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Assignment of C1q-binding HLA antibodies as unacceptable HLA antigens avoids positive CDC-crossmatches prior to transplantation of deceased donor organs☆

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ABSTRACT

Soon, a virtual crossmatch shall replace the complement-dependent cytotoxicity (CDC) allocation crossmatch in the Eurotransplant region. To prevent positive CDC-crossmatches in the recipient centre, careful definition of unacceptable antigens is necessary. For highly sensitized patients, this is difficult by CDC alone. Assignment of all antibodies detected by sensitive assays, however, could prevent organ allocation. To assess the usefulness of the Luminex C1q-assay to prevent positive CDC-crossmatches, all CDC-crossmatches performed prior to deceased kidney transplantation in a 16-month-period were reviewed. Sera causing positive crossmatches were investigated by the C1q-assay. 31 out of 1432 crossmatches (2.2%) were positive. Sera involved in 26 positive crossmatches were available. C1q-binding donor-specific antibodies were detected in 19 sera (73.1%). The other sera were from recipients without any HLA antibodies detectable by CDC or common solid phase assays. Three patients had known Non-HLA antibodies causing positive CDC-results. Four crossmatches were only weak positive. Therefore, avoidance of donors with HLA antigens against whom C1q-binding antibodies were detected would have prevented all positive crossmatches due to HLA antibodies. Provided that all HLA specificities against which antibodies are detected by the Luminex C1q-assay are considered as unacceptable antigens, CDC-crossmatches prior to transplantation might safely be omitted in many patients. They should be maintained in highly immunized patients, however, for whom assignment of all C1q-positive antibodies as unacceptable antigens could lead to a significant delay or even prevention of transplantation.

1. Introduction

Already in the 1960s it has been demonstrated that hyperacute rejections after transplantation of solid organs could be prevented, if only transplantations with negative results of complement dependent cytotoxicity (CDC) crossmatches were performed [1]. Since that time, in Germany and many other countries performance of a CDC-crossmatch is an essential procedure prior to transplantation of solid organs to exclude the presence of harmful donor-specific antibodies in the recipient.

During recent years, several solid phase immunoassays (SPI) for the detection of HLA antibodies have been developed. One of these is the Luminex bead array-technology: fluorescent-dyed beads coated with several HLA antigens or only a single specific HLA molecule [2]. Bead array assays have a higher sensitivity compared to CDC-assay, and enable a better differentiation of antibody specificities, especially if single antigen beads are used [3]. That way, unacceptable antigens can be defined and by consideration of these unacceptable antigens during the organ allocation, positive crossmatches can be significantly reduced (so-called "virtual crossmatch" [4,5]).

In the Eurotransplant region, there are currently two CDC-crossmatches performed for immunized patients prior to kidney transplantation: The allocation crossmatch in the donor laboratory, and the transplantation crossmatch in the recipient laboratory. Soon, the allocation crossmatch, performed by CDC, shall be replaced by a virtual crossmatch, while the transplantation crossmatch still will be performed by CDC. To prevent positive CDC-crossmatches in the recipient laboratory and avoid unnecessary shipment of organs, careful

Abbreviations: CDC, complement dependent cytotoxicity; SPI, solid phase immunoassay; UAG, unacceptable antigen; ET, Eurotransplant.

☆ The authors declare that they have no conflicts of interest.

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definition of unacceptable antigens is an essential prerequisite. For sensitized patients, this is difficult by CDC alone. Assignment of all antibodies detected by sensitive SPI, however, could prolong the patients' waiting time.

Common bead array assays detect IgG antibodies against HLA specificities irrespective of their complement-binding capability. The clinical relevance of antibodies, which are detectable by SPI only, is still a matter of debate [6–13], as they are not always associated with a worse graft outcome [14,15]. However, meanwhile assays are available, which are based on the sensitive bead array technology, and also allow the assessment of the complement binding capability of HLA antibodies. In these assays, the secondary antibody is not directed against the Fc-fragment of human IgG, but against complement factors (e.g. C1q or C3d) added after incubation of the HLA-coated beads with the patient serum [16–19].

The usefulness of such assays for the prediction of both, acute as well as delayed allograft rejection has already been demonstrated [17,18,20,21].

The aim of our study was to assess the ability of the C1q-single antigen-assay to determine unacceptable antigens for the prevention of positive CDC crossmatches retrospectively.

2. Material and methods

2.1. Selection of potential recipients

Recipients for organs from deceased donors were selected by Eurotransplant (ET) according to detailed regulations [22]. If the results of donor HLA typing were known at the moment of allocation, only patients for whom none of the donor's HLA antigens had been assigned as unacceptable antigen (UAG) were considered as recipients. According to the regulations of ET [22], "HLA antigens, towards which the recipient has formed alloantibodies defined with the CDC in the current serum, *must* be reported as unacceptable mismatches. Depending on the policy of the transplant center, additional antibodies can be defined as UAG" [22].

