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Review

HLA Typing and Donor-Specific Antibody Screening in Kidney Transplantation: Bridging the Past to the Future

Mostafa T. Mohammed [1, 2, 3], Esmat Al Sharkawy [3], Amel M. Kamal Eldin [3], Emad Allam [3], Manal M. Saber [3], Anthony Nassar [1], Nour Younis [1], Christa Deban [1], Ahmad Jaser [4], Indira Guleria [5, 6], Basset El Essawy [4, 7], Anil Chandraker [1], Mahmoud Nasr [2, 4], Jamil R. Azzi [1, *]

1. Transplantation Research Center, Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; E-Mails: mtawfeekmohammed@bwh.harvard.edu; mostafarafik@yahoo.com; anthonynassar96@gmail.com; nyounis@bwh.harvard.edu; christa.deban@gmail.com; achandraker@bwh.harvard.edu; jazzi@bwh.harvard.edu
2. Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; E-Mail: mahmoud_nasr@hms.harvard.edu
3. Department of Clinical Pathology, Faculty of Medicine, Minia University, Minia, Egypt; E-Mails: esmatalsharkawi57@gmail.com; amal.mahmoud@mu.edu.eg; Emadallam_lab@yahoo.com; manal.saber@mu.edu.eg
4. Renal Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; E-Mails: ajaser@bwh.harvard.edu; aelessawy@bwh.harvard.edu
5. HLA Tissue Typing Laboratory, Renal Transplant Program, Division of Renal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
6. Pathology Department, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; E-Mail: iguleria@bidmc.harvard.edu
7. Department of Medicine, Al-Azhar University, Cairo, Egypt; E-Mail: belessawy@gmail.com

* **Correspondence:** Jamil R. Azzi; E-Mail: jazzi@bwh.harvard.edu

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Abstract

Human leukocyte antigens (HLA) are unique proteins expressed on the surface of human cells, playing a pivotal role in the immune system, particularly in the contexts of infection, cancer, and transplantation. The widespread adoption of HLA typing methods has become an essential component in assessing donor-recipient compatibility, a crucial limiting factor in solid organ transplantation. In general, the greater the disparity between a donor's and recipient's HLA types, the higher the likelihood of provoking an alloimmune response, which frequently results in alloimmune graft rejection. With significant advancements in organ transplantation techniques, immunosuppressive medications, and surgical procedures, attention has increasingly turned toward understanding and managing humoral rejection processes. Pre-transplant antibody screening plays a critical role in identifying individuals with elevated levels of antibodies against potential donor antigens. This screening aids in risk assessment and planning to mitigate the risk of antibody-mediated rejection (AbMR). Several methods are available for assessing circulating antigen-specific antibodies and HLA tissue typing, including cell-based assays like serological assays, complement-dependent cytotoxicity, and flow cytometry. However, non-cell-based approaches, such as molecular methods, HLA imputation techniques and high-throughput HLA-matchmaker assays have gained significant popularity due to their ability to provide higher resolution and robust donor-recipient matching. Despite the advancements in precision and sensitivity observed in HLA cutting-edge technologies, numerous challenges still persist. These challenges involve complexities linked to allelic ambiguities, the differentiation of closely related alleles, and the ongoing effort to establish a standardized HLA testing methodology across diverse laboratories. Additionally, correlating the HLA crossmatch results with the clinical outcomes for transplant donors poses another important aspect that warrants attention and requires expert analysis. In this review, we will enumerate the different methods of HLA typing and DSA screening and discuss the unmet needs and future directions for HLA typing methods.

Keywords

Human leukocytic antigen; donor-specific antibody; antibody-mediated rejection; kidney transplantation; Luminex; HLA-matchmaker; virtual cross matching

1. Implications of HLA Typing in Kidney Transplantation

Kidney transplantation stands as a lifesaving medical intervention, offering individuals suffering from end-stage renal disease (ESRD) a newfound opportunity for a healthier and more enriching life. Kidney transplantation is intimately tied to achieving compatibility between the donor and the recipient, a process in which Human Leukocyte Antigens (HLA) play an essential role [1]. HLA molecules are complex proteins present on the surface of human cells. These molecules serve as markers, assisting the immune system in discriminating self from non-self-antigens [2]. HLA typing involves the identification and characterization of these antigenic markers in the tissues of both the donor and the recipient [2]. The degree of compatibility or disparity in HLA types between the donor

kidney and the recipient's immune system significantly influences the outcome of the transplantation graft [3, 4].

