ARSHI

الجمعية العربية لتطابق الأنسجة
و الجينات المناعية

ARSHI Newsletter, Volume 2 Issue 1,
February 2022

A Special COVID-19 Issue

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ARSHI Arabian Society for Histocompatibility and Immunogenomics

Volume 2 Issue 1

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From the Editor-in-Chief

Dr. Ahmed A. Mostafa MD, MSc, Ph.D., F(ACHI)

Assistant Professor of Pathology and Lab Medicine-University of Saskatchewan-Canada. Director of the Histocompatibility and Immunogenetics Laboratory, St Paul's Hospital, Saskatoon, SK Canada

Welcome dear ARSHI members and ARSHI newsletter readers. It is my great pleasure to introduce you to the 3rd ARSHI newsletter which features a special COVID-19 chapter. In the beginning, I would like to thank all the ARSHI members who dedicated their valuable time to share their scientific views and articles for publication in this newsletter. Second, I would like to introduce you to the new ARSHI editorial board members, who have done outstanding work in putting these articles together in the best format and flow; Dr. Sally Elfishawy, Ms. Marion Alvares, Ms. Kenana AlAjlan, Mr, Emad Al khaldi, and Dr. Jamshaid Siddiqui.

The ARSHI newsletter aims to share scientific data, reports, and case studies in the field of Histocompatibility and Immunogenetics (HI) in the Middle Eastern and North African (MENAF) region. In addition, we aim to share updates from the ARSHI board of directors, achievements, and awards of ARSHI members. We will do our best to include write-ups about method validation and the development of new labs in the region. The readers will also get updates from the international ASHI committee's previous and upcoming webinars. We will also stimulate discussions and exchange of ideas and further the progression of science in the HI discipline in the MENAF region. The primary criteria for acceptance are that the work is original, scientifically, and technically sound and provides valuable knowledge to life sciences research.

We welcome cutting-edge science and in-depth studies, as well as promising though more preliminary, descriptive, and small-scale results if they contribute to the aim of the newsletter. We also encourage research reports covering negative findings, re-analyses of previous datasets, and studies leading to new insights or hypotheses. At this time, the newsletter is not limited to novel articles but also to open access publications. Abstracts may be published from subscription journals.

The COVID 19 pandemic section highlights the contributions, expertise, research, and insights that occurred in the MENAF regions. In this inaugural section, we are pleased to feature one case study that was presented at the 47th ASHI Annual Meeting, 2021 by Dr. Ahmad Abu Khader under the supervision of Dr. Noureddine Berka. The work was later published in the HLA journal. Dr. Rania Bakry studied and wrote about the effect of HLA-B*15 in predicting survival in Egyptian patients with COVID-19. The third study is an opinion study by Dr. Ashraf Dada and Mr. Mohammad Al Hroub about the effect of the COVID-19 pandemic in the Middle East

Region. The fourth study, from Dr. Rabab Al Attas and her team, is about the impact of the Covid-19 pandemic on Transplantation Laboratories, in King Fahad Specialist Hospital, Dammam, Saudi Arabia. An informative scientific article from Dr. Ashraf Dada and his colleagues is about how SARS-COV-2 infection triggers the development of HLA Class I and II antibodies in recovered convalescent plasma donors. The final article describes common manifestations of SARS-COV-2 infection in a quarantine facility: Saudi Arabia by Alanoud AlshamiI et al. We also featured most of the COVID-19 publications form our region in the same chapter.

In this edition, we will present a few case studies and new method validation. We will also highlight awards, recognitions and certifications accomplished by our ARSHI members in the last year.

In addition, Dr. Abdulhalem A. Jan wrote a nice article about the fully established services for the HLA department in King Fahad Hospital, Madinah, Saudi Arabia. In the end, we will give some highlights about the 2021 ASHI annual meeting.

Finally, I would like to close by inviting all ARSHI members to consider contributing articles, reviews, commentaries, technical reports, and interesting cases to future issues of the ARSHI newsletter. Please do not wait to be asked and share your thoughts and expertise with our community, as it is essential to our continued success!

Kind regards,

Ahmed Mostafa

Editorial-Board

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ARSHI President Message

Dr. Ashraf Dada, MD, Ph.D

Dear distinguished ARSHI members, dear colleagues, ARSHI looks back on a short but proud history, which was founded, shaped, and developed by respected and committed experts with a vision for the development of Histocompatibility and Immunogenetics (H&I) in the Arab World and Middle East region. The importance of ARSHI as a flagship derives from its role to improve and promote the training, education, and research in the fields of Transplant Diagnostics (SOT & BMT), Pharmacogenetics, Disease Associations and Transfusion Support. For these outlined noble goals, I and the Board of Directors (BOD) of ARSHI feel honored to head this large society with members from many countries in the MENA region and we keenly feel the weight of this responsibility. Unfortunately, I took over the presidency of ARSHI at a difficult time, when the COVID-19 pandemic in our region was peaking. Nevertheless, the new ARSHI BOD was able to meet regularly and we completed an extensive work plan and set ambitious goals for the development of ARSHI. It is a pleasure for me to mention that I came across a highly motivated and experienced proactive team that actively supports the progress of our aspiring society. So, we finalized a new organizational structure for ARSHI and created a 4-year strategic plan with defined goals, which are announced in this newsletter. While we discussed this plan on the board, we understand that it might be difficult to achieve all targeted points.

However, the plan will pave the way to make even modest progress and will provide a stable basis and roadmap for the current and future BOD. I think this is an essential approach and a minimal concept that should be followed to achieve sustained development over the next few years.

Therefore, the exercise book is definitely well filled and we will tackle it with your support. The BOD met with corporate law experts in order to strengthen the structures of ARSHI as a professional society and expand its activity to include and support the African continent. Among others, we were advised to raise annual fees from ARSHI members, as fees are an integral part of each professional society. However, this needs an official registration of ARSHI, which we are currently doing. A broad discussion on this point among ARSHI members will certainly take place before any fees implemented. We have also remarkably intensified ARSHI cooperation with international societies; planned agreements are in the pipeline and will be announced later. In addition, it is my pleasure to inform you that we are now in the final step to launch an official Journal of ARSHI with the name JAI "Journal of Applied Immunogenetics", which will serve as an international publication platform.

In 2020, the 1st ARSHI conference was postponed due to the COVID-19 pandemic, that started in our region at that time. Now, the decision was made to hold a virtual meeting, which will take place in the upcoming month from 04 to 05 March 2022 (see below announcement).

Finally, I would like to thank the editorial board members for issuing this newsletter under the leadership of Dr. Ahmad Mustafa. This special COVID-19 issue contains plenty of exciting scientific, personal and social contributions, just enjoy t!

Kind regards, *Ashraf Dada*

Save the date!

EARN 6 CME HOURS! From "AMERICAN COLLEGE OF HISTOCOMPATIBILITY AND IMMUNOGENETICS"

Greeting!

A gentle reminder that we are going live soon for the "ARSHI" that will take place on 4 - 5 March 2022 at the webinar - Virtual Scientific Meeting

Event Details:

Event Name: "**1st Arabian Society for Histocompatibility & Immunogenomics" Conference**

Attending: **Two Days Conference**

Start Date: **Friday, 4 March 2022,03:30 PM 2022, 06:00 PM (Riyadh Time)**

End Date: **Sunday, 5 March 2022, 08:80 PM,7:00 PM (Riyadh Time)**

Coming soon!

Be ready to publish your article

ARSHI
Arabian Society for Histocompatibility and Immunogenomics

JOURNAL OF APPLIED IMMUNOGENETICS

Official Journal of ARSHI

Who is our ARSHI president?

Dr. Ashraf Dada is a well-known German pathologist with focus on immunohematology, transplant immunology, blood banking & transfusion medicine, stem cell collection & processing, histocompatibility & immunogenetics, and molecular virology.

Dr. Dada was born in Bani Suhaila/Palestine, where he visited Alawda secondary school in Abasan/Khanyounis/Gaza. Dr. Dada completed the high school diploma in Münster/Germany. Thereafter, he studied medicine at the University of Erlangen-Nuremberg/Germany and later he finished his doctorate at the same university. He completed the German Board in Transfusion Medicine and Immunohematology in Stuttgart/Germany.

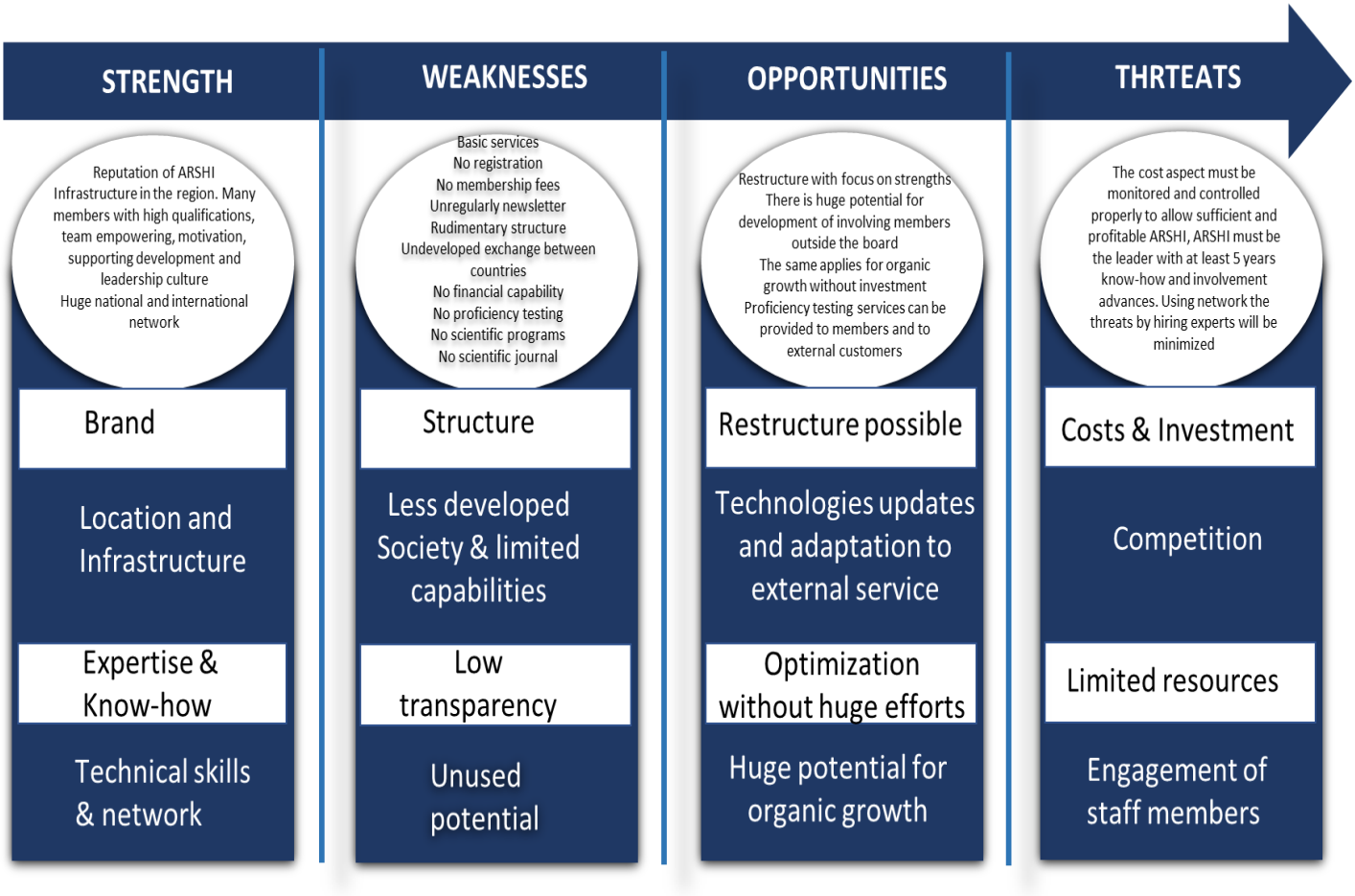
2001, Dr. Dada was appointed as a senior consultant and Chairman of the Department of Transfusion Medicine and Immunohematology at the University Hospital Regensburg/Germany, where he also chaired the Transfusion Committee of this famous academic Hospital for long years. Later on, he became the Medical Director and General Manager in the same position of SYNLAB Institute for Transfusion Medicine, Munich/Germany. In 2010, Dr. Dada was nominated as the Chairman of SYNLAB European Advisory Board for Transfusion Medicine and Immunohematology with branches in almost all European countries. Dr. Dada served also as the President of the German Physician Society PalMed (2012-2019), for three consecutive elections periods and recently, Dr. Dada was honored to be elected as the President of ARSHI.

