

DIAGNOSTIC METHODS IN THE DETERMINATION OF IMMUNOLOGICAL RISK IN A PATIENT BEFORE KIDNEY TRANSPLANTATION

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Abstract

Kidney transplantation is the treatment of choice in patients with an end-stage kidney disease (ESKD). Before kidney transplantation, it is important to assess the patient's immunological risk, which has an impact on graft survival after kidney transplantation. Human leukocyte antigens (HLA), also known as major histocompatibility complex (MHC) are responsible for the rejection of genetically different tissue by recognizing and distinguishing foreign proteins from self-proteins. HLA-typing and anti-HLA antibody screening are crucial to determine the donor/recipient mismatching. Diagnostic methods used for anti-HLA antibody detection and immunological risk assessment are complement-dependent cytotoxicity (CDC), flow cytometry crossmatch (FCXM), bead-based assays (Luminex technology), calculated panel-reactive antibody (cPRA), and algorithm "predicted *Indirectly Recognizable HLA Epitopes*" (PIRCHE). The aim of these diagnostic tests is to minimize HLA mismatch between the donor and the recipient and prevent an early graft failure by the presence of HLA donor-specific antibodies (DSA).

Key words: kidney transplantation, immunological risk, acute rejection

INTRODUCTION

The HLA system is located on the short arm of chromosome 6 and consists of three regions designed as class I (HLA-A, HLA-B, HLA-C), class II (HLA-DR, HLA-DP, HLA-DQ), and class III (complement system and tumor necrosis factor family genes) (1). As of March 2024, the current HLA allele database reports 39 886 classical HLA class I and class II alleles, encoding over 25 430 unique HLA proteins (2). Kidney transplant recipients and donors must undergo HLA typing to determine the donor/recipient mismatching/immunocompatibility. HLA typing can be performed by DNA-based molecular methods, such as sequence-specific primer polymerase chain reaction (SSP-PCR), Sanger-based sequencing, next-generation sequencing (NGS), and real-time polymerase chain reaction (RT-PCR). The most widely used method is RT-PCR-based HLA. It is based upon the use of sequence-specific primers, in which if the specific allele is present, DNA extracted from a blood sample is amplified by PCR and detected in real-time by fluorescent dyes or probes (3). The development of anti-HLA donor-specific antibodies (DSAs) directed against HLA molecules of the donor is a leading cause of allograft rejection after kidney transplantation (3, 4). Nearly one-third of patients on the waiting list have anti-HLA antibodies, as a result of sensitization. These antibodies may appear after previous transplantation, blood transfusions, or pregnancies. Patients with anti-HLA antibodies are disadvantaged in finding a suitable donor and are at higher immunological risk requiring intensive post-transplant monitoring and

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effective immunosuppression therapy. The aim of the antibody tests against HLA proteins is to detect pre-existing DSAs which are associated with a higher risk of rejection and graft loss. The history of anti-HLA antibody detection dates back to the 1950s when cell-based cytotoxicity assays began to be used. In the early 2000s, Luminex screening was introduced and showed higher sensitivity and specificity compared to cell-based cytotoxicity assays. Over the past 15 years, cPRA and PIRCHE-II methods have been included in the diagnostic process for determining immunological risk in a patient before kidney transplantation (5).