Traditionally, the fundamental concept of the donor-specific antibody (DSA) ability to fix complements was primarily utilized and was mainly enhanced by antibody subclasses within the IgG class, as IgG1 and IgG3 were powerful activators of the classical complement system. This process produces chemokines that attract inflammatory cells to the graft, opsonize donor endothelium cells, making them targets for neutrophils and macrophages, and promote membrane attack complex formation, which causes immediate lysis of antibody-coated cells [5, 6]. However, in certain circumstances, there is a group of individuals who have detectable DSA but no indication of inflammation or allograft injury on histological examination. They reflect some form of tolerance, and the explanation is yet unknown [7]. The ability to use DSA screening methods to detect the presence of preformed DSA and avoid transplantation when DSAs were present resulted in the eradication of hyperacute rejection and led to a considerable decrease in the prevalence of acute accelerated rejection [8]. Although it has been established that DSA could be present in preexisting or de novo antibodies, both have been associated with reduced kidney allograft survival and can lead to chronic antibody-mediated rejection (AbMR) of transplants [8, 9]. Additionally, in certain cases, de novo antibodies might be more immunogenic than pre-existing antibodies, as some subsets of de novo antibodies possess a high complement-finding affinity. The diagnosis of AbMR rejection is based on clinical history and a combination of histological and laboratory findings, and nowadays, it is well established that class I antibodies are correlated with early AbMR, whereas class II antibodies are linked to late AbMR and chronic rejection [10, 11]. Despite advancements in screening tools and treatment strategies to limit AbMR, such as immunoadsorption, approximately 33% of de novo DSA-positive transplant recipients continue to undergo AbMR in kidney transplant settings [12].

2. Insights into the Major Histocompatibility Complex and HLA Imputation: Enhancing Kidney Transplantation Understanding

The major histocompatibility complex (MHC) is a closely integrated set of genes found in all mammalian species. This complex serves as a fundamental element in the recognition and differentiation of cells as self or non-self. Class I MHC glycoproteins, which are encoded by the A, B, and C loci, are situated on the surface of nearly all nucleated cells. These glycoproteins comprise a 45 kDa polymorphic α chain and a 12 kDa constant B2-microglobulin, facilitating the display of intracellular peptide antigens to CD8+ cytotoxic T cells. In contrast, Class II MHC glycoproteins are expressed on antigen-presenting cells like dendritic cells, B-cells, and macrophages. Encoded by DP, DQ, and DR regions, they encompass a constant 33 kDa α chain and a 28 kDa polymorphic β chain for DR, and polymorphic α and β chains for DQ and DP. The Class II MHC glycoproteins play a vital role in presenting extracellular peptide antigens to CD4+ helper T cells [13].

HLA detection methods have been employed to identify and characterize a variety of diseases, including autoimmune diseases, infectious diseases, and transplant rejection. HLA was initially reported in the field of kidney transplantation, where serum from patients who had received a blood transfusion or were pregnant caused donor lymphocytes to agglutinate, resulting in a positive cytotoxic crossmatch between the recipient's serum and the donor's lymphocytes [14]. The polymorphic nature of HLA is believed to provide a population survival benefit due to the extensive

array of potential non-self-peptides that can be presented to T cells, generating robust immunological responses to infection. The polymorphism of HLA genes in the human population has led to the presence of a multitude of possible HLA alleles. This polymorphic characteristic makes finding an exact match between donors and recipients exceedingly challenging. Moreover, even when a perfect match cannot be achieved, the understanding of HLA compatibility has evolved beyond the concept of strict antigen matching [15].