Currently, Dr. Dada holds different professional leadership positions; He is the Director of the Histocompatibility and Immunogenetics lab at King Faisal Specialist Hospital & Research Centre Jeddah/KSA (CAP&ASHI accredited) and the Director of Immunoserology, Immunopathology and Apheresis Collection Facility Section at same Hospital. Dr. Dada is a certified Director by the American Society for Histocompatibility and immunogenetics (ASHI) with a comprehensive authorization including transfusion support.

Moreover, Dr. Dada is academically active in teaching and research. He holds an Associate Professor position at College of Medicine/Alfaisal University. Beside considerable number of short reports and abstracts (>150), Dr. Dada published a significant number of original papers in international peer-reviewed journals with a cumulative impact factor of more than 100. Dr. Dada received multiple national and international awards and recognitions, including an award for long-life scientific achievement. Dr. Dada is a principal investigator of different ongoing research projects, including a phase II clinical trial.
Finally, Dr. Dada is a considerable and popular media interview partner for awareness about the COVID-19 pandemic and the immunological effects of vaccination against SARS-CoV-2 virus.

ARSHI SWOT, Organizational Structure and Strategic Plan

Arabian Society for Histocompatibility and Immunogenomics
-SWOT Analysis-



Vision

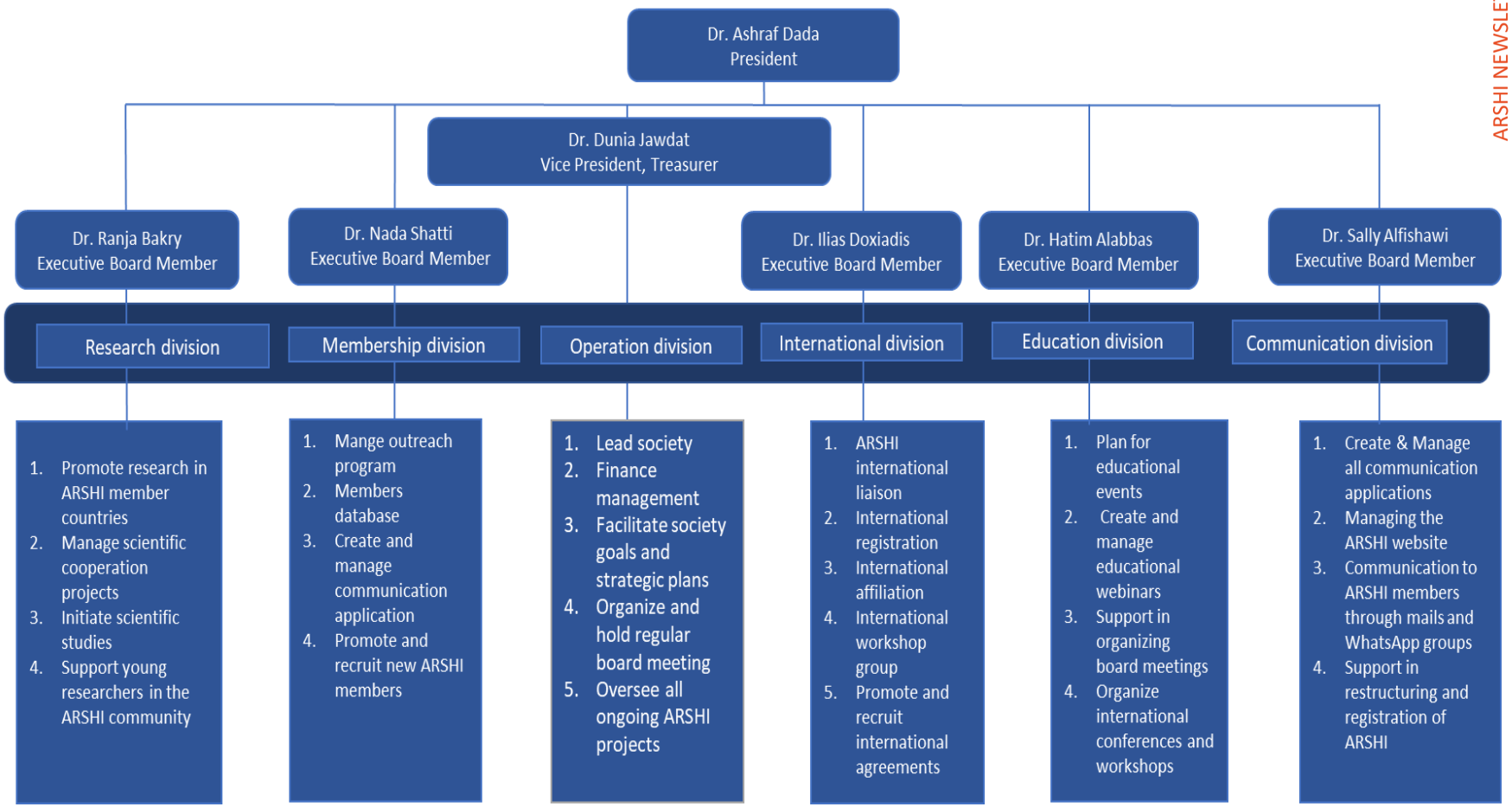
Provide state of the art education and training via exchange of information and promote technical skills and research opportunities in the fields of immunogenetics, transplant diagnostics and transfusion immunology

Mission

To improve and disseminate the knowledge of histocompatibility and immunogenetics in the MENA region, that impact on the quality of human life.

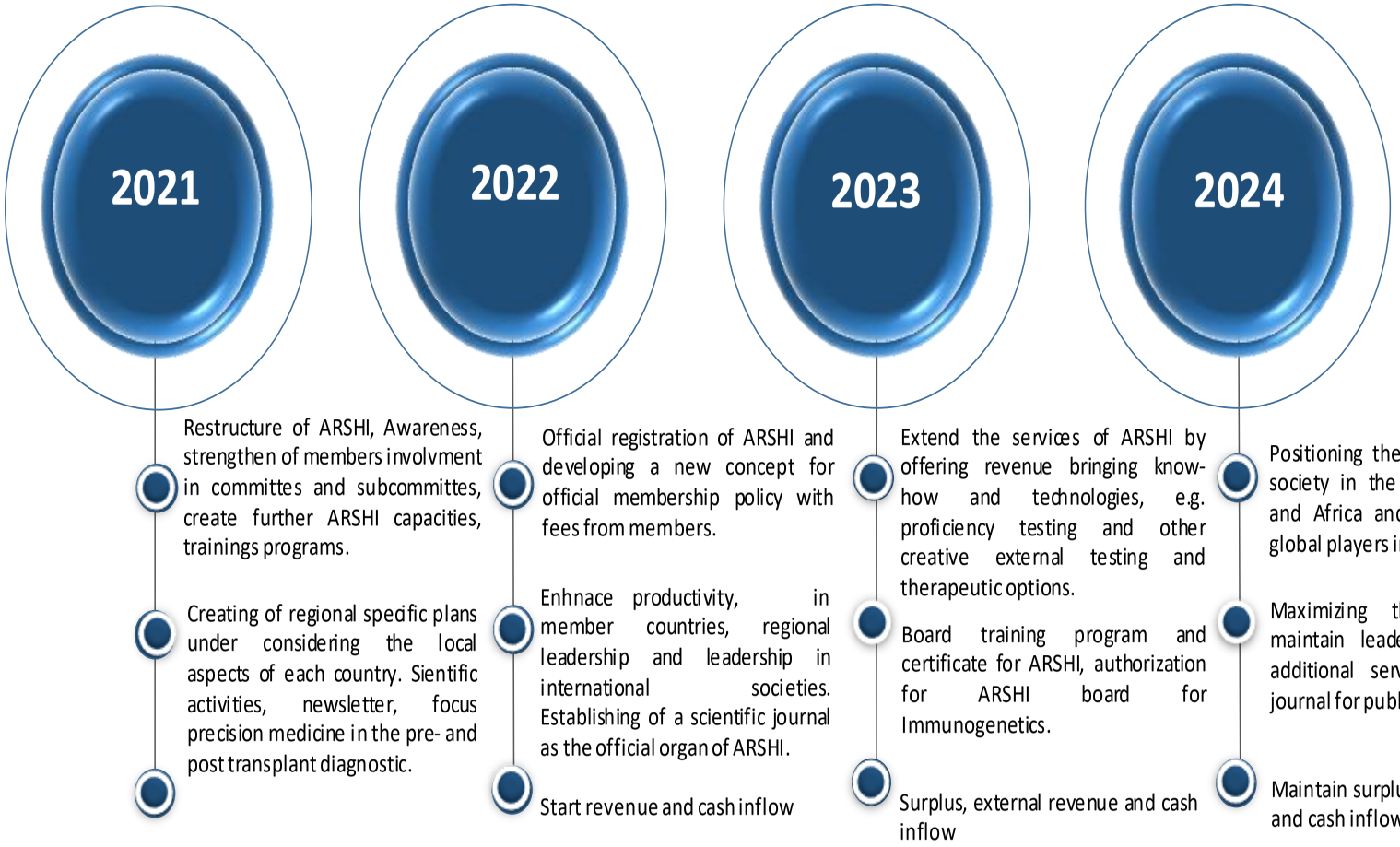
ARSHI
Arabian Society for Histocompatibility and Immunogenomics

Arabian Society for Histocompatibility and Immunogenomics
-Organization Chart-



ARSHI
Arabian Society for Histocompatibility and Immunogenomics

Arabian Society for Histocompatibility and Immunogenomics
- Four Years Strategic Plan-



COVID-19 Where are we at?

COVID-19 Pandemic in the Middle East Region

Dr. Ashraf Dada and Mr. Mohammad Al Hroub Department of Pathology and Laboratory Medicine, Infection Control, King Faisal Specialist Hospital & Research center (KFSH&RC-Jeddah)/Saudi Arabia.

With the start of the year 2020, new SARS-COV-2 infections spread like wildfire around the globe, surprised and shocking the whole world. Consequently, the collective response and adoption of preventive measures to stop the global spread were implemented too late, after COVID-19 had already penetrated all continents. The whole world was not ready and no one could expect a global infectious disease with such an unpredictable gigantic scale. On March 11, 2020, the World Health Organization (WHO) declared this novel coronavirus as a pandemic (Corona Virus Disease or COVID-19 pandemic) and advised all countries to take immediate and appropriate measures to contain the spread of the COVID-19 pandemic. According to the proposal of the WHO, the response to the pandemic should be firm, fast, and must include well-defined, well-structured, and effective measures. Despite the important and central role of the WHO in containing the pandemic, each country is considered as the natural responsible body in national risk management for establishing effective preventive measures, overall coordination, awareness campaigns, and communication tools. This fact represents a challenge and a stress test for many countries in the world, especially for less developed ones, like is the case in the Middle East region. The great hope at the beginning of the pandemic that the summer heat in the Middle East would reduce the activity of the SARS-CoV-2 virus turned out to be a wrong assessment. COVID-19 pandemic hits the Middle East Region in all seasons of the year independent of climate conditions. The results are increased burdens and growing challenges on health systems in this part of the World. However, it is also a fact that the pandemic-related tasks and the strategic response to COVID-19 vary remarkably from one country to another one in the Middle East, in terms of prevention, response, and operational readiness. Particularly, in the WHO Eastern Mediterranean Area, trends in the COVID-19 pandemic, like virus transmission, number of COVID-19 cases, deaths, and hospital admissions differ significantly across the region (http://www.worldometers.info/coronavirus/).

In our opinion, one of the not trivial hurdles and challenges in the Middle East Region has been the continuous spread of rumors and misinformation. In addition, the region was not excluded from conspiracy theories not only about the SARS-CoV-2 virus and its origin but also about the corona vaccination and its sense! For this reason, we urge individuals and everyone, especially with contact to influencing social-, print- and television media, to verify any information

through reputable sources such as WHO or CDC before spreading any information that can be at best confusing, and at worst deadly. We think that experts and distinguished members of the Arabian Society for Histocompatibility and Immunogenomics (ARSHI), as professionals in immunogenetics and immunology in the Middle East Region may play a role in this regard by reporting the most up-to-date and evidence-based information about any thesis related to COVID-19 pandemic. However, this may be the minimal task of every one of us. The ideal role is besides public awareness, to be proactive by initiating and participating in committees or scientific groups and research projects that, include the development of COVID-19 diagnostic tests, reagents for PCR, NGS, clinical trials and developing of therapeutic approaches, drugs, and vaccines. This will surely help the Middle East region to better control the pandemic. Our role in defeating the virus is vital!

