ACHI is one of the smaller certification clients they manage. Like many other industries, PSI has experienced staffing shortages through the pandemic, particularly in the customer service department. We have heard feedback that wait times on the phone are often extremely long and the scheduling process isn't clear. We are working with PSI to improve this for 2022. Please call PSI's main customer service number **888-519-9901** for assistance with anything to do with the exam.

## Congratulations to the following people who passed the December 2021 exam

| CHS | CHT |
|---|---|
| Kaitlyn Bergman | Leigh Fonda Allen |
| Michelle Brennan | Kevin Billand |
| Rickee Buczynski | Dratin Castlin |
| Catherine Lee | Jerome Ryan Chavez |
| Donna Lee | Tara Keller |
| Yinxing Liu | Meghan Kremer |
| Ryan Maas | Emilie Masterson |
| Janel Manwaring | Andrew Niepow |
| Ann Pole | Timothy Petzold |
| Shravida Shetty | Jahfarie Wayde Wisdom |
| Amy Younie | Lisa Yong Wu |

## There are just two things that everyone has do to keep their certification active

1. Pay the annual certification fees each year
2. Collect CE credits to submit at the end of your three (3)-year cycle.

All certification activities can be managed at our LearningBuilder "CE Center" website: https://abhi.learningbuilder.com/

You can add a variety of activities to your personal continuing education credit (CEC) record — you can add an unlimited amount of ACHI approved courses that provide extra credit (1 hour = 0.15 CECs, as opposed to non-ACHI approved courses where 1 hour = 0.10 CECs).

Other types of activities that can be used for CECs are listed below:

**Author Credit**

Be listed as an author, within your three (3) year cycle, of a paper or chapter in a recognized journal or book pertaining to histocompatibility and/or immunogenetics. A maximum of 1 CEC may be claimed for each chapter or publication and appropriate documentation (e.g., copy of abstract, etc.) is required for credit. For Diplomates, a maximum of 40 contact hours or 4 CECs can be claimed.

**College & Academic Courses - QUARTER**

Earned academic credit in career-related college or university course work calculated at the rate of 1 semester hour = 1.5 CECs and 1 quarter hour = 1.2 CECs. Course descriptions and official transcripts of successful completio of academic course work are required for credit. A maximum of 4.5 CECs may be claimed per three (3) year period.

**College & Academic Courses - SEMESTER**

Earned academic credit in career-related college or university course work calculated at the rate of 1 semester hour = 1.5 CECs and 1 quarter hour = 1.2 CECs. Course descriptions and official transcripts of successful completion of academic course work are required for credit. A maximum of 4.5 CECs may be claimed per three (3) year period.

**Histocompatibility & Immunogenetics Courses – NOT ACHI Approved**

Programs that meet the above criteria but are not ACHI approved may be submitted for CE credit. However, credit will be calculated at the rate of 0.1 CEC per contact hour. Documentation of attendance must be submitted to ACHI for credit. All documentation must include the amount of CEC achieved.

**Inspections**

The performance of an on-site laboratory inspection for the ASHI and CAP laboratory inspections program can be submitted for CEC. A maximum of 0.8 CEC may be claimed per inspection with a maximum of eight (8) inspections per three (3) year period. Documentation from ASHI and CAP is required for credit.

**Laboratory work/analysis for purpose of presentation**

Performance of laboratory work or analysis for, but not actual attendance or presentation of, data at any national or international histocompatibility workshop. Signature of the laboratory director, supervisor or sponsor will be required as verification of participation to receive credit. Credit will be calculated at the rate of 0.1 CEC per contact hour. A maximum of 1 CEC may be claimed per three (3) year period. For Diplomates, a maximum of 40 contact hours or 4 CECs.

**Presentations: Paper, Poster, Workshop**

Presentation of a paper, poster or workshop at any meeting or workshop pertaining to the field of histocompatibility and immunogenetics. Maximum credit for each presentation is 0.3 CEC. Additionally, a maximum of 1 CEC may be claimed for the time spent on the preparation of the presentation. For Diplomates, a maximum of 40 contact hours or 4 CECs. A copy of the program or other appropriate documentation is required for credit.

**Safety, Management, IT & QA Courses**

Programs which include the areas of safety, management, computer technology and quality assurance may be submitted for CE credit. Credit will be calculated at the rate of 0.1 CEC per contact hour. Signature of the laboratory director, supervisor or sponsor will be required as verification of participation to receive credit. A maximum of 1.5 CEC may be claimed per three (3) year period. For Diplomates, a maximum of 40 contact hours or 4 CECs.

**Self-Instruction**

Self-instruction of career-related topics can be claimed at the rate of 0.1 CEC per contact hour for the following:

1. Any audio/visual materials.

2. Open or closed circuit television and radio broadcasts and instruction using telephone networks (i.e., teleconferences).

3. Reading of publications on histocompatibility and immunogenetics and other related medical literature.

Signature of the laboratory director, supervisor or sponsor will be required as verification of participation to receive credit. A maximum of 1.5 CEC may be claimed per three (3) year period for CHS, CHT, and CHA. For Diplomates, a maximum of 25 contact hours or 3.75 CECs.