Traditionally, multiple HLA loci play varying roles in transplant outcomes. HLA-A, -B, and -DR loci are crucial for HLA matching due to their high polymorphism [16]. Research studies demonstrate that increasing mismatches at these loci heightens sensitization and graft loss risks, particularly at HLA-DR. Although HLA-DQ is less polymorphic, it contains underestimated diversity and mismatches that increase the risk of de novo DSA formation and acute rejection. The current allocation prioritizes HLA-DR matching, potentially disadvantaging non-Caucasian candidates [17]. Although several studies suggest that assessing whole antigen-level mismatches may not precisely reflect the degree of the mismatch, and specific loci mismatching is associated with a higher risk of sensitization, currently, HLA-DP/DQ and HLA-C mismatches are not factored into the allocation process, emphasizing the need for precise HLA matching [18].

Current research on HLA molecules has shifted towards the biological domain, focusing particularly on the tertiary structure of HLA and its delineated epitopes. These epitopes are crucial segments of antigens that intricately bind to the B-cell receptor, intensifying immune recognition [2]. Intriguingly, within these epitopes lies a smaller unit known as the "functional epitope," composed of 2–5 amino acid residues with the potential to trigger antibody production. These functional epitopes are now acknowledged as eplets [19]. A deeper consideration of eplet configuration has revealed that the "triplets" within a protein sequence may not follow a continuous pattern, but they cluster closely (within 3 Angstroms) on the protein's three-dimensional structure, forming an antigenic surface. While the eplet determines antibody specificity, the larger structural epitope delineates its binding characteristics, illuminating the varying reactivity of an antibody towards HLA molecules sharing the same functional epitope [20]. Intriguingly, exposure to a foreign HLA molecule can generate antibodies recognizing multiple other HLA molecules sharing the same functional epitopes. Consequently, there's a growing curiosity about discerning if HLA mismatches at the eplet or epitope level offer a more accurate representation of the actual dissimilarity between a donor and recipient [21].

HLA-Matchmaker is a unique tool that was initially designed to identify eplet mismatches using linear sequences of three amino acid residues on exposed parts of class I HLA molecules. By comparing eplet repertoires in donors and recipients, specific mismatches can be identified, aiding in calculating eplet mismatches for each HLA molecule or locus [19]. A recent research study using eplet mismatches found that kidneys mismatched at HLA-A or B but compatible at the triplet level had comparable graft survival rates as kidneys that were fully matched at the A and B loci, suggesting that triplet compatibility within the class I alleles could be used as a better prognostic marker of graft outcome than traditional whole antigen mismatch criteria [22]. To help determine optimal immunosuppressive strategies, researchers suggested utilizing an "eplet load" threshold to stratify individuals based on eplet mismatches. However, it's important to note that not all mismatches are equally significant, and certain highly immunogenic epitope mismatches hold greater importance than a higher eplet mismatch load [23]. The ultimate objective is to predict

mismatches that the immune system may accept versus those that should be avoided due to provocation.

3. Cell-Based HLA Typing Methods: Tracing Their Evolution and Impact in Kidney Transplantation

HLA typing methods and DSA screening tools have enabled significant progress in kidney transplantation, including the ability to define the relative expression and existence of anti-HLA antibodies in the recipient's blood, either directly or by tracking the potential consequences of binding. Serological techniques, such as ELISA and Micro cytotoxicity assays have been the most established and long-used procedures in HLA typing as they involve a relatively simple approach that manipulates cell typing samples with a series of laboratory tests that reveal the expressed antigens within the sample; this allows clinicians to check for antigens in the sample, in conjunction with anti-HLA antibody data, to determine donor eligibility for a given recipient [24].