Panel reactive antibody testing

Complement-dependent cytotoxicity is a type of cell-based assay that identifies preformed complement-fixing antibodies. The serum of the recipient is mixed with lymphocytes from an HLA-typed panel (presenting donor cells frequently found in the donor population) and the complement is sequentially added with a viability dye. Antibody present in patients' serum allows the formation of immune complexes by binding to the donor cells and activating the complement cascade which results in cell lysis. In a positive reaction, a viability dye enters the lysing cell and binds DNA whereas in a negative reaction is normally excluded from live cells. The reactivity is assessed as panel reactive antigen (PRA) value, which is defined by the percentage of cells from an HLA-typed panel causing a positive reaction. CDC assay may display a false positive reaction which is caused by the presence of non-HLA antibodies or autoantibodies, such as complement-activating IgG and IgM antibodies. To reduce the risk of a false positive reaction, the dithiothreitol (DTT) is added to eliminate the presence of the IgM antibodies. In contrast, the false negative reaction may be seen in the case of a low titer antibody. As a major limitation, it is difficult to define the specificity of the anti-HLA antibody, especially for highly immunized individuals, also CDC assay results may be interfered by cytolytic or pre/posttransplantation therapy. Furthermore, the detection of anti-HLA antibody class II is complicated by the localization of both class I and class II on the surface of B-lymphocytes. Nevertheless, the advantage of the CDC assay is reflecting the situation *in vivo* since HLA antigens are naturally present on the cell membrane and a positive CDC assay predicts a higher risk of hyperacute or acute rejection and is still considered as a contraindication to transplantation in many transplant centers (3, 6, 7).

Solid-Phase Assays (LUMINEX)

Solid-phase assays are diagnostic methods with higher specificity and sensitivity using purified HLA molecules. Soluble HLA antigens are affixed to microparticle beads which are analyzed via flow cytometry-based platform – Luminex.

Using Luminex technology, fluorescent anti-human globulin is added to the patient serum and illuminates when it binds to anti-HLA antibodies. A screening test (consisting of color-coded microbeads coated with purified HLA Class I, Class II, and MICA (non-HLA) antigens) is provided by xMAP™ technology (Luminex) with a high sensitivity of antibody detection. In case of a positive screen result, a single antigen identification can be followed to determine the precise specificity of DSA by a single-antigen bead assay. The Luminex technology provides a high-throughput sample analysis and is essential in risk stratifying patients based on assessment of detected DSAs and cPRA. The amount and strength of a specific antibody can be expressed in terms of median fluorescence intensity (MFI) but the threshold above which an antibody is classified as positive is not standardized, as each HLA laboratory uses different MFI cut-offs. It is important to note that the MFI value is not equal to a concentration or titer of a specific antibody and pre-transplant DSA values are not constantly predictive of graft outcomes. MICA antibodies (anti-major histocompatibility complex class I-related chain A) present before kidney transplantation are also associated with a worse prognosis of graft survival in the posttransplant period (5).

The calculated panel reactive antibody (cPRA)

The calculated PRA (cPRA) estimates the percentage of deceased donors with unacceptable antigens with whom a transplant recipient may be incompatible. cPRA involves the use of single antigen beads assay results (reflecting the specific anti-HLA antibodies) and HLA antigens frequencies of the donor population (5, 8). As an example, according to the Canadian cPRA Calculator which uses Canadian Transplant Registry (CTR), anti-HLA-A2 antibody is present in 46 percent of the deceased donors, which means that the chances of finding a compatible donor for patients with antibodies to common HLA antigens (such as A2) may be approximately 54 percent based on the donor pool. If the cPRA is ≥ 80 percent, the patient has antibodies against many HLA antigens and is considered a highly sensitized patient with 80 percent incompatible donors (10).

Crossmatch testing in kidney transplantation

The aim of the crossmatch testing is to detect any anti-HLA DSA in the recipient's serum that is targeted against a particular donor's HLA alleles expressed on the surface of T and B lymphocytes. The diagnostic methods performed before transplantation are CDC crossmatch and more sensitive flow crossmatch and virtual crossmatch (5).

Complement-dependent cytotoxic crossmatch (CDC crossmatch, CDC-XM)

For a better understanding of crossmatch tests, it is important to know that T-cells express HLA class I molecules and B-cells express both HLA class I and HLA class II molecules, but usually B-cells are associated with HLA class II. A positive T-cell crossmatch result confirms the presence of HLA class I DSAs. A positive B-cell crossmatch result usually means the presence of HLA class II DSAs. CDC crossmatch consists of the use of the donor lymphocytes, the recipient's serum and complement. The donor lymphocytes are isolated from the spleen, lymph node, or peripheral blood and then incubated with the recipient's serum and complement. If DSA is present, cell lysis occurs, and CDC crossmatch is considered positive. The addition of anti-human globulin (AHG) to CDC-XM increases the sensitivity compared to standard CDC-XM. Crossmatch test may have a false positive result if the non-HLA IgG (or IgM) antibody is present because it is targeted against antigens localized on lymphocytes (5).