In recipients on the waiting list for a pancreas or in the ET senior program, organ allocation took place already before HLA typing was finished. That way, in some recipients, UAG have not been considered by the organ allocation procedure.

2.2. CDC crossmatch

Crossmatches were performed with stored frozen (≤ −30 °C) sera from all potential recipients. Unseparated lymphocytes were extracted from EDTA-blood samples or from the spleen of the deceased organ donor using Rosette Sep (Cellsystems Biotechnoloy, Troisdorf, Germany) and stored in storage medium (Lymphostabil, BioRad Laboratories GmbH, München, Germany). 1 μl of the patient's serum as well as 1 μl cell-suspension were incubated in a Terasaki plate for 30 min at room temperature. Then 5 μl complement were added. After incubation at room temperature for 60 min, the dye (Fluoroquench, OneLambda Inc., Los Angeles, CA, USA) was added and the crossmatch was analyzed after 15 min. The crossmatch was considered borderline positive at a cell death rate of >10%, and positive at a rate of >20% of dead cells.

To exclude positive reactions due to IgM antibodies, a crossmatch with Dithiotreitol (DTT, Sigma-Aldrich, München, Germany) treatment was performed in parallel: for this, 0.01 M DTT was added to the cell-suspension before the first incubation step [23]. The crossmatch was analyzed like that without DTT treatment.

If both – untreated and DTT-treated – crossmatches were positive, the overall result of the crossmatch was positive, leading to the denial of the graft offer for the corresponding recipient. If both crossmatches were negative or if at least the DTT crossmatch was negative, the overall result of the crossmatch was negative.

2.3. Determination of HLA antibodies

Presence, specificity and complement-binding capacity of HLA antibodies in patients were determined retrospectively in sera showing to have borderline positive or positive crossmatches according to definition above. In the case of four positive crossmatches, a stored (≤ −30 °C) older specimen, obtained several weeks earlier from the same recipient was used due to lack of further material from the original sample.

Antibodies were determined using the Luminex Labscreen Single Antigen C1q test (OneLambda, Canoga Park, CA, United States). The test was performed according to the manufacturer's instructions. A serum was considered positive for a specific HLA antigen if the corresponding bead had a mean fluorescence intensity (MFI) of at least 500, a value frequently chosen as cut-off [24,25]. The virtual panel-reactive antibody (vPRA) values were determined using the calculator provided by the ET reference laboratory (assessed at http://www.etrl.org/Virtual%20PRA/Default.aspx).

To assess differences in the vPRA obtained by the C1q test and the standard Luminex IgG test, sera of other 18 patients were investigated by single antigen C1q test as well as by the standard Luminex single antigen IgG test (Labscreen Single Antigen. OneLambda, Canoga Park, CA, United States).

2.4. HLA-typing

According to the regulations of ET, every donor must be typed at least for HLA class I A, B and C and class II DR and DQ [22]. In our Institute, DNA extraction for HLA typing was done by a semiautomatic device using magnetic beads (Prepito, PerkinElmer chemagen Technologie GmbH, Baesweiler, Germany).

HLA-A, -B, -C, -DRB1, -DRB3/4/5 and -DQB1 alleles of the deceased organ donors were typed by SSP-PCR with endpoint fluorescence detection (Fluogene, inno-train, Kronberg, Germany) using the device Fluovista (inno-train) and by SSP (MicroSSP, OneLambda, Canoga Park, CA, United States). DNA isolation and HLA typing were performed and evaluated according to the manufacturer's recommendations. In case of insufficient donor typing [e.g. for HLA DP] by the donor HLA laboratory, additional typing was performed in our institute to determine whether the detected antibodies were donor specific using the same method.

3. Results

3.1. Crossmatch results and results of C1q-testing

Between October 2012 and January 2014, crossmatches for 155 deceased organ donors were performed with between three and ten potential kidney recipients per donor, as well as up to ten pancreas recipients. This resulted in a total of approximately 1432 crossmatches during the observation period. Out of those 1432 crossmatches, 31 (2.2%) were positive or borderline positive.

Those 31 crossmatches were obtained with the sera of 23 different recipients: in four recipients, two crossmatches were performed with lymphocytes of two different donors, each with a positive result. In two further recipients, even three crossmatches with positive results, each with lymphocytes from different donors, were performed.

For 19 positive crossmatches, C1q-positive donor specific antibodies (DSA) were detected in the patients' sera. In all those sera, DSA against HLA-class I specificities were detectable. In nine sera, additionally antibodies directed towards HLA class II, including antibodies against DPA1 and DPB1, were detectable. In one case, it was not possible to determine whether the C1q-positive antibodies were donorspecific, because the patient was not typed for DPB1 and no material was available to complete the HLA typing. In five out of the 19 positive crossmatches from two different recipients, at least one donor HLA antigen was already assigned as UAG for these recipients.