The complement-dependent cytotoxicity crossmatch (CDC) represents one of the classical standard tests in the HLA laboratory. The procedure involves collecting donor lymphocytes from blood or lymphoid tissue, incubating them with recipient serum and rabbit complement, and then adding a viability stain to differentiate between living and dead cells. This approach determines if an antibody-antigen interaction is formed on the cell surface, which activates complement, as well as the degree of cell death using cell dyes [25]. In fact, CDC crossmatch is very affordable and requires only a centrifuge and fluorescence microscope and has been widely considered as the gold standard method for HLA typing; however, the test has many drawbacks, as it is very subjective and greatly varies according to the technical capabilities of the laboratory worker. Additionally, it is based mostly on complement binding strength and reliant on donor cell survival and viability, which are not always achievable in the case of deceased donors. Furthermore, because CDC crossmatch is not IgG-specific and can detect IgM antibodies, and most recipient autoantibodies are IgM, the risk of a false positive result is significantly high. Because the test can only detect complement fixing DSA, it can produce a false negative result in cases of serum complement inhibition [26]. Serial modifications have been made over time to improve sensitivity, such as incubation for longer periods of time, the use of anti-human globulin as a secondary antibody, and the use of DTT treatment to disperse RBCs agglutination caused by heavy IgM autoantibody coating. Despite these improvements, the false positive rate is still up to 20%, and the test still has a few technical limitations [24, 27]. The number of laboratories using CDC crossmatch is decreasing every year, as they need to perform another method of HLA detection to confirm the results and offset the drawbacks, but this requires equipment upkeep and staff competency.

Flow cytometry utilization for HLA antibody identification and crossmatching has altered perceptions and long-term transplant outcomes in general, particularly when a negative donor CDC crossmatch exists [24, 27]. In the flow crossmatch, donor cells are incubated with recipient serum, but instead of complement, a fluorochrome-labeled anti-human IgG is added, which will bind to corresponding antibodies bound on the donor cell surface. In addition, fluorescently tagged antibodies specific to B and T lymphocytes are added to the donor cells. Utilizing a flow cytometer, laser excitation identifies the lymphocytes, and the presence of the detector antibody on the cell surface correlates with the number of bound antibodies [7].

The cut-off value for a positive flow crossmatch is extremely variable, as HLA antigens distribution are commonly defined according to the normal population that does not have known

anti-HLA antibodies, and a positive test is defined if the mean fluorescent intensity (MFI) of the serum of the tested patient is 2 to 3 SD stronger than the MFI with negative controls. As a result, a positive flow crossmatch is established statistically, however, it may not necessarily be physiologically relevant in all cases. Similarly, the false negative rate of the test might be significantly higher when testing rare antigens with low densities. In fact, the low MFI might not be reflecting the absence of the antigen; but rather might be low due to the rarity of the antigen [24]. Although flow cross-matching has a higher detection sensitivity than the CDC and may identify non-complement-binding antibodies, it may exclude matched individuals who are not necessary and may be overly sensitive in some cases. In the majority of cases, a positive crossmatch confirms DSA binding to the donor cells [28], which is not always the case, as false positive flow cytometry results can be caused by non-HLA antibodies, which would not necessarily contraindicate a transplant. On the other hand, there are several reasons for false negative outcomes, such as the presence of low-quality donor material, HLA downregulation by drugs like statins or steroids, and weak anti-HLA antibodies that are not strong enough to elicit a positive flow cytometry. Furthermore, DSA specificity cannot be determined using this technique, as donor cells typically express more than one HLA [7].

Nonetheless, cell-based HLA typing tests continue to be important tools for understanding the complexities of the immune system and optimizing transplant outcomes. Technological improvements are constantly addressing their technical limitations, promising increased accuracy, resolution, and efficiency in HLA typing, boosting their value in a variety of clinical and research applications. These tests are becoming faster and more effective as a result of innovative laboratory methods and automation. Furthermore, advancements in imaging and data analysis have improved the resolution and precision of cell-based HLA typing, allowing for more complete and comprehensive HLA compatibility evaluations.

4. Luminex Bead-Based Assay: A Robust Advancement in Kidney Transplantation Field

Over the last decades, the Luminex system has been considered one of the most widely used methods for DSA detection and HLA typing. It is primarily based on the affixation of HLA onto polystyrene microbeads, which are loaded with a unique mixture of two fluorescent dyes that are simultaneously activated by a red laser at 635 nm. A specialized flow cytometer can detect emitted light at wavelengths of 660 nm (red) and 730 nm (infrared). Up to 100 different beads with unique HLA antigens can be recognized simultaneously by monitoring the composition of the emission intensities for both channels. The binding is then detected by a PE-conjugated secondary antibody specific for human IgG [29]. The combination of the fluorescence signals from each bead indicates HLA specificity, and the secondary reagent will indicate bound HLA-specific antibodies; both signals are acquired by the Luminex system using appropriate lasers and detectors [30]. This semi-quantitative test identifies the presence, the relative strength, and the specificity of HLA antibodies. Therefore, in solid-phase technology, the HLA source is made up of manufactured beads coated with multiple HLA class I or II antigens (phenotype beads or PRA beads), or a single HLA antigen single antigen bead (SAB) that does not rely on live cells [24, 30].