With the beginning of the year 2021, many countries in the Middle East began to vaccinate the population. The success of the COVID-19 vaccine campaign varies significantly from one country to another, as a considerable number of Middle East countries depend on international aid for vaccine procurement. Consequently, some Middle East countries joined the COVAX program, which was launched by the World Health Organization (WHO), to ensure that fewer wealthy countries have access to free vaccines or subsided ones. This program has helped many countries to get the vaccine either free of charge or at very low purchase prices, which has reduced the economic pressure of these countries. Nonetheless, the COVAX program progresses slowly and countries that depend on it have a low rate of vaccinations and they cannot increase the rate by purchasing vaccines for regular prices due to economic considerations and limited financial resources. Another economic burden is the costly funding of precaution measures, which are considered as one of the major challenges that the Middle East region has been faced. From an economic point of view, passable information is available about the expected economic and health costs of infectious disease outbreaks, but some countries in the Middle East has failed to fund adequately or simply are not able to invest in preventive and preparedness measures to mitigate the risks of such major pandemic. Hence, in some countries in the Middle East, the private sector has become an increasing role in providing health services. On one hand, the private sector could help and support in managing and containing the pandemic. On the other hand, this sector created in some Middle East countries social selectivity and exclusivity, which might be not the ideal option to fight a global pandemic affected all individuals independent of social status. According to published reports, the economic impact across the Middle East region was mainly due to decreased productivity, business closings and trade disruptions, and decimation of the tourism industry, especially in countries that depend heavily on tourism, like Egypt, Tunisia, and Morocco.

Other countries in the Middle East, especially in the Gulf region with advanced economic resources and sufficient healthcare systems have tolerated the burden of the pandemic better than others. Saudi Arabia for example has a remarkable achievement and is considered as the most successful country in the Middle East in containing the COVID-19 pandemic. The reasons for this success are multifactorial, e.g. well established advanced digitization programs, practical apps, experienced employees, technical skills, know-how & acquired experiences and expertise from the Middle East Respiratory Syndrome (MERS-CoV). The experience with MERS-CoV is one of the main reasons for the remarkable quick response and rash control of the pandemic in KSA. Also the adequate and well-established health care system is a key in containing the pandemic. Saudi Arabia has about 500 hospitals with a capacity for a huge number of intensive care beds (about 13000) and offers vaccination

and access to healthcare care services for free for all COVID-19 patients. Further, KSA is the only country in the region with safety laboratory level III, which provides experts with detailed information about the pathogenicity and immunogenicity of the SARS-CoV-2 virus. This makes the country at the forefront of understanding different viral phenomena, e.g. virulent grade and escape mutations, which are very helpful in fighting any viral pandemic. Other countries in the Middle East region might try to establish similar health care systems to be better prepared for a possible future pandemic, as Hospitals have to be ready and functional and they need to provide sufficiently qualified specialists, nurses, and health workers, protective equipment, testing capacities, and effective treatment options. In general, affected Middle East countries may benefit from exchanging technological innovations, knowledge, expertise, and learning from each other to manage this and future outbreaks more effectively. COVID-19 may be a "wake-up" call for all countries in the Middle East, but also worldwide, to intensify cooperation, collective actions and strategic approaches to avoid a possible pandemic disaster and limit the harm to a minor level.

(Table 1): WHO measures and challenges facing the Middle East region during the COVID-19 pandemic

WHO measures	**Challenges faced Middle East region**
Active surveillance measures	Lack of sufficient awareness Campaign
Continuous prevention and control of infection	Fragile health care system in some countries
Effective plans for health care facilities	Spreading of the emergence of new variants
Postponement of mass gathering events	General COVID-19 fatigue
Raising public awareness and understanding of the disease.	The failure of many people to adhere he public health and preventive measures

I- The Impact of Covid-19 Pandemic on Transplantation Laboratories, Single Center Experience

Rabab Alattas; Zuhoor Al Qahtani; Mohammed Awaji; Amani Mohammed; Ahmad Otaibi; Rafah Bamrdouf; Saber Alzahrani; Nora Quhaidan, Kenana; Ajlan

Aim: During assessment for transplantation, all patients are tested for HLA- sensitization (PRA) & donor / recipient compatibility by crossmatch testing. A positive flow cytometry crossmatches (FCXM) due to the o presence of DSA is often considered a contraindication for transplantation. On the other hand, a positive crossmatch in the absence of DSA is challenging and needs further investigation for relevance particularly in the absence of a history of previous sensitization such as blood transfusion, previous transplantation or pregnancies. It is well known that some viral infections can trigger the formation of polyclonal antibodies that cross-react with HLA- antigens creating false-positive crossmatch and make risk assessments difficult. The global pandemic of COVID-19 have affected all aspects of our life. However, the impact of this pandemic on transplantation laboratories have not been assessed yet. During the epidemic, we have noticed frequent positive FCXM, some of them due to true HLA-antibodies in non-sensitized individuals while others with no detectable HLA antibodies. When we searched, further we found recent history of CVID-19 infection and or vaccination in either the donors or the patients. Therefor we decided to see the relation between COVID –infection and emergence of HLA-antibodies or other autoantibodies that may interfere with laboratory transplant workup. In our laboratory, we sought the highest accuracy by investigating all discrepant XM-results to give clear guidelines to transplant clinicians. The significant financial and technical burden imposed by these investigation procedures on our laboratory stimulated us to validate a way to get rid of these noises while saving the cost of repeating tests and ensuring timely results. In this brief, we will share our experience as a reference laboratory supporting transplantation in all MOH transplant centers in SA.

Methods:

1-Testing prevalence of HLA- antibodies in COVID- 19 infected patients:

40 male COVID-19 infected patients never transplanted or transfused who screened, positive for SARS-CoV-2 Ab (IgG/ IgM antibodies) were tested for HLA-Ab. HLA antibodies performed by LSM and SAB using One Lambda® on Luminex platform. A standard three color FCXM was performed and analyzed on FACSCanto II. SARS coV2 antibody detected by Liaison SARS-CoV-2 S1/S2 IgG/IgM (DiaSorin). 14 random samples from original samples were cross-matched with surrogate cells & the concordance of presence or absence of antibody with XM-results was estimated.

2- Flow crossmatch

All transplant candidates with their corresponding donors referred during the period 20/3/2020 - 20/9/2021 who screened positive for SARSCoV-2 antibodies post either vaccination or actual infection were cross matched. 75 Discrepant crossmatch results (virtually negative crossmatch due to negative or very low DSA) that gave positive actual FCXM were investigated. Similarly, when the XM result was not valid due to abnormal negative control results, the issues related to donor were also investigated and

the protocol modified to remove cross-reacting / nonspecific bindings.

Results:

Considering the possibility of a prevalence of 10% natural antibody in Non-sensitized individual, we found significant prevalence of 81% & 73% positive HLA antibody screening & antibody detection respectively in COVID-19 infect patients. Table 1 and table 2 showed the characteristic of these antibodies. When some of these sera were cross matched with surrogate cells , only few sera demonstrated positive XM with the corresponding cells , however due to few number of cross matched performed the actual significant cannot be inferred at this stage.

Initial 75 discrepant were repeated to confirm this discrepancies / or to clear the positivity with protocol modifications giving rise to total of 246 FCXM performed with an average number of repeats of 2.5 per sample giving rise to threefold increase in technical work and reflected in increase of financial cost and increase in turnaround time from conventional 3 days/ single XM test to 9 working days. (figure1).

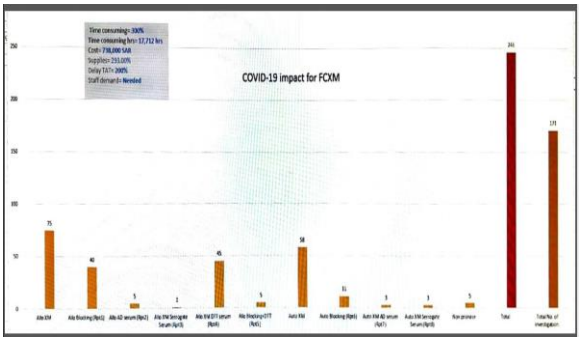


Figure1: Investigation performed for discrepant XM- results during COVID- 19 Pandemic and overall increment in technology and cost.

Figure 2 showed some crossmatch behaviors due to donor COVID-19 vaccination where donor cells reacted positively with all including negative control sera used in the procedures.

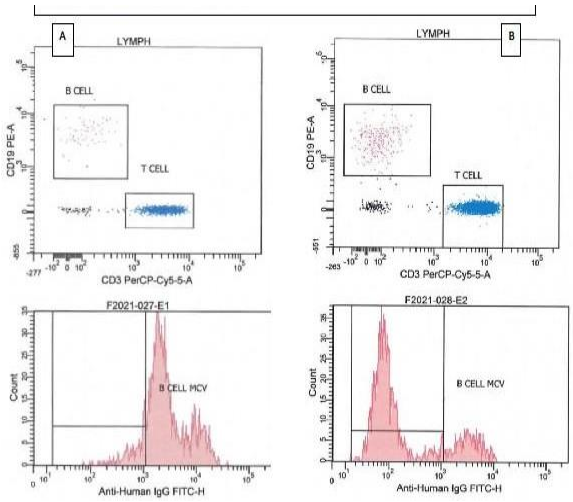


Figure 2: SARS-CoV-2 positive Donor cells reacting with NCS before and after blocking donor cells.

Conclusion:

Our preliminary study showed that COVID infection had affected HLA laboratories significantly by creating false positive crossmatch results & emergence of HLA-antibodies in non-sensitized patients. Accordingly, each laboratory must be vigilant about positive crossmatch in non-sensitized patients infected with COVID-19 to avoid unjustifiable denial of TX. The investigation procedures we did have led to significant financial & technical burden in our laboratory. Therefore, validating the best way to get rid of these discrepancies while saving resources are highly recommended. The impact of such HLA sensitization on transplant outcomes cannot be exactly inferred from this study and should be monitored on large scale.

We will continue to monitor the development of HLA antibodies following vaccination and COVID-19 infection to validate the relevance of such sensitization on compatibility testing & the clinical impact of such sensitization post-transplantation.

II- SARS-COV-2 triggers the development of class I and class II HLA antibodies in recovered convalescent plasma donors

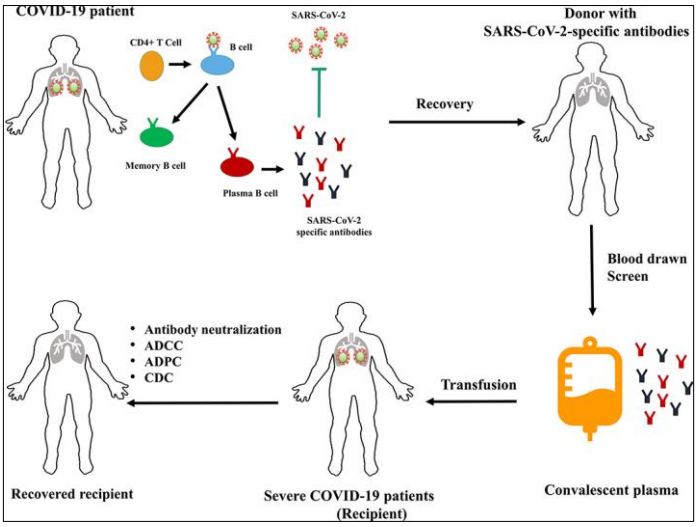
Ashraf Dadaa, Khalid Elhassana, Rayan Bawayana, Ghadeer Albishia, Lama Hefnic, Sawsan Bassic, Turki Sobahyc, Edward Cuplerc, Nabeela AlBaza, Ghassan Walib, Basem Alraddadib, Abeer Alshukairib

[a]Department of Pathology and Laboratory Medicine/King Faisal Specialist Hospital and Research Center Jeddah/Kingdom of Saudi Arabia [b]Department of Internal Medicine of King Faisal Specialist Hospital and c-Research Center Jeddah, Section Infection diseases/ Kingdom of Saudi Arabia [c]Research Center of King Faisal Specialist Hospital and Research Center Jeddah/ Kingdom of Saudi Arabia

Convalescent plasma is considered one of the available investigational immunotherapies evaluated for the management of COVID-19 disease. However, given that numerous studies have shown the development of HLA antibodies after viral infections, there is a concern that the SARS-CoV-2 virus could induce the development of HLA antibodies in convalescent plasma donors. HLA antibodies contribute significantly to the development of TRALI, a life-threatening complication of transfusion affecting the respiratory tract severelyand associated with a high mortality rate. This risk is particularly high in COVID-19 patients, who have an already affected respiratory system due to the COVID-19 disease itself. In this prospective single-center study, we tested and evaluated the presence of class I and II

HLA antibodies in 34 convalescent plasma volunteers. All included donors have no history of sensitization such as blood transfusions, pregnancy, or previous transplants.

