Successful kidney transplantation across a positive complement-dependent cytotoxicity crossmatch by using C1q assay-directed, bortezomib-assisted desensitization

A case report

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Abstract

Rationale: Human leukocyte antigen (HLA) is the major immunologic barrier in kidney transplantation (KT). Various desensitization protocols to overcome the HLA barrier have increased the opportunity for transplantation in sensitized patients. In addition, technological advances in solid-phase assays have permitted more comprehensive assessment of donor-specific antibodies. Although various desensitization therapies and immunologic techniques have been developed, the final transplantation decision is still based on the classic complement-dependent cytotoxicity (CDC) crossmatch (XM) technique. Some patients who fail to achieve negative XM have lost their transplant opportunities, even after receiving sufficient desensitization therapies.

Patient concerns: A 57-year-old male with end-stage renal disease secondary to chronic glomerulonephritis was scheduled to have a second transplant from his son, but CDC XM was positive.

Diagnoses: Initial CDC XM (Initial T-AHG 1:32) and flow-cytometry XM were positive. Anti-HLA-B59 donor specific antibody was detected by Luminex single antigen assay.

Interventions: Herein, we report a successful case of KT across a positive CDC XM (T-AHG 1:8 at the time of transplantation) by using C1q assay-directed, bortezomib-assisted desensitization. After confirming a negative conversion in the C1q donor-specific antibody, we decided to perform KT accepting a positive AHG-CDC XM of 1:8 at the time of transplantation.

Outcomes: The posttransplant course was uneventful and a protocol biopsy at 3 months showed no evidence of rejection. The patient had excellent graft function at 12 months posttransplant.

Lessons: The results of XM test and solid-phase assay should be interpreted in the context of the individual patient.

Abbreviations:
- AHG = anti-human globulin
- CDC = complement-dependent cytotoxicity
- DSA = donor-specific antibodies
- DTT = dithiothreitol
- HLA = human leukocyte antigen
- IVIG = intravenous immunoglobulin
- KT = kidney transplantation
- MFI = mean fluorescence intensity
- NIH = National Institute of Health
- PP = plasmapheresis
- XM = crossmatch

Keywords: bortezomib, C1q-binding antibody, crossmatch, desensitization, donor-specific antibody, hyperacute rejection, kidney transplantation

1. Introduction

The presence of donor-specific antibodies (DSA) has been considered a contraindication for renal transplantation, as such its presence has been associated with hyperacute rejection and immediate graft loss.\cite{1} A complement-dependent cytotoxicity (CDC) crossmatch (XM) was developed to identify DSA directed against donor lymphocytes, which are associated with hyperacute rejection. Consequently, various XM techniques were introduced to enhance test sensitivity. Furthermore, application of a solid-phase test for DSA has allowed detection and characterization of the relevant antibodies.\cite{2,3}

With recent advances in immunologic tests, desensitization protocols to overcome the human leukocyte antigen (HLA) barrier have increased the opportunity for transplantation in sensitized patients with renal failure.\cite{4,5} New interventions, such as bortezomib, are used for desensitization under the expectation of increasing transplantability.\cite{6} However, decisions to perform kidney transplantation (KT) after desensitization were based on...
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### 2. Case review

A 57-year-old male with end-stage renal disease secondary to chronic glomerulonephritis underwent his first KT from his brother in 1996. He lost the allograft due to chronic rejection and returned to hemodialysis in 2011. He was scheduled to have a second transplant in another center from either his two sons or his wife, but all were CDC XM positive. A desensitization protocol comprising plasmapheresis (PP), intravenous immunoglobulin (IVIG), rituximab, and bortezomib, the patient’s C1q-positive antibodies were converted to C1q-negative status.

#### 2.1 Early experiences obtained prior to the introduction of solid-phase tests or new interventions.

In this study, we report a successful KT across a positive CDC XM (1:8 T anti-human globulin [AHG]) at the time of transplantation. After receiving a desensitization protocol comprising plasmapheresis (PP), intravenous immunoglobulin (IVIG), rituximab, and bortezomib, the patient’s C1q-positive antibodies were converted to C1q-negative status.

#### 2.2 Case review

A 57-year-old male with end-stage renal disease secondary to chronic glomerulonephritis underwent his first KT from his brother in 1996. He lost the allograft due to chronic rejection and returned to hemodialysis in 2011. He was scheduled to have a second transplant in another center from either his two sons or his wife, but all were CDC XM positive. A desensitization protocol comprising plasmapheresis (PP), intravenous immunoglobulin (IVIG), rituximab, and bortezomib, the patient’s C1q-positive antibodies were converted to C1q-negative status.

#### 2.3 Three-dimensional HLA models demonstrated that desensitization had a different effect on epitope reaction between IgG and C1q assays (Fig. 2).

After confirming a negative C1q-binding DSA, we decided to perform KT accepting a positive AHG-CDC XM of 1:8 at the time of transplantation. The kidney appeared well perfused and did not show evidence of hyperacute rejection.

The patient did not receive posttransplant PP + IVIG treatment, and his postoperative course was uneventful. The protocol biopsy performed at postoperative month 3 showed mild tubular dilatation with a few casts. Peritubular C4d staining was negative.