Several advantages of the Luminex system have been demonstrated in the field of organ transplantation, including its higher sensitivity and specificity, as well as its capacity to detect a broad range of HLA antibodies, non-HLA antibodies, and immunological complexes.

Notwithstanding the relevance of the Luminex technology in HLA typing and crossmatching, there are still significant limitations that must be considered when analyzing the data [7]. Precision and clinical relevance are two of the most critical challenges in HLA typing because laboratory results are based on serotyping, which employs indirect evidence of an antigen's presence to identify its existence and has a limited ability to detect low-level antigens, which might lead to false results. Underestimation of HLA antibodies detected by Luminex technology could be secondary to the presence of prozone-like phenomena. The serum donor-specific antibodies may appear negative, making the relationship between Luminex beads and the Prozone-like phenomenon appear unclear [31]. But several procedures can be used to overcome the prozone effect, such as the addition of EDTA, heat inactivation, the addition of dithiothreitol, and dilutions [7]. More importantly, the prevalence of cross-reactive antibodies, which typically appear as a result of disruption of the HLA molecule's tertiary structure following impingement on Luminex beads, reveals some of the hidden cryptic epitopes, which can result in the identification of nonspecific antibodies and the generation of diverse responses against non-HLA antigens [24]. On the other hand, Luminex results are primarily presented as qualitative MFI measurements; these measurements cannot be converted into quantitative antibody titers and are only presented as a correlation. They also cannot be defined against a single specific antigen or group of antigens from a large, pooled panel of antigens. Furthermore, MFI measurement is influenced by a variety of parameters, such as antibody orientation, density, and concentration. Nevertheless, determining a universal MFI cutoff value across various institutions and laboratories is almost impossible [32].

5. Genetic Analysis: "Exploring Future Horizons in Non-Cell-Based HLA Typing Methods"

The non-cell-based HLA typing comprises techniques used to determine HLA profiles without the need for live cell samples. Clinical HLA laboratories utilize genotypic techniques, such as next-generation sequencing (NGS), to provide HLA typing for patients undergoing solid organ transplantation. These approaches depend on obtaining DNA from a potential donor and Polymerase Chain Reaction (PCR)-based techniques to identify sequence variants in antigen-coding areas [25]. Various PCR-based approaches, like sequence-specific primer PCR (SSP-PCR), sequence-specific oligonucleotide PCR (SSO-PCR), and sequence-based typing (SBT), are commonly used for high-resolution HLA typing. SBT provides detailed allele-level typing by directly sequencing the HLA genes [30]. Additionally, Sequence-Specific Oligonucleotide Probing (SSOP) is another widely utilized non-cell-based method that involves hybridizing labeled oligonucleotide probes to specific HLA alleles, allowing for the identification of alleles based on the hybridization pattern. As a result, clinicians can gain a more comprehensive and accurate understanding of the donor's antigenic profile and reducing the risk of mismatched transplants [31].

Recently, a clinical study focused on living kidney transplant donors and recipients explored the application of NGS for genomic HLA typing, comparing it to traditional pre-transplant serological or low-resolution HLA typing (referred to as one-field typing). The study utilized NGS-derived two-field HLA typing data to reclassify HLA antibodies as "donor-specific." The findings revealed that the two-field HLA re-typing approach identified additional HLA mismatches (MM) in a significant proportion (64.1%) of cases for crucial HLA loci (-A, -B, -C, -DRB1, and -DQB1) that were missed by the one-field typing. Particularly in patients with biopsy-confirmed AbMR, the two-field HLA typing demonstrated a notably higher MM count compared to the one-field method. This suggests that two-field HLA