**Teaching & Instruction**

Teaching of histocompatibility and immunogenetics or career-related subjects to allied health professionals, quality control managers, histocompatibility and immunogenetics students, medical students and residents, nurses, and other health professionals. Credit is awarded at the rate of 0.1 CEC per contact hour with a maximum of 1.5 CEC per three (3) year period for CHS, CHT, and CHA. For Diplomates, a maximum of 40 contact hours or 4 CECs. Signature of the laboratory director, supervisor or sponsor will be required as verification of participation to receive credit.

**Volunteer Service – Committees & Colleges**

The time spent serving in ASHI, ACHI or other professional organizations or associations (e.g. AFDT, UNOS) relating to histocompatibility and immunogenetics. A maximum of 1 CEC per year may be claimed for each committee membership, or a maximum of 2 CECs per year may be claimed for each committee chaired. A maximum of 2 CECs per year may be claimed for each position held on the College of Directors that is independent of committee chairmanship. Documentation is required for credit.

**Webinars & Teleconferences**

Attendance at an ACHI approved audio/visual recorded presentation of any ASHI scientific meeting or other symposia of career-related material. Signature of the laboratory director, supervisor or sponsor will be required as verification of participation to receive credit. Credit will be awarded at the rate of 0.15 CEC per contact hour with a maximum of 1 CEC per three (3) year period. For Diplomates, a maximum of 40 contact hours or 4 CECs.

## CONTACT US!

American College of Histocompatibility & Immunogenetics
1120 Route 73, Suite 200
Mt. Laurel, NJ 08054
856-335-3299 x 7004
Fax: 651-305-3838
Email: MWeeks@ashi-hla.org

# ARB Update – Accreditation News

John Schmitz, *PhD, F(ACHI) – ARB Program Director*

It is amazing how time flies! As we begin 2022, the ARB wishes everyone a productive, prosperous and happy new year. In this quarterly update I will wrap up ARB activities at the end of 2021and update you on current ARB activities.

The ARB held an in-person meeting December 3-4 in San Diego, CA. It was really wonderful to have the face-to-face interaction with the ARB members. While virtual meetings are convenient, flexible, and have played an important role during the ongoing pandemic, being together in one room reinforced the benefits of personal interaction. In depth discussion is easier and being able to visualize reactions to topics is really beneficial. It was also refreshing to be able to share non-ARB discussion during dinner and watch David "Tex" Kiger wrangle the bull (think urban cowboy).

We were able to accomplish our primary duty of laboratory accreditation reviews as well as discuss a number of other topics. The primary non-review activity currently is completing our CMS accrediting organization review. After submitting our crosswalk between ASHI and CLIA standards, we had a virtual inspection by CMS staff. We subsequently responded to a first and second round of written questions from CMS. We are now awaiting their review of our responses to their second set of questions. We anticipate a final decision in March 2022.

## New Vaccination Requirement for Inspectors:

Starting in cycle 1, 2022 (April-June inspection window), the ARB will be requiring our inspectors visiting laboratories onsite to be fully vaccinated* for COVID-19. The ASHI Board of Directors Executive Committee discussed this recommendation from the ARB and voted to approve the requirement for fully vaccinated* inspectors for in-person inspections. It is recommended that they stay up to date with booster dose(s) as well, given their enhanced efficacy against the Omicron variant. Inspectors should prepare to provide proof of vaccination at time of entering medical campuses and foreign countries. The ARB will keep guidance in line with current CDC recommendations and update as needed.

*Fully vaccinated means a person has received their primary series of COVID-19 vaccines.

## UNOS & ASHI Crosswalk Task Force:

A new project the ARB has undertaken is to review ASHI standards in the context of UNOS policies. The ARB has formed a new task force this year with the goal of performing a crosswalk of the ASHI Standards with UNOS Policies & Regulations. We are grateful for Julie Houp to be leading this group consisting of ARB commissioners, a representative from QAS and the UNOS histocompatibility committee. A crosswalk has not been done in many years, so this project is essential for our laboratories accredited for solid organ transplantation.

Other issues discussed during the December ARB meeting are being worked on and I will address them in subsequent quarterly updates.

## Laboratory Cycle Updates:

**CYCLE 1:** Laboratories in cycle 1 were sent links to their accreditation applications on January 1, 2022 due back on March 1, 2022. Onsite labs in this cycle will be inspected between the dates of April 15 and June 15, 2022. Laboratories in this cycle received their new accreditation letters and/or certificates at the end of August 2022.

**CYCLE 2:** Laboratories in cycle 2 received their accreditation letters & certificates in December 2021. The ARB met face-to-face in San Diego in December to discuss this cycle. Cycle 2 will be inactive until May of 2022 when the application & inspection process starts up again.

