

CHAPTER 1.6 Transplantation immunology work-up

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1 Introduction

The registration of a patient on the waiting list for a deceased donor kidney transplantation requires certain immunological diagnostic tests. The immunological work-up requirements for transplant recipients of kidneys allocated by Eurotransplant (ET) are described in the ET Manual. The recommendations released by ET must be implemented after being approved and published in guidelines by the respective national authorities (e.g. the German Medical Association “BÄK” in Germany). As all European tissue typing laboratories providing histocompatibility data in this framework must be accredited by the European Federation for Immunogenetics (EFI), EFI Standards also apply. This chapter summarises the pre-transplant immunological work-up of a recipient for deceased donor kidney transplantation, mainly based on the latest valid versions of the ET Manual, Chapter Histocompatibility [1], the BÄK Guideline for Organ Recipient Safety [2] and the EFI Standards, Section Renal and/or Pancreas Transplantation [3]. Other relevant literature is also considered (see references).

Four key pre-transplant immunological tests are required for a recipient to be placed on the waiting list:

- ABO blood group (section 2)
- Human leukocyte antigen (HLA) typing (section 3)
- HLA antibody screening (section 4)
- Autologous crossmatch (section 5)

Other serological tests (e.g., detection of non-HLA antibodies such as anti-endothelial cell antibodies [4], MICA antibodies [5] or the measurement of soluble CD30 levels [6]) are not widely used in routine practice.

Based on the results of HLA antibody screening, unacceptable antigens are defined and reported to ET (section 6). If a donor is available, potential

recipients are selected by virtual crossmatching (section 7). For recipients who receive a kidney offer, an additional physical crossmatch is performed (section 8).

2 ABO Blood Group Determination

Kidney transplants from deceased donors with ABO-incompatibility are not permitted. The recipient's blood group must be determined and verified in two independent blood samples before being placed on the waiting list. Recipient and donor blood groups must be confirmed immediately before transplantation.

3 Human Leukocyte Antigen (HLA) Typing

For the registration with ET, each recipient must be typed for at least HLA-A, -B, -C, -DRB1 and -DQB1 (in Germany, also HLA-DPB1) using DNA-based typing techniques. Preferably, HLA-DRB3/4/5, -DQA1 and -DPA1 should also be typed (a total of 11 HLA loci), as the potential donor is usually typed at 11 HLA loci for allocation, and the commercially available antibody kits are able to detect antibodies against all these loci. Typing resolution is usually one-field, but for certain HLA subgroups or patients with allele-specific antibodies, two-field unambiguous typing for the respective HLA locus should be obtained. HLA-Bw4/Bw6 are reported on the basis of the HLA-B antigens. HLA typing of the recipient should be performed on two different blood samples. The HLA typing data are translated into matching determinants by ET algorithms for allocation.

4 HLA Antibody Screening

4.1 Screening schedule

For wait-listing, the patient must be tested for HLA-specific antibodies. While on the waiting list, the patient must be screened for HLA antibodies every three months. The frequency of the repeated HLA antibody screening is necessary to avoid the use of an outdated serum for crossmatching at the time of organ offer. According to the ET Manual [1], a serum is outdated if it is

older than 180 days from the date of blood drawing. In Germany, however, the national guidelines [2] consider a serum to be outdated if it is more than 150 days old.

The responsible physicians (dialysis centres, transplant centres) must inform their HLA laboratories about previous immunising events (transfusions, pregnancies, previous transplants) or graft removal. This information is important not only for the plausibility check of the test results, but also for the immunological risk stratification based on which detected HLA antibodies are evaluated for reporting as unacceptable antigens (see section 6). HLA antibody screening should be performed after each immunising event or graft explantation (in addition to regular screening). In these cases, ET recommends a repeat antibody screening two and four weeks after the event.

4.2 Antibody testing methods

Regarding the method used for HLA antibody screening, ET requires different applications depending on the time point and the sensitisation status of the patient. The degree of sensitisation is determined using virtual panel-reactive antibodies (vPRA) (see section 6).

- The complement-dependent cytotoxicity (CDC) test, also known as the lymphocytotoxicity test (LCT), is used to detect complement-fixing antibodies, which are known to correlate with hyperacute/acute rejection. A CDC-PRA of > 5% is considered positive. HLA specificities to which the patient has preformed CDC-positive IgG alloantibodies must therefore be reported as unacceptable antigens. The addition of dithiothreitol (DTT), which disrupts the disulfide bonds of IgM, may help to recognise an IgM-related positive test result. IgM antibodies may be transplant-irrelevant antibodies (e.g. IgM autoantibodies) or IgM HLA alloantibodies. The impact of pre-transplant IgM HLA alloantibodies on graft rejection and failure is controversial [7, 8]. CDC antibody screening must be performed for each new patient on the waiting list. If the patient is classified as immunised (vPRA > 0%), CDC antibody screening must be repeated at least annually. For non-immunised patients (vPRA = 0%), annual CDC screening is not required by ET. In Germany, CDC antibody screening is usually performed at least once a year for all patients on the waiting list for a kidney and pancreas transplantation.