Flow cytometry crossmatch (FCXM)

Unlike CDC crossmatch, FCXM is independent of complement activation and is considered to be a more sensitive method. In FCXM, the donor lymphocytes are mixed with the recipient's serum and fluorescent-labeled reagents. Donor-specific anti-HLA antibodies bind to the HLA antigens presented on lymphocytes. This complex /antigen-antibody/ allows a fluorescein-labeled

anti-human IgG antibody to bind and cause positive FCXM result due to detection by flow cytometry. The result is expressed by MFI value (5, 7, 15).

Virtual crossmatch

The purpose of virtual crossmatch is to evaluate two tests' results (anti-HLA screening and HLA typing of the donor) and estimate the result of actual crossmatch (if performed). Using virtual crossmatch, it is possible to define immunocompatibility between the donor and the recipient and consider all the detected DSAs with lower MFI values measured by Luminex. Virtual crossmatch is extremely useful and beneficial over FCXM and CDC. The sensitivity of FCXM is lower compared to Luminex and CDC sensitivity and specificity is inferior to Luminex as well (11, 12).

The possible crossmatch test results

There are situations where CDC crossmatch results may not correlate with the FCXM and any discrepancy between the assays should be discussed with the HLA laboratory. In many

HLA laboratories worldwide, a positive CDC crossmatch due to the presence of DSAs is still considered a contraindication to transplantation because of the higher risk of hyperacute graft rejection.

A negative CDC crossmatch and a positive FCXM result represent an intermediate risk of developing an antibody-mediated rejection (ABMR). Although it is not an absolute contraindication to transplantation, a positive FCXM result is associated with a higher risk of an acute graft rejection.

The combination of a negative CDC crossmatch, negative FCXM, and the presence of DSAs in a single-antigen bead assay (virtual crossmatch) can be confusing and its impact on graft survival may be uncertain. However, the presence of DSAs relates to prior exposure to the donor-specific antigen which may subsequently lead to a latent memory response (5).

PIRCHE-II (Predicted Indirectly ReCognizable HLA Epitopes)

PIRCHE-II is an algorithm that can predict alloimmune reactivity to HLA mismatches and estimate a patient's immunological risk after kidney transplantation. In addition to the fact that the number of detected HLA alleles is steadily increasing, a specific amino acid sequence has also been extensively identified. Epitopes are described as parts of HLA molecules that can cause an alloreactive response in case of a mismatch between the recipient and the donor. With this finding, epitope-based HLA matching is rather used than counting the HLA mismatches. Eplets are described as small amino acid polymorphisms localized on the external domain of the HLA molecule. If the eplets are different between the recipient and the donor, they are seen as an alloreactive target. According to research by Geneugelijk from 2018, patients with a high number of mismatch eplets at the time of transplantation, are at higher risk of developing DSA after kidney transplantation. Mismatched HLA-derived peptides are identified by CD4⁺ T-cells through an indirect pathway but only those that can bind to HLA class II molecules. The higher the PIRCHE-II value, the higher the possibility of CD4⁺ T-cell alloreactivity to donor-derived HLA peptides. According to multivariate analyses, the PIRCHE-II algorithm can predict DSA formation. The probability for DSA formation was higher at higher PIRCHE-II numbers, predominantly for HLA-DRB1 and HLA-DQ (3, 13, 14).