We found a significantly increased HLA antibody formation rate, the frequency of positivity for HLA antibodies for class I, class II, class I, and II, and the overall reactivity was 23%, 31%, 46,% and 76%, respectively. 6 donors (18%) were excluded from donation, due to high titer HLA antibodies. The presented data suggest a strong association between SARS-CoV-2 infection and the development of HLA antibodies in recovered convalescent plasma volunteers. This finding could be of clinical relevance for the establishment of pre-donation HLA diagnostic strategies before the transfusion of convalescent plasma to enhance the safety of convalescent plasma transfusion and reduce the mortality rate in COVID-19 patients who require convalescent plasma.



The figure is courtesy of Front. Immunol., 23 September 2020

III- Some of the COVID-19 featured publications

Abu-Khader A, Wang W, Berka M, Galaszkiewicz I, Khan F, Berka N.

SARS Cov-2 vaccination induces de novo donor-specific HLA antibodies in a renal transplant patient on waiting list: A case report. HLA. 2021;1-6. doi:10.1111/tan.14 492

https://onlinelibrary.wiley.com/doi/10.1111/tan.14492

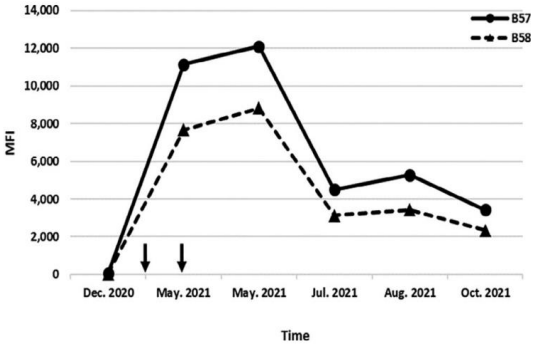
Abstract

Background. Due to the ongoing SARS-CoV-2 (COVID-19) pandemic, vaccination is increasing in healthy subjects and renal patients on transplant waiting lists, which both have competent immune systems that can develop antibodies (Abs). The ability of COVID-19 vaccination to induce anti-HLA Abs formation in renal transplant candidates is not well studied, especially the donor-specific HLA Ab (DSA), which is associated with Ab-mediated rejection.

Case Presentation. Herein we describe the case of a waitlisted renal patient scheduled for transplant with no previous history of sensitization developing class I de novo DSA (dnDSA) after COVID-19 vaccination. The patient is a 42-year-old man that has consistently tested negative for the COVID-19 virus and was routinely worked up for a renal transplant. With no previous history of sensitization, the patient received two doses of a COVID-19 mRNA-based vaccine. Flow cytometry crossmatch (FCXM) and anti-HLA Abs monitoring were compared before and after vaccination.

Results. Before vaccination, preliminary FCXM for intended donor was negative for both T and B cells with no anti-HLA Abs detection. Eighteen days after the first dose of COVID-19 vaccination, patient developed dnDSA against B57 and de novo non-DSA against B58 These Abs were associated with strong positive FCXM results in both T cell and B cells panels. This event prompted the transplant team to cancel the surgery, depriving the patient of a living donor graft. Conclusions. As waitlisted renal patients have competent immune systems, COVID-19 vaccination could be associated with anti-HLA Abs formation that could affect future transplantability and this potential risk should be explored.

Mean fluorescence intensity (MFI)



monitoring for anti-HLA Abs before and after vaccination. Arrows indicate time of COVID-19 vaccination doses.

Ahmed Samir Abdelhafiz, A. Ali, M.A. Fouda Douaa M. Sayed, Mahmoud M Kamel, Lamyaa Mohamed Kamal, Mahmoud Ali Khalil, Rania M. Bakry

HLA-B*15 predicts survival in Egyptian patients with COVID-19, Human Immunology.

https://doi.org/10.1016/j.humimm.2021.09.007

Abstract

Genetic differences among individuals could affect the clinical presentations and outcomes of COVID-19.

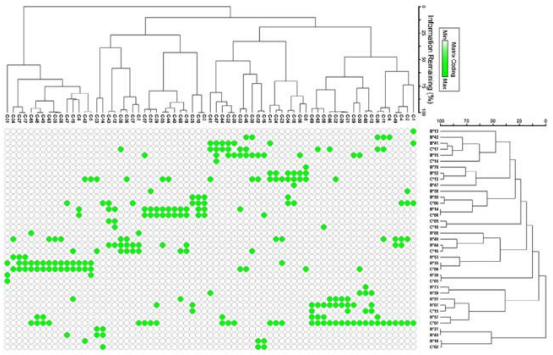
Human Leukocyte Antigens are associated with COVID-19 susceptibility, severity, and prognosis. This study aimed to identify HLA-B and -C genotypes among 69 Egyptian patients with COVID-19 and correlate them with disease outcomes and other clinical and laboratory data. HLA-B and -C typing was performed using Luminex-based HLA typing kits. Forty patients (58%) had severe COVID-19; 55% of these patients died, without reported mortality in the moderate group. The alleles associated with severe COVID-19 were HLA-B*41, -B*42, -C*16, and -C*17, whereas HLA-B*15, -C*7, and -C*12 were significantly associated with protection against mortality. Regression analysis showed that HLA-B*15 was the only allele associated with predicted protection against mortality, where the likelihood of survival increased with HLA-B*15 ($P < 0.001$). Patient survival was less likely to occur with higher total leukocytic count, ferritin, and creatinine levels. This study provides interesting insights into the association between HLA class I alleles and protection from or severity of COVID-19 through immune response modulation.

This is the first study to investigate this relationship in Egyptian patients. More studies are needed to understand how HLA class I alleles interact and affect Cytotoxic T lymphocytes and natural killer cell function.

Table 2
Predictors of survival from COVID-19.

Factors	OR	95% Confidence interval	P
HLA-B*15	1351.06	(4.5021–405445.1879)	**<0.001**
TLC	0.56	(0.3792–0.8196)	**<0.001**
AST	0.93	(0.8481–1.0199)	**0.12**
Ferritin	0.98	(0.9746–0.9943)	**<0.001**
Creatinine	0.36	(0.1733–0.7441)	**<0.001**
IHD (yes)	0.01	(0.0002–0.7158)	**0.03**

Goodness-of-fit test: Hosmer-Lemeshow, $\chi^2 = 6.14$, $P = 0.63$. $P < 0.05$ is considered significant.
ALT: alanine aminotransferase, TLC: total leucocytic count, AST: aspartate amino-transferase, IHD: ischemic heart disease.



Two way cluster dendrogram for association between HLA-B and -C alleles. HLA-B*41, HLA-C*17, HLA-B*15, and HLA-B*42 formed one cluster with a similarity index of >80%. HLA-B*49, HLA-B*44, and HLA-C*16 formed another cluster.

Alanoud Alshami, Rabab Al Attas, Ahmad Azzam, Amani Mohammed and Norah Al-Quhaidan

Detection of SARS-CoV-2 antibodies in pediatric kidney transplant patients. BMC Nephrology (2021) 22:123

https://doi.org/10.1186/s12882-021-02325-x

Abstract

Background: The sero-prevalence of SARS-CoV-2 infection has been studied in immunocompetent children. However, data in the pediatric kidney transplant population (PKT) are lacking.

Methods: Using two commercial immunoassays that measured IgG antibodies against SARS-CoV-2 spike protein and IgG against the nucleo-capsid (N) protein, we screened 72 PKT recipients who attended the outpatient clinic for routine blood work. The majority of patients with positive serology underwent an additional serology test at least once during subsequent clinical follow-up. Patients were confirmed to have SARS-CoV-2 infection if they had two positive tests.

Results: Eight patients out of the 72 screened (11.1%) had positive results for SARS-CoV-2 IgG antibodies in both serological tests. Of those who tested positive, 4 had positive SARS-CoV-2 PCR results before screening. All patients were asymptomatic or had a history of mild symptoms. All tested patients had persistently positive antibodies at a median follow-up time of 75 days (IQR, 44.5, 86.5 days). One patient had a positive PCR test at 75 days and a positive serology test at 120 days post infection.

Conclusion: The sero-prevalence of SARS-CoV-2 was relatively high (11.1%) in our population. Although all patients were asymptomatic or mildly symptomatic, they mounted a strong humoral immune response that persisted for a few months despite being on triple immune-suppressants. These findings have positive implications regarding vaccination efficacy in this group.

Table 1 Demographics and Clinical Characteristics of the 72 transplant patients enrolled

Characteristics	N (%)
Sex	
Male	44 (61.1%)
Female	28 (38.9%)
Age ($\mu \pm SD$)	9.83 ± 3.87
Primary disease	
Congenital Nephrotic Syndrome	22 (30.6%)
Hypoplastic/Dysplastic kidney disease	19 (26.3%)
Obstructive Uropathy	10 (13.9%)
Nephronophthisis	5 (6.9%)
Idiopathic FSGS	4 (5.6%)
ARPKD	3 (4.2%)
Interstitial Nephritis	3 (4.2%)
Unknown	3 (4.2%)
IgA Nephropathy	1 (1.4%)
SLE	1(1.4%)
RPGN	1(1.4%)
Induction	
ATG	21 (29.2%)
Basiliximab	51 (70.8%)
Maintenance immunosuppressants	
Tac + MMF + Prednisone	70 (97.2%)
Tac + Azathoprime+Prednsione	1 (1.4%)
Tac + MMF	1 (1.4%)
Positive Serology Test Results?	
Yes, positive anti-SARS-CoV-2 spike protein IgG (Diasorin) + positive anti-SARS-CoV-2 N protein IgG (Abbot)	8(11.1%)
No, positive anti-SARS-CoV-2 spike protein IgG (Diasorin) + negative SARS-CoV-2 N protein IgG (Abbot)	1(1.4%)
No, negative for both tests	63(87.5%)
Total population	72(100%)

Alanoud Alshami, Rabab Alattas, Hadeel Anan1, Abdulbary Alhalimi, Ahmed Alfaraj, Hadi Al Qahtani

Silent disease and loss of taste and smell are common manifestations of SARS-COV-2 infection in a quarantine facility: Saudi Arabia in PLOS ONE | October 30, 2020

https://doi.org/10.1371/journal.pone.0241258

Abstract

Objectives: In this study, we aimed to study the clinical presentations, and viral clearance of SARSCOV-2 positive quarantined individuals.

Design: Cross-sectional study.

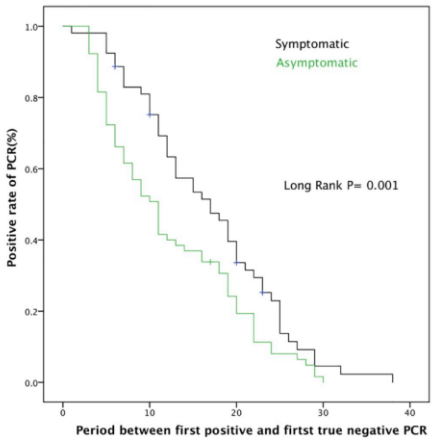
Setting: Governmental- designated facility in the eastern province, Saudi Arabia.

Participants: 128 laboratory confirmed COVID-19 quarantined individuals who had a history of travel abroad in the last 14 days before the quarantine or were in direct contact with laboratory confirmed cases. The study was from March 18th-till April 16th.

Primary and secondary measures: The clinical presentation, prevalence of asymptomatic carriers among SARS-COV-2 positive quarantined subjects, and the difference between virus clearance among symptomatic and asymptomatic individuals.