**CYCLE 3:** Applications for the cycle 3 group of laboratories were due in the accreditation office on November 1, 2021. Onsite laboratories in this cycle were inspected over the winter through February 15, 2022. The ARB will be meeting during the first weekend in April to review this group of laboratories – expect to receive new letters & certificates by the end of April 2022.

# ASHI Educational Workshop


Attention ASHI members! We have a stellar program for the 2022 ASHI Educational Workshop. This event was previously known as the ASHI Regional Workshop but other than the name update, this meeting is familiar in its popular format. It provides a wonderful educational opportunity and the change to connect with your histocompatibility and immunogenetics colleagues. It's the perfect meeting for newer ASHI members, too. The in-person meeting will be held in Fort Lauderdale from June 23-25th. The meeting will also be held virtually on July 22nd and 29th with live Q&A portions.

The speakers include:

John Lunz, PhD, F(ACHI)
LifeLink Transplant Immunology Laboratory

Robert Liwski, MD, PhD, FRCPC, A(ACHI)
Dalhousie University

Brett Loehmann, CHS, CTBS, BS
Mid-South Transplant HLA Laboratory

Lynden Gault, CHT(ACHI)
Gift of Hope Histocompatibility & Immunogenetics Laboratory

Prakash Rao, PhD, MBA, FACHE, HCLD
New Jersey Organ & Tissue Sharing Network

For more detailed information regarding the program and how to register, visit http://www.ashiregionals.org/

We hope to see you there!

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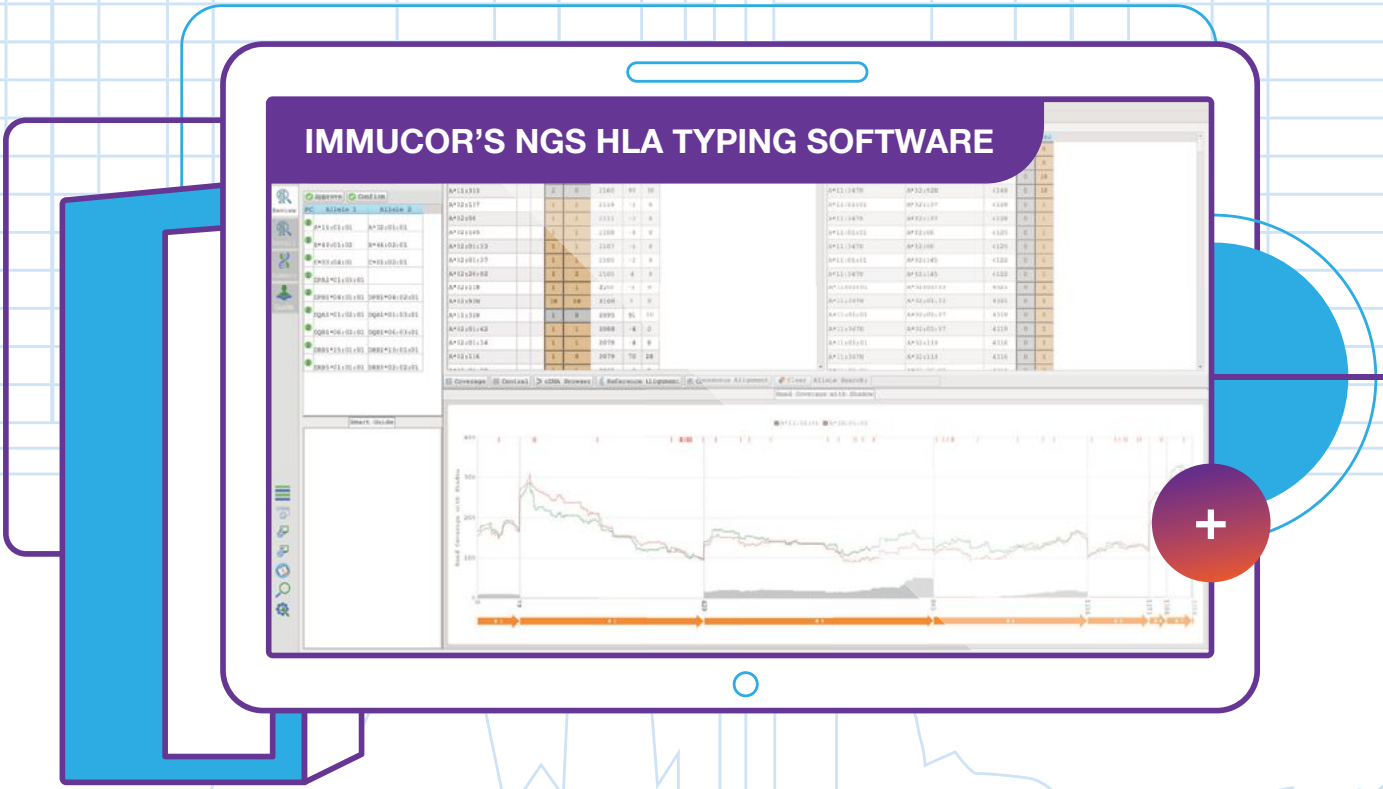


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