CASE REPORT

We present a case report of a 33-year-old female patient with a chronic kidney disease KDIGO G5d due to lupus nephritis type IV who required regular hemodialysis treatment from October 2021. 21 months later, in July 2022, there was a patient diagnosed with a brain death (standard criteria donor) who was being examined as a potential kidney donor for our patient. At the time of the kidney allocation, donor-specific antibody (DSA) anti-A2 HLA class I was detected by the Luminex method with 12 000 MFI, cPRA before the transplantation was 67,56%. Before the kidney transplantation a crossmatch test was performed by flow cytometry (FCXM) with a negative result. Since FCXM was negative, we proceeded with the transplantation despite the presence of DSA, but with an extension of the induction immunosuppressive protocol. According to the patient's immunological risk assessment, we chose an induction immunosuppressive protocol consisting of Thymoglobuline (3,5 mg/kg cumulative) and an anti-CD20 monoclonal antibody Rituximab (1000 mg) which targets CD20-expressing B cells on their surface. Rituximab was administered on day 4 after the kidney transplantation. Maintenance immunosuppressive therapy consisted of a calcineurin inhibitor (tacrolimus), antimetabolite (mycophenolate), and glucocorticoid (prednisone). During the first week after the kidney transplantation there was a significant decrease in serum creatinine. Two weeks later, there was an increase in serum creatinine with an overall worsening of the patient's clinical condition. Based on the results and general condition, the patient was admitted to the Transplant-Nephrology Department for a

suspected acute humoral graft rejection. By the Luminex method, de novo DSA HLA class II (DQB1*06:03) was detected with 21 864 MFI, DSA anti-A2 HLA class I (present at the time of the transplantation) was in significant decrease with 400 MFI. The PIRCHE algorithm also confirmed a high immunological risk of initial de novo DSA formation against donor HLA class I and II antigens; the PIRCHE score of de novo DSA DQB1*06:03 was 16 (the cutoff value at which potential de novo DSA formation can be predicted is more than 15). Based on the result, the patient was indicated for complex antirejection therapy consisting of plasmapheresis, immunoadsorption, intravenous immunoglobulins, and intravenous corticosteroids. After completion of the treatment, a control Luminex testing showed a decrease in MFI of DSA (DQB1*06:03) to 11508, and also decrease in serum creatinine was present. cPRA after the transplantation was 23.89 %. Despite the completion of the therapy and the decrease in MFI of the DSA, the patient's general condition did not improve. The patient's symptoms included general weakness, fatigue, headache, abdominal pain, diarrhea, muscle cramps, tremor in the arms and hands, and loss of diuresis. During the hospitalization, the patient also underwent a graft biopsy, the result of which was without the presence of rejection changes (without acute antibody-mediated rejection/cellular rejection). Three weeks later, the patient was hospitalized for dyspeptic syndrome and examined to determine the cause of the complaints. We hypothesized calcineurin toxicity as one of the possible causes that may be manifesting these symptoms and therefore decided to change the immunosuppressive therapy (calcineurin inhibitors for mTOR inhibitors), even though the tacrolimus concentration was not much above the therapeutic levels. After this change in immunosuppression there was a significant improvement in the patient's clinical condition as well as in renal parameters.

CONCLUSIONS

Kidney transplantation is the best therapeutic way to replace kidney function in patients with ESKD. Before the procedure, both the donor and the recipient must undergo HLA typing, which is important to determine the immunocompatibility of the pair. Patients awaiting kidney transplantation may be disadvantaged by the presence of anti-HLA antibodies that have developed as a result of sensitization, e.g. following a blood transfusion, pregnancy, or previous transplantation. The sensitization of a recipient is evaluated by cPRA, if the cPRA > 20% the patient is at increased immunological risk with the need for more intensive monitoring and effective immunosuppression therapy. A crossmatch test is performed just before kidney transplantation to detect the presence of antibodies specific to a particular donor. A positive result of CDC crossmatch is still considered a contraindication to transplantation in many HLA laboratories worldwide. A virtual crossmatch test represents a newer diagnostic method with higher sensitivity and specificity than FCXM or CDC. Advances in kidney transplantation and transplant immunology also include the PIRCHE algorithm, which can predict the production of DSA and thus assess a patient's immunological risk after kidney transplantation.

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