Results: Sixty-nine of the 128 residents (54%) were completely asymptomatic until the end of the study. The remaining 59 residents (46%) had only mild symptoms. The most common symptom was a sudden loss of smell and taste, accounting for 47.5%. The median time to virus clearance was significantly different between the two groups. Symptomatic residents cleared the virus at a median of 17 days (95% CI, 12.4–21.6) from the first positive PCR vs. 11days (95% CI, 8.7–13.3) in the asymptomatic group (P = 0.011). False-negative test results occurred in 18.8% of the total residents and false-positive results in 3%.

Conclusion: The prevalence of asymptomatic carriers among quarantined travelers and those identified by contact tracing is high in our study. Therefore, testing, tracing, and isolating travelers and contacts of laboratory-confirmed cases, regardless of symptoms, were very effective measures for early disease identification and containment. Loss of taste and smell were the most common presentations in our mild symptomatic residents and should be used as a screening tool for COVID-19. The persistent positive PCR beyond 14 days observed in the mild symptomatic residents despite being symptoms free, warrant further studies to determine its implications on disease spread and control.



Eman Farid, Kannan Sridharan, Ola AM Alsegai, Safa Al Khawaja, Eman J Mansoor, Noor A Teraifi1, Manaf Al Qahtani & Jameela Al Salman

Utility of inflammatory biomarkers inpatients with COVID-19 infections: Bahrain experience, Biomark. Med.(2021) 15(8), 541–549

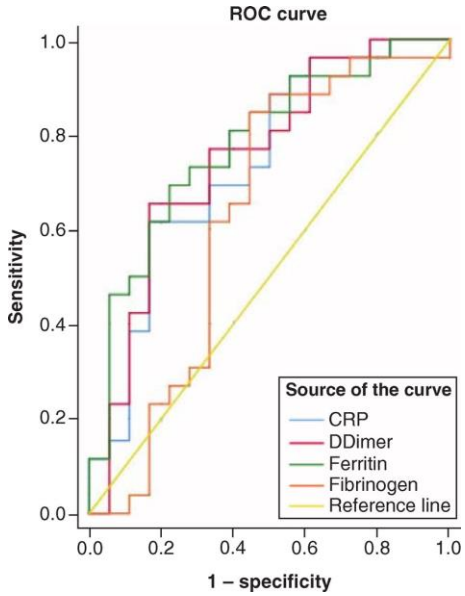
https://doi.org/10.2217/bmm-2020-0422

Aim: COVID-19 pandemic continues and dearth of information remains considering the utility of various inflammatory biomarkers. We carried out the present study to delineate the roles of these biomarkers in various strata of patients with coronavirus infection.

Materials & methods: A retrospective study was carried out after obtaining approval from the relevant Ethics Committee. Patients established with COVID-19 infection as shown by positive real-time quantitative PCR test were included. Details on their demographics, diagnosis, whether they received tocilizumab, and the values of the following biomarkers were obtained: IL-6, C-reactive protein (CRP), serum ferritin, D-dimer, procalcitonin, fibrinogen, lactate dehydrogenase and creatinine kinase. Receiver operating characteristic curves were plotted and correlation of biomarkers with IL-6 were estimated.

Results: One-hundred and three patients were recruited. We observed that serum ferritin followed by D-dimer had better predictive accuracy in identifying patients with pneumonia compared with asymptomatic; and CRP in addition to the earlier markers had better accuracy for predicting severe illness compared with mild–moderate. Serum IL-6 levels were significantly higher in patients with severe illness admitted in intensive care unit. Significantly, higher levels of IL-6 and serum ferritin were observed in patients receiving tocilizumab. A trend of increased IL-6 levels was observed immediately following the initiation of tocilizumab therapy followed by a drop thereafter.

Conclusion: We observed serum ferritin, D-dimer and CRP to accurately predict patients developing severe COVID-19 infections as well as those at risk of developing COVID pneumonia. A trend in IL-6 levels was observed in patients on tocilizumab therapy.



Receiver operating characteristics curve for the predictive ability of biomarkers in differentiating pneumonia from asymptomatic state.

Serum ferritin followed by D-dimer had the best ability to predict pneumonia from asymptomatic state.

Dunia Jawdat, Ali Hajeer, Salam Massadeh, Nora Aljawini, Malak S. Abedalthagafi, Manal Alaamery

Correlation between ABO Blood Group Phenotype and the Risk of COVID-19 Infection and Severity of Disease in a Saudi Arabian Cohort. J Epidemiol Glob Health (2022).

https://doi.org/10.1007/s44197-021-00023-3

Abstract

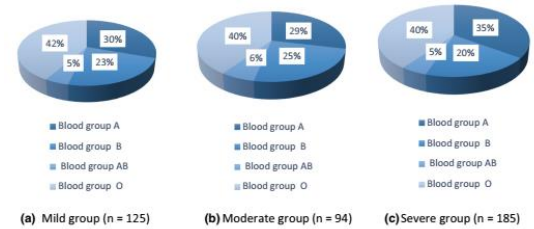
Background Disease severity among patients infected with SARS-CoV-2 varies remarkably. Preliminary studies reported that the ABO blood group system confers differential viral susceptibility and disease severity caused by SARS-CoV-2. Thus, differences in ABO blood group phenotypes may partly explain the observed heterogeneity in COVID-19 severity patterns, and could help identify individuals at increased risk. Herein, we explored the association between ABO blood group phenotypes and COVID-19 susceptibility and severity in a Saudi Arabian cohort.

Methods In this retrospective cohort study, we performed ABO typing on a total of 373 Saudi patients infected with SARSCoV-2 and conducted association analysis between ABO blood group phenotype and COVID-19 infection severity. We then performed gender-stratified analysis by dividing the participating patients into two groups by gender, and classified them according to age.

Results The frequencies of blood group phenotypes A, B, AB and O were 27.3, 23.6, 5.4 and 43.7%, respectively. We found that blood group phenotype O was associated with a lower risk of testing positive for COVID-19 infection (OR 0.76 95% CI 0.62–0.95, p = 0.0113), while blood group phenotype B was associated with higher odds of testing positive (OR 1.51 95% CI 1.17–1.93, p = 0.0009).

However, blood group phenotype B was associated with increased risk in the mild and moderate
group but not the severe COVID-19 infection group. Blood group phenotype O was protective in all severity groups.

Conclusion Our findings provide evidence that blood group phenotype B is a risk for COVID-19 disease while blood group phenotype O is protective from COVID-19 infection. However, further studies are necessary to validate these associations in a larger sample size and among individuals of different ethnic groups.



Blood group distribution of the a mild COVID-19 patient cohort b moderate COVID-19 patient cohort and c severe COVID-19 patient

Nawal Al Kaabi, Abderrahim Oulhaj, Farida Ismail Al Hosani, Shamma Al Mazrouei, Omer Najim, Salah Eldin Hussein, Jehad Saleh Abdalla, Mohammed Saifuddin Fasihuddin, Afnan Abdellatif Hassan, Gehad Elghazali, Ahmed Al Rumaithi, Jumana Al Azazi, Stefan Weber, Rami Beiram, Khatija A. Parekh, Mohamud Sheek-Hussein, Yunkai Yang, Yang Xiaoming, Jenny Quliang, Islam Eltantawy9, Sally Mahmoud, Ashish Koshy, Peng Xiao, Subhashini Ganesan, Wael Elamin & Walid Zaher

The incidence of COVID-19 infection following emergency use authorization of BBIBP-CORV inactivated vaccine in frontline workers in the United Arab Emirates. Scientific Reports (2022) 12:490

https://doi.org/10.1038/s41598-021-04244-1

Based on the findings from the Phase III clinical trials of inactivated SARS COV-2 Vaccine, (BBIBPCORV) emergency use authorization (EUA) was granted for the vaccine to frontline workers in the UAE. A prospective cohort study was conducted among frontline workers to estimate the incidence rate and risk of symptomatic COVID-19 infection 14 days after the second dose of inoculation with BBIBP-CORV inactivated vaccine. Those who received two doses of the BBIBP-CORV vaccine in the period from 14th of September 2020 (first dose) to 21st of December 2020 (second dose) were followed up for COVID-19 infections. 11,322 individuals who received the two-dose BBIBP-CORV vaccine were included and were followed up post the second dose plus fourteen days. The incidence rate of symptomatic infection was 0.08 per 1000-person days (95% CI 0.07, 0.10). The estimated absolute risk of developing symptomatic infection was 0.97% (95% CI 0.77%, 1.17%). The confirmed seroconversion rate was 92.8%. There were no serious adverse events reported and no individuals suffered from severe disease. Our findings show that vaccinated individuals are likely to remain protected against symptomatic infection or becoming PCR positive for SARS COV 2 following the second dose of the vaccination.

Characteristics	SARS-COV-2 negative or Asymptomatic (*n*- 11,228) *n* (%)	SARS-COV-2 positive and Symptomatic (*n*- 94) *n* (%)	Total (*n*- 11,322) *n* (%)
Age category			
< 50 years	9716 (86.53%)	84 (89.36%)	9800 (86.56%)
≥ 50 years	1512 (13.47%)	10 (10.64%)	1522 (13.44%)
Gender			
Male	9227 (82.18%)	70 (74.47%)	9297 (82.11%)
Female	2001 (17.82%)	24 (25.53%)	2025 (17.89%)
Nationality			
Arab	6904 (61.49%)	84 (89.36%)	6988 (61.72%)
Asian	3799 (33.84%)	6 (6.38%)	3805 (33.61%)
Other	286 (2.55%)	2 (2.13%)	288 (2.54%)
Missing	239 (2.13%)	2 (2.13%)	241 (2.13%)
Associated comorbidities			
Any comorbidity	1054 (9.39%)	15 (15.96%)	1069 (9.44%)
Diabetic, *n*(%)	442 (3.94%)	9 (9.57%)	451 (3.98%)
Hypertension	617 (5.50%)	9 (9.57%)	626 (5.53%)
Cancer	109 (0.97%)	0 (0.00%)	109 (0.96%)
Pulmonary diseases	20 (0.18%)	0 (0.00%)	20 (0.18%)
Immunosuppression	9 (0.08%)	0 (0.00%)	9 (0.08%)
Transplant	3 (0.03%)	0 (0.00%)	3 (0.03%)
Other comorbidities	183 (1.63%)	3 (3.19%)	186 (1.64%)
Number of comorbidities			
None	10,174 (90.61%)	79 (84.04%)	10,253 (90.56%)
1	717 (6.39%)	11 (11.70%)	728 (6.43%)
≥ 2	337 (3.00%)	4 (4.26%)	341 (3.01%)

Table 2. Demographic and clinical characteristics of the study individuals.

Case Studies

Case 1: Seasonal Influenza vaccine influenced HLA antibody formation in a kidney transplant recipient with history of blood transfusion: A case study.

By: Dr. Ahmed Mostafa MD, PhD, F(ACHI)

Aim: It is critical for each laboratory to validate and optimize their assays for their centers. Indeed, the performance of multiple assays should be considered, as no single test is without its own limitation.

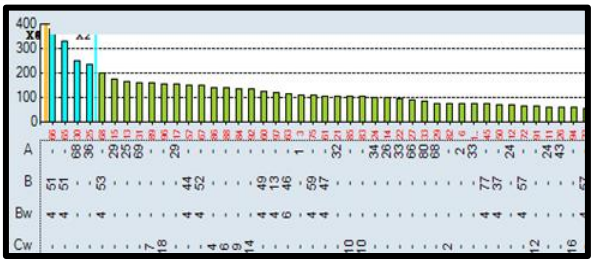
Methods: 59-year old male Asian Canadian was diagnosed with end-stage renal disease secondary to both obstructive nephropathy as well as hypertension. He was referred to the kidney transplant clinic for an upcoming living donor renal transplant from his sister. The patient had a history of blood transfusion in 2016. The initial flow cell crossmatch (FCXM) was performed in May 2018. FCXM was negative for both T and B cells. In order to confirm the FCXM results and to properly assess the patient, SAB (LABScreen single antigens beads, One Lambda) was done on patient sera and the results were negative for both HLA class I and II antibodies with 0% PRA. The transplantation of the patient was delayed due to some medical concerns that required the patient to be hospitalized and his transplantation was temporary suspended. One year later, the patient was fit to be added to the transplantation list. The patient received the influenza seasonal vaccination and the initial FCXM was repeated.