typing is both viable and substantially enhances the sensitivity of HLA MM detection in living-donor kidney transplants. Furthermore, the study emphasized the pivotal role of accurate HLA typing in graft management, aiding in distinguishing between donor and non-donor HLA-directed alloreactivity over the long term. The inclusion of additional HLA loci, such as HLA-DRB345, -DQA1, -DQB1, -DPA1, and -DPB1, enables a more precise virtual crossmatch and improved prediction of potential DSA. The study also emphasized the potential utilization of two-field HLA typing to help identify the most suitable donors for living kidney transplantation. However, the study's limitations, such as its single-center nature and modest cohort size, and indirect factors affecting HLA matching, such as variations in cold ischemia time and immunosuppressant therapies, can influence the multifaceted nature of success in kidney transplantation. Thus, while HLA matching remains a critical aspect, it should be considered as one of the diverse contributors to successful living kidney transplantation [33].

6. Virtual Cross Match in Kidney Transplantation: Enhancing Donor-Recipient Compatibility Assessment

Virtual crossmatching (vXM) is an innovative method for histocompatibility risk assessment that has gained increased recognition in recent years, especially in emergency-related cases. It relies on utilizing an in silico immunologic compatibility assessment tool that combines a detailed recipient alloantibody profile defined by Luminex with molecular HLA typing of the donor's HLA [34]. This eliminates the need for in vitro cross-matching and enables physicians to determine the most suitable donor more quickly and with greater certainty without the necessity for on-site sample presence. vXM is a very specific method of evaluating the intensity and existence of HLA antibodies against a potential donor. Many advantages have been linked to vXM, including an increased ability to detect donor-specific HLA antibodies, increased access for sensitized patients to appropriate organs, and significantly reduced cold ischemia time while eliminating the complexity of in vitro laboratory crossmatching [35]. Studies have shown that virtual crossmatching can produce outcomes with the same degree of accuracy as physical crossmatching, enabling better organ allocation and increasing allograft survival, which can lessen the need for in vitro laboratory crossmatching in the future and make it retroactive [36].

Together with the development of the new techniques used for antibody identification evaluation, particularly through solid-phase single antigen bead (SAB) testing and more in-depth, high-resolution donor typing, the accuracy and predictability of the vXM have increased. Despite the fact that the percentage of deceased donor kidney offers declined due to positive vXM has dropped to nearly 0.5%, many reasons can cause vXM outcomes to be inconsistent; this can lead to false negative results, including incomplete HLA typing of the donor, lack of a standard threshold level for a positive MFI, SAB manufacturing challenges using non-identical kit batches, and the presence of an HLA antibody against a very rare allele not covered by the beads [37]. Additionally, false positive vXM results could be secondary to using medications like high-dose IVIG, anti-thymocyte globulin, and anti-CD20, as well as the presence of non-HLA antibodies binding to HLA alleles on the bead due to the denatured, non-native conformation of HLA peptides (exposing cryptic epitopes not accessible in vivo) [38]. However, some of these problems can be resolved by using a pretreatment reagent that absorbs non-specific antibodies and utilizing acid to set apart antibodies against denatured class I HLA [39].

7. Conclusion

Kidney transplantation greatly enhances the quality of life and survival for individuals with severe chronic kidney disease compared to dialysis. However, the shortage of available organs and the need to avoid clinically insignificant alloantibodies limit the pool of compatible donors. Current HLA typing and crossmatching, which rely on traditional cell-based assays, serve as the standard point of care globally. Non-cell-based HLA typing approaches have significantly advanced our comprehension of HLA diversity, positively impacting transplantation outcomes and disease association studies. However, interpreting the complex genetic data generated by these methods is not always easy. Moreover, challenges such as equipment costs, integration with existing systems, and technical complexities pose significant obstacles to the widespread adoption of non-cell-based HLA typing in clinical laboratories. Hopefully, continuous research and technology improvements will increase the accessibility of these tools, reinforcing their vital place in medical and scientific endeavors. Finally, this will help to reduce wait times and the risk of death for transplant candidates.

Author Contributions

All authors have contributed equally.

Competing Interests

The authors declare no conflict of interest.

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