- Solid phase assays (e.g. bead microarray assays on a Luminex platform) show higher sensitivity and specificity than CDC tests [9]. The commercial test formats offer methods for detection (screening for the presence or absence) and identification (differentiation of the specificities) of HLA antibodies. Currently, the Luminex Single Antigen Bead assays are the most sensitive methods for the detection of HLA class I and class II antibodies. All sera used for solid phase assays must be pre-treated by EDTA, heat inactivation, DTT, or dilution to avoid complement interference/prozone effect. HLA antibody screening with solid phase assays is required every three months. In Germany, it is recommended that at least all patients with positive screening results at the time of registration and at annual follow-up be tested with the Single Antigen Beads assay. In case of changes in antibody profile/signal strength or implausibility of the results, additional tests should be performed. New antibody testing is required in all patients after a sensitising event (see section 4.1).

5 Autologous Crossmatch

Autoantibodies may cause false positive results in CDC antibody screening and/or crossmatching. Therefore, the detection/exclusion of autoantibodies must be performed for each patient by autologous CDC crossmatch (incubation of the patient's lymphocytes with his/her own serum) with and without DTT, usually prior to wait-listing. Autologous cross-matching is also useful during the waiting period for patients who show reactivity in CDC antibody screening tests (often without accompanying specific HLA antibodies detected in the solid phase assays). In patients known to have IgM autoantibodies, the allogeneic CDC crossmatch (incubation of donor lymphocytes with patient serum) with and without DTT must be performed at the time of a kidney offer.

6 Unacceptable HLA Antigens

If HLA antibodies are detected, they will be evaluated whether they should be reported to ET as "unacceptable HLA antigens". Unacceptable HLA antigens are prohibited donor HLA mismatches (see section 7). The assignment of an HLA antibody specificity as an unacceptable HLA antigen is centre- and patient-dependent. Therefore, the criteria for defining unacceptable antigens

must be discussed between the transplant centre and the affiliated HLA laboratory.

After entering the unacceptable antigens in the ET software ENISNext, the virtual PRA (vPRA) value is automatically calculated, indicating the frequency of the unacceptable HLA antigens in the ET donor pool. A vPRA of $> 0\%$ indicates that a patient is sensitised. In addition, based on the unacceptable antigens and ABO blood group of a patient, the donor frequency calculator in ENISNext can also calculate the likelihood of receiving an offer for an ABO identical or compatible kidney transplant.

There are some general considerations published by ET [1] and the German Society for Immunogenetics [10] regarding the determination of unacceptable HLA antigens. All HLA antigens to which antibodies are found in the CDC screen must be reported as unacceptable antigens. Often antibodies are found in the solid phase assays, but not in the CDC test. Therefore, a careful plausibility check of these antibody reactions in the solid phase assays (including non-specific response patterns due to antibodies against denatured antigens or “natural antibodies”) and individually adapted stratification of immunological risks (taking into account the patient’s history of alloimmunisation) must be integrated into the evaluation. The disadvantage of reporting antigens as “unacceptable” and thus prolonging the waiting time for an organ offer must be weighed against the risk of not reporting them and thus resulting in an HLA incompatible transplant with subsequent short- and long-term post-transplant complications.

Highly sensitised patients (vPRA $\geq 85\%$ in two different sera) often have a low chance of receiving a crossmatch negative kidney offer. Such patients may be eligible for inclusion in the Acceptable Mismatch (AM) programme of ET.

7 Virtual Crossmatch

If a kidney donor is available and expresses HLA antigens that are indicated as unacceptable antigens in a patient, ET will not make a kidney offer to that patient. This exclusion during the allocation process is called a positive virtual crossmatch.

Recipients with a negative virtual crossmatch may be offered the kidney and, if accepted, a physical crossmatch must be performed by the HLA laboratory affiliated with the transplant centre. The virtual crossmatch is performed for all kidney, pancreas and combined kidney-pancreas transplantations.

8 Physical Crossmatch

A physical crossmatch is performed as a decisive crossmatch (also called transplantation crossmatch) after the virtual crossmatch (see section 7) is negative. It is usually performed with the CDC technique, using recipient serum and donor lymphocytes (isolated from either the donor's peripheral blood, lymph nodes or spleen). To increase the sensitivity of the CDC crossmatch, B lymphocytes can be used in addition to T lymphocytes or unseparated lymphocytes. It is important that the serum is representative of the current immunisation status of the recipient: either the most recent serum in the quarterly screening scheme (while avoiding outdated sera – see above) from a non-immunised patient, or a fresh serum if the patient is immunised or has had a recent immunising event. In immunised patients, transplantation can only be performed if the prospective decisive crossmatch is negative. In Germany, for first-transplant, non-immunised recipients (with confirmed negative HLA antibody tests and with no immunising event since the last antibody screening), a decisive crossmatch may be performed either prospectively or in parallel with the transplantation.

A decisive crossmatch must be performed for all kidney, pancreas and combined kidney-pancreas transplantations. Due to the limited tolerance to ischaemia, crossmatching for a pancreas recipient is usually performed at the donor centre and for a kidney recipient at the recipient centre by the affiliated HLA laboratory.

References

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- 3 European Federation for Immunogenetics – Standards for Histocompatibility & Immunogenetics Testing; version 8.1, effective from January 1st 2024.
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