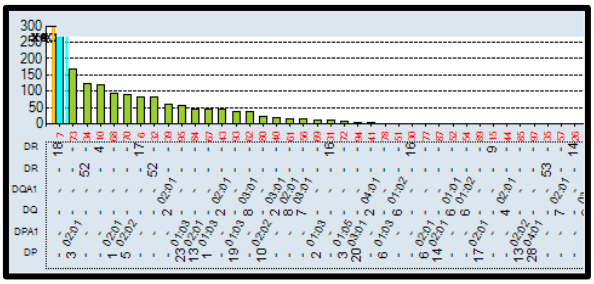
Results: Surprisingly the patient reacted strongly positive for both T and B cells FCXM. SAB for HLA class II did not show any change from previous and the PRA was 0%. For HLA class I antibodies we noticed the presence of A1, 23, 24, 80; B44, 45, 76 with a PRA of 51%. A1 and B44 were DSA's. We performed epitope analysis using the matchmaker in the Fusion 4.4 software to see if there is any shared epitope(s) that explained the pattern of antibodies. We found that two epitopes, 166DG and 166ES explain the entire specificity. The increase in the breadth of HLA antibodies was mainly within the same cross-reactive antigen group (CREG), indicating an expansion of existing specificities without development of new specificities. A1 is a DSA with an MFI of 1200, but it shares the same epitope of A80 with an MFI of 15000. This could explain the strong positivity of the T cell and B cell FCXM.

Conclusion: In conclusion, HLA antibody formation after the seasonal vaccination are due to activation of preexisting memory B cells, which result in increase of strength of HLA antibodies.

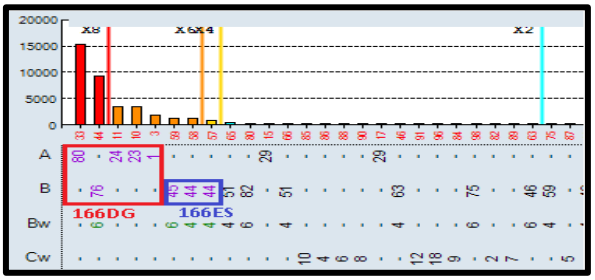
	Haplo type	A*	B*	C*	DRB1*	DRB 3/4/5	DQA1*	DQB1*	DPA1*	DPB1*
Patient	a	03:XX	07:XX	02:XX	11:XX	3*02	01:XX	*03(7)	01:XX	04:01
	c	03:XX	40(61)	07:XX	15:XX	5*01	05:XX	06:XX	01:XX	04:01
Sibling	b	01:XX	44:XX	05:XX	04:XX	4*01	03:XX	*03(7)	01:XX	04:01
	d	02:XX	44:XX	05:XX	04:XX	4*01	03:XX	*03(7)	01:XX	04:01

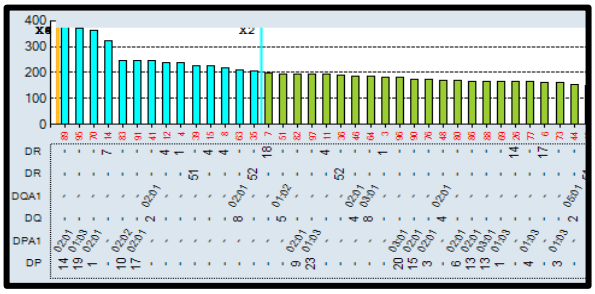
In 2018

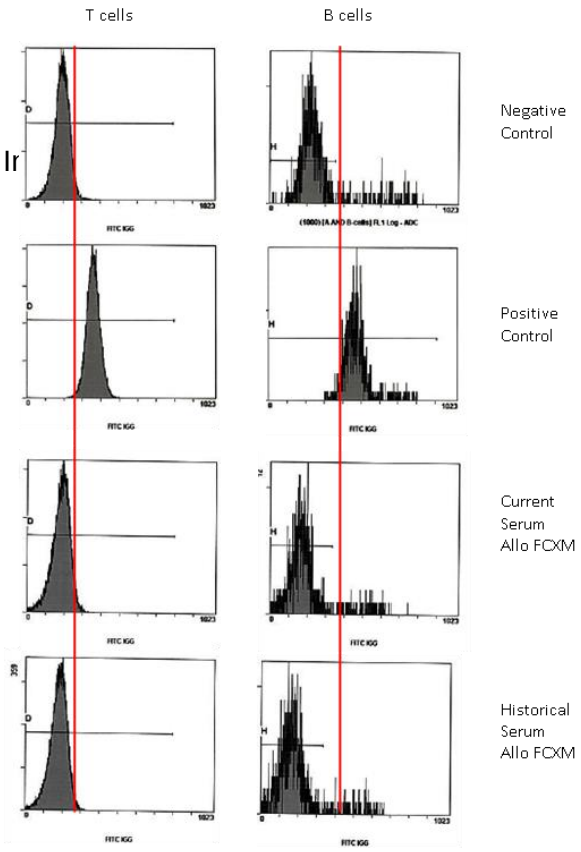


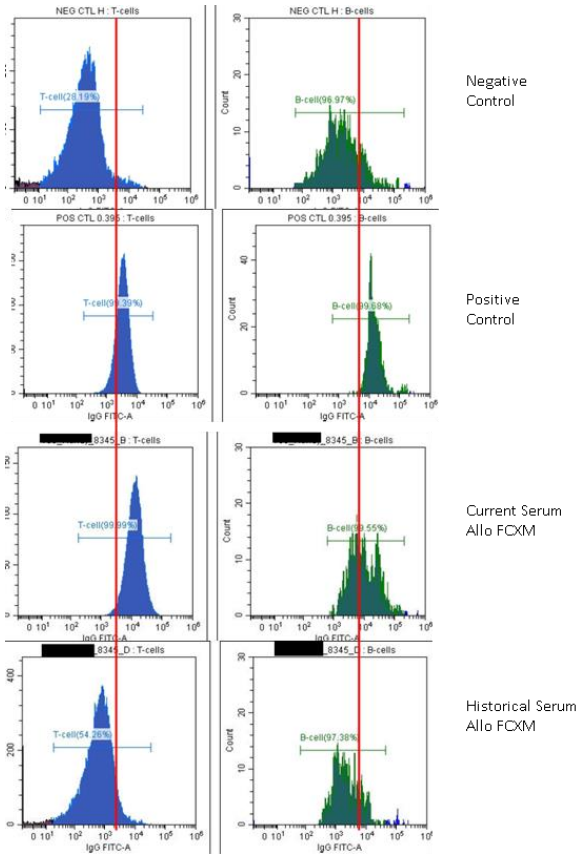


In 2020









Case 2: FCXM results in SLE patient

By: Dr. Ali Hajeer PhD, FRCPath, D(ABHI)

Deputy Chairman, Head of Immunopathology, Department of Pathology, Ministry of National Guard Health Affairs, King Abdulaziz Medical City, Prince Meteb Road, P.O.Box 22490 Riyadh 11426, KSA. +966118011111 Ext 54314, hajeera@ngha.med.sa, www.ngha.med.sa

Patient history: 41-years-old female with ESRD, SLE (Systemic Lupus erythematous), and Diabetes.

Positive PRA Screen by Luminex method since March, 2020.

Donor: unrelated, male 26 years old, non-directed donation.

HLA	A	B	C	DRB1	DRB3/4/5	DQA1	DQB1	DPA1	DPB1
Patient	*02:01	*07:02	*07:01	*08:04		*01:02	*03:01	*01:03	*02:01
	*03:01	*39:10	*12:03	*15:01	5*01:01	*05:05	*06:02	*01:03	*04:01
Donor	*02:05	*44:03	*04:01	*03:01	3*02:02	*03:03	*02:01	*01:03	*02:01
	*74:01	*50:01	*06:02	*04:10:01G	4*01:01	*05:01	*03:03	*01:03	*04:02
Mismatch	2	2	2	2	2	2	2	0	1

Luminex PRA Screening:
HLA Class I: positive
HLA Class II: positive

FXM

	MCFS*	Results	Interpretation
T cell (Allo)	624	Positive	Positive T cell &
B cell (Allo)	1167	Positive	B cell FXM
T cell (Auto)	39	Negative	Negative T cell &
B cell (Auto)	1074	Positive	Positive B cell Auto- CXM

Results:
The positive FCXM cutoffs value used by the HLA laboratory were >76 and >140 MCFS (Median Channel Fluorescence shift) for T and B cells, respectively.
FCXM Positive auto B FXM
Running LAB Screen (Screening)

Running both LABScreen Single Antigen Class I & Class II

LABScreen Single Antigen Class I & Class II:
DSA:
B*44:03 =5911 MFI (cutoff 2000 MFI)
DRB4*01:01= 3595 MFI (cutoff 2000 MFI)
DRB1*03:01 = 3209 MFI (cutoff 2000 MFI)
Total DSA= 12715 MFI

Conclusions & Recommendation
Single Antigen Class I & Class II are necessary for interpreting FCXM. Class I and Class II single antigen data correlated with Flow XM. Positive auto B FXM can be explained by SLE (autoimmunity). Donor was used to start our first two-way paired kidney donation. A 6 years old (PRA negative, no history of sensitization) had a related (cousin) 6/6 MM. He was offered this donor one haplotype match with this non-directed donor. This initiated a 2 way PKD, where the donor of the pediatric case was used for another patient with 0 MM as DR.

Development of New Laboratory

Fully established services for HLA Department in King Fahad Hospital, Madinah, Saudi Arabia.

By Dr. Abdulhalem A. Jan

Consultant of Cellular and Molecular Biology.
General Director of Laboratory and Blood bank Madianh, Saudi Arabia.

Many people around the world are suffering from Kidney diseases. Especially, in Saudi Arabia, which has nearly 16,000 patients in renal failure, around 60% on dialysis and more than 6,000 awaiting kidney transplants. Several reasons play a role in this health concern contributing to high numbers of cases among the Saudi population, such as hypertension, genetics and diabetes.

King Fahad Hospital (KFH) in Al-Madinah city is a tertiary hospital since 1980 and acts as reference to other hospitals in the region, which has several departments; some are general while some are more specialized. The laboratory department of KFH has 12 specialized sub-departments, which is also acts as reference laboratory in Madianh region. Now and as part of an extension to the laboratory department, a new sub-department has been established, which is Human Leukocyte Antigens (HLA) for Kidney transplantation. HLA department was established partially since 2019, with the collaboration of other hospitals specialized in tissue typing test. Histocompatibility testing, including HLA molecular typing and HLA antibodies identification, is performed in our HLA Laboratory. However, in the past years, performed in house and samples were sent out to a reference laboratory to perform the test. Therefore, as improvement project in laboratory and expand the scope of service, we decided to implement flow cytometric crossmatching in our lab to decrease the turnaround time and expedite the transplant procedure. In 2021, we established three color flow crossmatching using plate method, halifaster protocol. The first step was preparation and optimizing the reagents, including negative and positive control serum, pronase concentration, wash buffer as well as antibody cocktail titration. After that, we established the positive cut off using different donors' cell against negative control serum, which was collected from non-sensitized male donor. The final step was the validation of flow crossmatching and the established positive cut off by the comparing

the results of flow crossmatch and virtual crossmatch, the presence and absence of donor specific antibody. Since April 2021 and up to date, we have performed 196 crossmatching as well as all test investigations for kidney transplantation in KFH for pre-transplantation, post-transplantation and flow-up with the patient. In conclusion, it can be said that HLA department in KFH is the only laboratory in the region that serves all patients in need. Statistically, the rate of patients that are waiting for kidney transplantation in Madinah region is high. Fortunately, KFH examined about 291 patients that are compatible for kidney transplantation, most of these cases' surgeries were completed successfully with post-surgery high quality of life, while the others still waiting for compatible donors. At the same time, there is improvement project to expand HLA for other organs.

Finally, all gratitude and appreciation goes to the hospital administration, kidney center in Madinah, Mr. Ahmad A. Bokhari, and Mr Emad Alkhaldi for their support in training the staff, without their contributions we could not have reached this level of optimal quality in health care and patients safety.

HLA Department at King Fahad Hospital, Madinah, Saudi Arabia

New Method Validation

Clinical Validation of AllType™ next-generation sequencing (NGS) using Ion Torrent platform –KFSH-D experience

By: Mr. Saber AlZahrani, CHS (ABHI)
NGS Team Leader.
Histocompatibility and Immunogenetics Lab
King Fahad Specialist Hospital –Dammam (KFSH-D).

Next-generation sequencing (NGS) technologies have been widely adapted by many histocompatibility and immunogenetics labs for HLA high-resolution typing. Here we describe our experience in validating NGS-based HLA typing. We evaluated and validated HLA typing by NGS methodology using AllType™ kit (One Lambda, Inc.) and TypeStream™ software 1.3.0 on the Ion torrent S5 sequencing platform. The AllType™ assay covers 11 HLA genes (HLA-A, -B,-C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1) all amplified in full gene length except for DRB1, DRB345, DQB1 & DPB1 that amplify in Exon 2 thru 3'UTR.

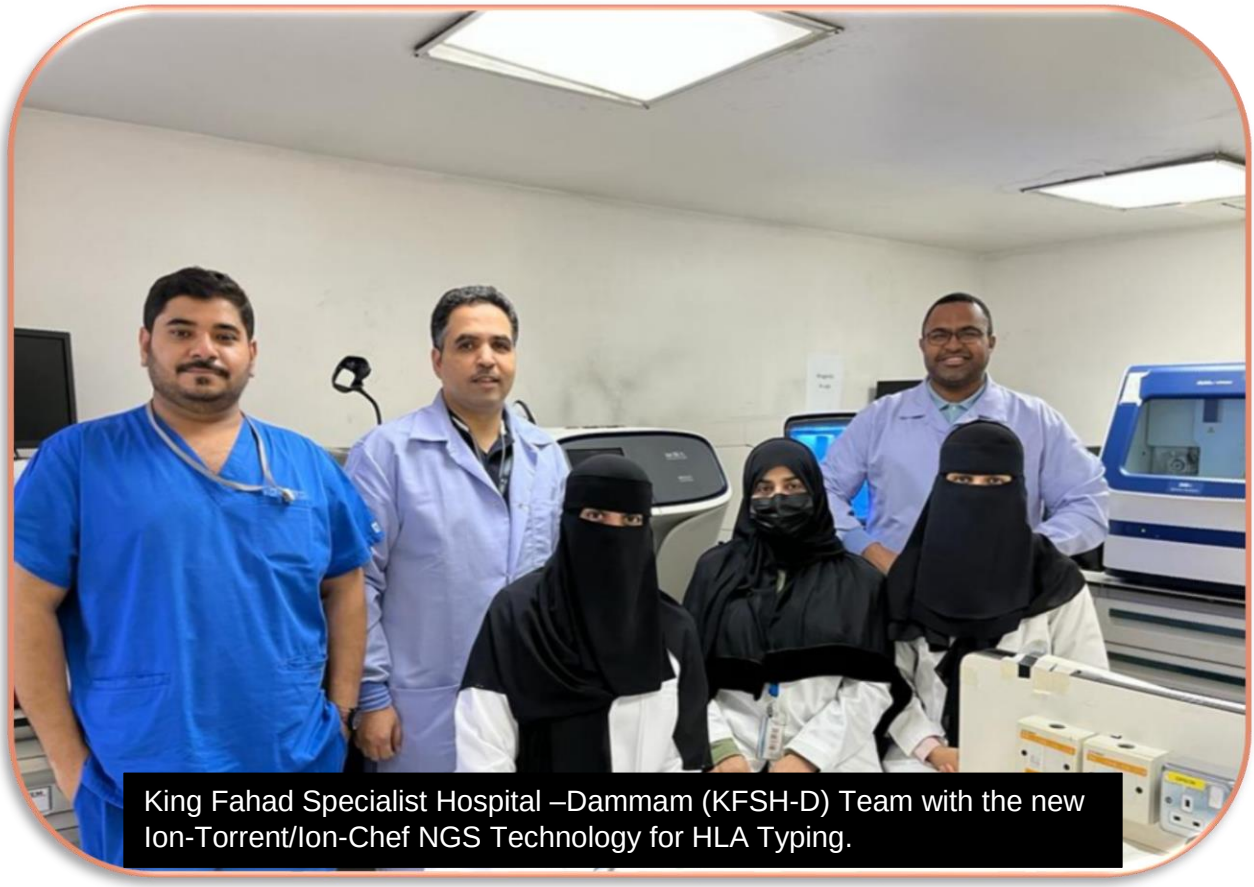
The validation panel consisted of 100 genomic DNA samples divided into three groups, 44 historic proficiency-testing samples (PT), 32 clinical samples, and 24 blind samples, generously provided by the KFSH&RC histocompatibility lab. The total number of alleles from the 11 loci interrogated by NGS was 1196 alleles.

To comply with ASHI standards and NGS validation guidelines, a wide range of HLA genotyping was included in the panel including 1085 heterozygous alleles, 111 homozygous alleles, and 7 rare alleles. The validation was performed in different run sizes includes 8, 16, 32, and 48 samples to mimic different routine run sizes. The fidelity of the barcoding to identify a specific sample was monitored by rotating the barcode index between samples that were used for reproducibility. The reproducibility of the technique was evaluated by testing three samples in three different runs (inter-reproducibility), and three samples were tested times time within one run (intra-reproducibility). Moreover, the tech-to-tech reproducibility was evaluated by testing eight samples by three different technologists in three different runs. All quality matrices were analyzed and calculated during the validation All the 100 samples tested with Alltype assay typing showed 100% concordance for all the 11 HLA loci (1196 HLA alleles) at six digits compared to Sanger sequencing and reference PT results. HLA ambiguity was observed mostly for HLA class II HLA-DRB1 (7%), DQB1 (48%) & DPB1 (13%) loci likely to be due to the absence of exon 1. However,

some ambiguities, which could not be resolved, were due to phase ambiguity.

The assay was found to be highly accurate, precise, reproducible and have adequate specificity and sensitivity to perform HLA high-resolution typing (6-digit typing). The successful implementation of NGS HLA typing helped to streamline the HLA typing workflow especially for stem cell matching and to provide better resolution for antibody evaluation in organ and tissue transplantation. Also, contributed to the discovery of novel alleles.

Special thanks to Dr. Rabab Al Attas, Dr. Mohammad Awaji, Dr. Amani Mohammed, Kenana AlAjlan, Dalal AlAbduladheem, Hassan AlHarbi, and Mariam AlZahrani for their valuable contribution to this validation.



King Fahad Specialist Hospital –Dammam (KFSH-D) Team with the new Ion-Torrent/Ion-Chef NGS Technology for HLA Typing.

AllType FASTplex NGS Assay

Intelligently Simple

Designed for simplicity and efficiency, the AllType™ FASTplex™ NGS Assay is the only single-test solution supported by streamlined software integrated with the HistoTrac® Software, which is now offered exclusively by Thermo Fisher Scientific.

The assay chemistry has been enhanced to increase performance, robustness, and reliability. With the new Class II exon 1 primer mix, the AllType FASTplex reagents reduce ambiguities, provide comprehensive gene coverage across 11 loci, and generate high concordant results at 99.8% or higher—all in a single PCR reaction. The protocol is optimized to run on the Ion Torrent™ and Illumina™ sequencers.

Sequencing data is automatically analyzed with our TypeStream™ Visual NGS Analysis Software. The interface includes bi-directional communication with the HistoTrac system, enabling the seamless integration of results to improve data transcription and reporting. Results can be combined with our HLA Fusion™ Software for antibody tracking, crossmatching, and epitope analysis.

AllType FASTplex NGS Workflow

- Fragmentation and Adaptor Synchronized Tagging combined
- Samples pooled early in the protocol so library preparation can be completed in one tube
- Turnaround time < 7 hours with < 90 minutes of hands-on time

Library Preparation	Sequencing	Analysis	Reported & Advanced Analytics
< 7 hours total ***< 90 minutes*** **hands-on time** *Single tube library preparation protocol	Ion GeneStudio™ S5 Illumina™ MiniSeq™ Illumina™ MiSeq™ Illumina™ iSeq 100™	Automated with TypeStream Visual NGS Analysis Software	Data integration with HistoTrac System & HLA Fusion Software

To learn more about our **AllType FASTplex NGS Assay**, visit **onelambda.com/ngs** or contact your One Lambda Channel Partner.

Congratulations!

I- Years to Reach a Prestigious HLA Fellowship

By: Dr. Ahmed Abu Khader Ph.D., MSc, MLT(CMLTO)

Histocompatibility Fellow
HIL- Alberta Precision Laboratories
Department of Pathology and Lab Medicine - University of Calgary

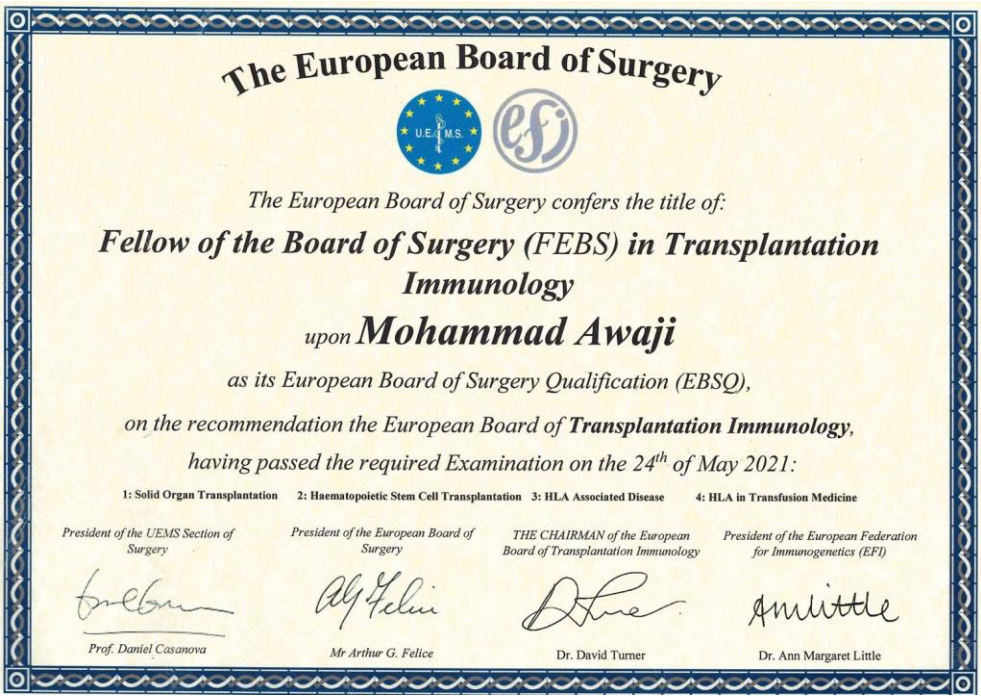
After obtaining a Ph.D. in bone marrow transplantation immunology from Germany, many years in academia, and joining the Center for Innovation at the Canadian Blood Services (CBS) and later the Cellular Therapy and Applied Genomics (CTAG) at King Hussein Cancer Center (KHCC), finally I got my dream being accepted in a prestigious fellowship program. The HLA fellowship program in Alberta Precision Laboratories in Calgary is one of the only three fellowship programs accredited by The American Society for Histocompatibility and Immunogenetics (ASHI) in North America. As the program, under Dr. Noureddine Berka, is too competitive and takes only one fellow every two years, I kept applying for this fellowship since 2016. I applied more than once, and I kept improving my curriculum vitae (CV) until finally, I got my dream of being accepted as the next fellow in 2020.

Due to the COVID-19, my fellowship journey started late, but as they say “Better Late than Never”. After starting my fellowship, registration with ASHI Directors' Training Review and Credentialing (DTRC) as a director-in-training was necessary. Now and after finishing more than half of my fellowship, I gained many values and expertise in transplanting solid organs as well hematopoietic stem cells (HSC). During my training, I was exposed to ASHI standards in HLA testing, laboratory accreditation and inspection, proficiency testing (PT), quality control (QC), quality assurance (QA), validation of new procedures, and reporting. Troubleshooting was a huge experience I gained as a director-in-training, as these scenarios connect many HLA practices, theories, and applications that any future director will need. Being trained in such an up-to-date laboratory, expose the trainee to the latest technologies in the field like next-generation sequencing (NGS) which is a necessity these days used in the context of epitopes and eplets analysis to resolve some antigen-antibodies reactivities. Being a fellow in a reference laboratory that receives external samples gave me insights into some financial or technical limitations that laboratories may face. During my bench rotation and training on overviewing patients’ files, I recognized how such an excellent program expose the trainee to ways of thinking in the field of HLA. It is not just results but results in the context of the patient’s and/or donor’s physiological status at the time of obtaining samples. Day to day workload and turnaround time (TAT) for tests make trainees appreciate the time and the effort of technologists and work in harmony to release trustful results in a timely manner. An excellent training program has bases approved by ASHI, and gives HLA fellows the best training experience they are looking for. Being persistent and keeping my dream to join a prestigious HLA fellowship program, took me four years that two fellows finished before I start My advice is to keep the hope and make your CV better every time you apply to compete in the next round of selection until you get your dream.

II- Passing the Diplomat of the American Board of Histocompatibility and Immunogenetics (ABHI) and the European Board of surgery in Transplantation Immunology (FEBS).

Dr. Mohamed Awaji Ph.D., D(ABHI), FEBS, TS(ABB), CHS(ABHI)

Clinical Scientist of Histocompatibility & Immunogenetics, King Fahad Specialist Hospital Dammam, University of Nebraska Medical Center Ad Dammām, Eastern, Saudi Arabia



The European Board of Surgery

The European Board of Surgery confers the title of:

Fellow of the Board of Surgery (FEBS) in Transplantation Immunology

upon ***Mohammad Awaji***

as its European Board of Surgery Qualification (EBSQ),

on the recommendation the European Board of ***Transplantation Immunology****,*

having passed the required Examination on the 24th of May 2021:

1: Solid Organ Transplantation 2: Haematopoietic Stem Cell Transplantation 3: HLA Associated Disease 4: HLA in Transfusion Medicine

President of the UEMS Section of Surgery	*President of the European Board of Surgery*	*THE CHAIRMAN of the European Board of Transplantation Immunology*	*President of the European Federation for Immunogenetics (EFI)*
Prof. Daniel Casanova	Mr Arthur G. Felice	Dr. David Turner	Dr. Ann Margaret Little

III- Passing the examination of the certified histocompatibility technologist (CHT) from the American board of Histocompatibility and Immunogentics (ABHI)

King Faisal Specialist Hospital and Research Centre (FSHRC)- HLA lab, Riyadh

1. Dana Alkanhal- passed the examination of CHT(ABHI)
2. Wadha Alsubaei – passed the examination CHT(ABHI)

ARSHI in ASHI 2021

Dr. Nezar Elshiek, Dr. Ahmed Mostafa, Mr Adel Shawatti and Dr. Hamid Liacini

Dr. Noureddine Berka, Dr. Ahmad Abu Khader, Dr. Moheeb Alwwami, Dr. Abeer Madbouly, Dr. Sondus El Skaridah, Dr. Reem Amin, Dr. Hamid Liacini, Mr Adel Shawatti, Dr. Reem Jan, Dr. Ahmed Mostafa, Dr. Nezar Elshiek and Dr. Medhat Askar

Dr. Annette Jackson, Dr.Medhat Askar and Dr. Rob Liwski.

By: Dr. Ahmed Mostafa MD, PhD, F(ACHI)

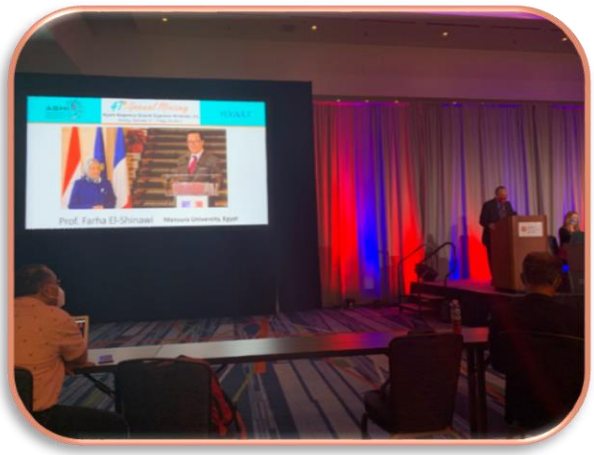
The 47th annual ASHI meeting took place in Orlando, Florida, USA from September 27th to October 1st. This year and for the first time ASHI meeting was a hybrid event. The reason was to avoid any surprises with the ongoing Covid pandemic restrictions means that we won't have a physical presence at the conference in Florida, we will be present with a virtual booth.

This year there were over 1150 attendees and ~ 300 of them physically attended the meeting. There were over 25 exhibits, 6 network reception, 150 posters, and 55 different countries were represented at the conference.

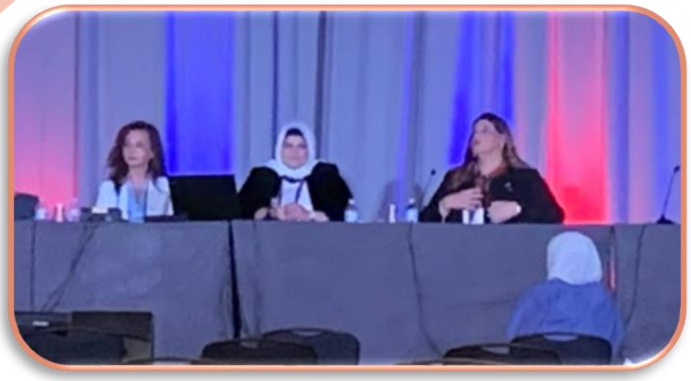
Offering the meeting to a global audience in a virtual format was visionary and truly in alignment with ASHI's mission pillar of Education, even in a pandemic. The educational material was excellent and provided the opportunity for much-needed CE without fear of travel. The ASHI 2021 hybrid meeting was a great opportunity to learn what is happening in our field of histocompatibility and immunogenetics and a very accessible online platform.

12 members from ARSHI were able to physically attend the meeting and also did some social gatherings.

On the first day, there was an option to attend the half-day of inspector workshop or the Basic Science Symposium on Immune Memory in Transplantation and Protective Immunity. This symposium highlighted experts in the field of T and B cell memory and featured lectures on HLA and COVID19. Several ARSHI members presented orally and presented posters. Dr. Medhat Askar was a speaker in the Opening Session: Labtopia: Design the Laboratory of the Future. An audience-driven discussion on designing an Immunogenetics laboratory of the future without organization restrictions.



Dr. Ahmed Mostafa presented in One Lambda workshop about the Alltype alternative protocol using the NGS.



Dr. Ameen and Dr. Madbouly had the pleasure of moderating such a diverse and dynamic set of presentations followed by an interactive panel discussion.Dr. Reem Jan was a speaker in this session and they described multiple challenges in HLA testing that existed in different areas of the world.

Dr. Rabab Alattas and her team presented 4 poasters virtuailly. Dr. Ahmad Abu Khader and Dr. Berka presented a case study.

Dr. Abeer Madbouly presented an oral presentation about the Validation of HLA matching performance for 300,000 domestic and international donor/recipient pairs for donor requests facilitated by the US Be The Match donor registry.

Abeer Madbouly, Pradeep Bashyal, Jennifer Novakovich, Jane Kempenich, Kim Wadsworth, Martin Maiers, Yung-Tsi Bolon

Aim:

The National Marrow Donor Program (NMDP) search algorithm, HapLogic®, used by transplant centers since 2006, has significantly advanced HLA-matching for transplantation. To maintain high predictive accuracy, frequent validations are conducted with updated data and statistical measures. Here, we describe a recent study to validate HapLogic's matching performance for domestic and, for the first time, all World Marrow Donor Association (WMDA) donors requested through the NMDP in the last 10 years.

Methods:

We studied a dataset of 164,000 domestic and 150,000 WMDA donors from 45 countries. Of the selected donors, 28% were domestic donors of color and 17% were WMDA donors from outside of Europe. These donors were projected by HapLogic to be potential 8-of-8 or 10-of-10 HLA matches and provided a confirmatory typing (CT) blood sample between 2010 and 2021. Only 5% of domestic donors were missing HLA-C and DQB1 in recruitment typing. These fractions increased to 37% and 47% for WMDA donors. Match status was defined using CT typing and performance was measured via Receiver Operating Characteristic Area Under the Curve (AUC), Brier Score, F-score and expected versus actual prediction probability distribution. We also evaluated the fraction of domestic donors with unimputed HLA genotypes or imputed by haplotype frequencies outside their reported race.

Results:

Table 1 shows matching performance for 10-of-10 and 9-of-10 HapLogic predictions for domestic and WMDA donors. Overall, high classification and prediction accuracy and low levels of prediction error were demonstrated based on AUC, F-score and Brier score respectively. These values are an improvement over older validations due to enhancements implemented in HapLogic and better HLA resolution in the newer validation dataset. Figure 1 depicts matching predictions compared to expectations for domestic and WMDA requests. HapLogic was unable to impute about 1% of the tested donor sample and used a haplotype frequency file to impute donor genotypes different from the self-identified race for 17% of domestic donors.

Conclusion:

Validations demonstrated improved HapLogic performance for domestic donors over previous results and show comparable performance for international donors. To date, this is the first study to evaluate

matching performance on a dataset of WMDA and domestic donors of this size and diversity. Work is underway to address some of the identified areas of improvement, including updating the population haplotype frequencies for better imputation and extending matching to include low-expression loci.

Quality measure	Domestic		International	
	10 of 10 predictions	9 of 10 predictions	10 of 10 predictions	9 of 10 predictions
Area Under the Curve AUC	0.98	0.95	0.98	0.96
Brier Score (mean square error)	0.056	0.07	0.05	0.06
F1 Score (harmonic mean of precision and recall)	0.94	0.81	0.95	0.8

Table 1: Matching performance for domestic and international donors for different match stringencies

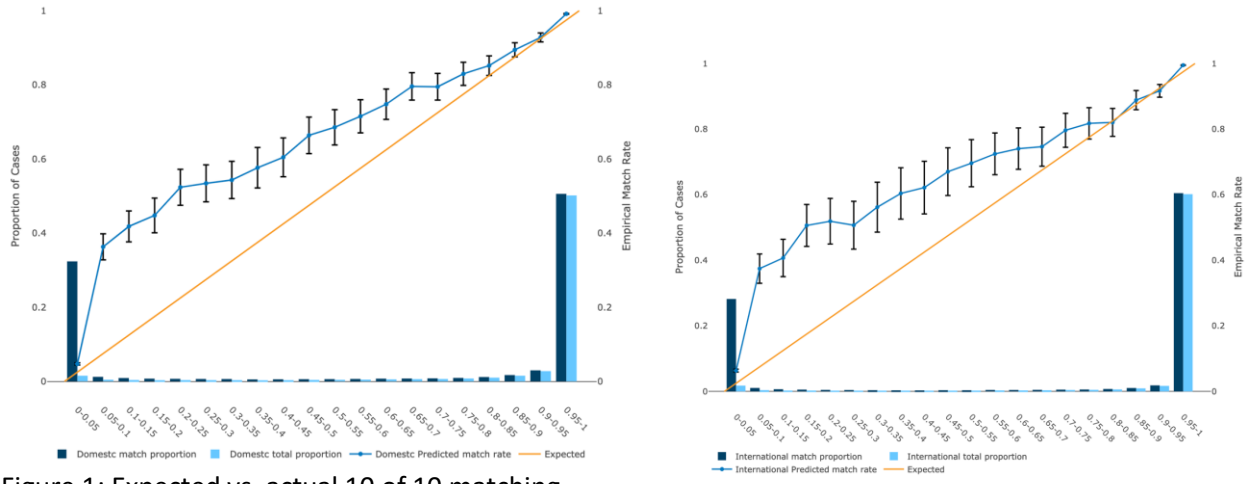


Figure 1: Expected vs. actual 10 of 10 matching predictions involving a) domestic and b) international donors

Upcoming events

04-05 March 2022

1st ARSHI Annual Conference

Virtual

19-23 March 2022

48th Annual Meeting of the BMT

Hybrid

11-15 May 2022

The 18th International HLA & Immunogenetics Workshop

Amsterdam/Holland

17-20 May 2022

35th EFI Annual Conference

Amsterdam, Holland

13-14 September 2022

32nd BSHI Annual Conference 2022
Birmingham, UK

10-14 September 2022

29th International Congress of The Transplantation Society (TTS 2022)

Buenos Aires, Argentina

23-28 October 2022

49th ASHI Annual Meeting

Las Vegas, NV, USA

ARSHI Newsletter Guidelines

✓ Frequency

ARSHI Newsletter is published twice in the year by the ARSHI Editorial Board, special editions may vary.

✓ Goal

To provide scientific and general information about HLA and related topics

✓ Submission

Kindly consider the following for submission:

- Document should be submitted in word format
- Submitted article should be a half or maximum one page (A4) with 12 font size
- If possible, article might be supported with photos
- To submit an article for publication in ARSHI Newsletter please contact us through the following mail.

info@arshi-hla.org