### Table 1

| Informative epitopes based on donor-specific epitope reaction | Pre-desensitization | SABA IgG | SABA C1q | Post-desensitization | SABA IgG | SABA C1q |
|-------------------------------------------------------------|----------------------|----------|----------|----------------------|----------|----------|
| 69TNT                                                       | Positive             | Positive | Positive | Positive             | Negative | Negative |
| 66IF+163TEW                                                 | Positive             | Positive | Positive | Positive             | Positive | Negative |
| 80I+65Ql                                                    | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 131I+163T                                                  | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 163TEW+65Ql                                                | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 45EE                                                       | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 62RNQ                                                      | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 66IF                                                       | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 71TNT                                                      | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 9TVE                                                       | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 97T                                                        | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 113HN                                                      | Positive             | Positive | Positive | Positive             | Positive | Positive |
| 116L                                                       | Negative             | Positive | Positive | Negative             | Negative | Negative |
| NIH-CDC-XM                                                  | Positive (1:1)       |          |          | Negative             |          |          |
| AHG-CDC-XM                                                  | Positive (1:32)      |          |          | Positive (1:8)       |          |          |

AHG = anti-human globulin, CDC = complement-dependent cytotoxicity, NIH = National Institute of Health, SABA = single antigen-bead assay, XM = crossmatch.

Epitope reactions were analyzed using HLA matchmaker (HLA-ABC antibody analysis [v02] updated on June 2016, http://www.epitopes.net/) and informative epitopes are defined based on HLA epitope registry (http://www.epiregistry.utp.br).
under immunofluorescence, and no endothelial swelling or peritubular capillary changes were present on examination via electron microscopy. Additional kidney injury molecule-1 immunohistochemistry for the evaluation of acute tubular injury showed negative staining in proximal tubules (Fig. 3). Currently, at 12 months post-transplantation, the patient continues to do well with a baseline creatinine level of 1.0 mg/dL and a urinary protein-to-creatinine ratio of less than 0.1 (g/gCr). Anti-HLA-B59 DSA remains at MFI 7815, but no HLA antibody reaction has been detected by C1q assay. The patient did not suffer acute rejection or infectious complication during follow-up.

The study procedures were in accordance with the Declaration of Helsinki. All procedures were performed after obtaining informed consent. A single patient case report does not require institutional review board approval according to our board policy.

3. Discussion

Historically, several cases of transplantation with a positive CDC XM at transplantation were performed in the past prior to the introduction of solid-phase tests or new interventions. In those cases, most patients had poor outcomes, but some cases did have acceptable outcomes. The reasons for the different courses remain unknown, but a positive CDC XM is a contraindication to transplantation. Afterward, despite advances in desensitization protocols and immunologic testing, most KTIs are performed after achievement of a negative CDC XM. In this report, we present a successful case of KT with a persistently positive CDC XM (T-AHG 1:8 at the time of transplantation) following C1q assay-directed, bortezomib-assisted desensitization.

KT is the treatment of choice for end-stage renal disease patients. However, over 30% of patients on a waiting list are sensitized to HLA due to pregnancy, blood transfusion, or previous transplantation. These sensitized patients are less likely to find compatible deceased donors and thus have a prolonged waiting time. In addition, they have a limited possibility of undergoing a paired kidney exchange. Desensitization protocols to overcome the HLA barrier have become increasingly popular because of organ shortages and have increased the opportunity for transplantation in sensitized patients.

Despite a low incidence of hyperacute rejection and acceptable short-term outcomes in sensitized patients, antibody-mediated rejection remains a significant challenge even after successful desensitization. Concern that relevant DSA are not being detected by less sensitive assays has led to the development of more sensitive XM techniques. Paradoxically, CDC XM has been used as a minimum requirement for KT due to its low sensitivity. However, recent studies have demonstrated that KT recipients from HLA incompatible donors exhibit a substantial survival benefit compared to that in patients who did not undergo transplantation and those who waited for
transplants from deceased donors, regardless of DSA levels.\(^{[12]}\) Therefore, accurate analysis of an individual’s DSA, which helps in the decision on whether to proceed with a transplantation, is critical for a patient. Additionally, rituximab is widely used for reducing B-cells.\(^{[4,9]}\) It is crucial to determine the role of DSA in the immune response to allografts, as it is difficult to determine an MFI cutoff value suitable for proceeding to transplantation.\(^{[13]}\) In fact, MFI values determined with conventional solid-phase assays do not accurately correlate with the complement-binding DSA. In contrast, the C1q assay directly discriminates the ability of a particular complement-binding DSA. It is quite important in a clinical setting considering that the DSA mediated complement cascade is the hallmark of in vivo graft injury.\(^{[14]}\) The classic CDC XM is neither sensitive nor specific enough to detect complement fixation.\(^{[15,16]}\) In our case, the AHG-CDC-XM result was 1:8 positive, but the NIH-CDC-XM and C1q DSA results were negative at KT. Hence, we decided to perform KT considering not only the CDC XM indicators, but also the results of the solid-phase tests. To the best of our knowledge, this is the first report of successful KT following C1q assay-directed desensitization.

Desensitization protocols have been based on PP and/or IVIG, which physically remove or inhibit the circulating DSA. In addition, rituximab is widely used for reducing B-cells.\(^{[4,9]}\) Nevertheless, prior desensitization protocols have demonstrated varying degrees of efficacy and durability. A potential explanation for the variation among results is the failure to inhibit plasma cells, the source of antibodies.\(^{[6]}\)

Bortezomib, a proteasome inhibitor, exerts an inhibitory effect directly on antibody-secreting plasma cells. Prior study using bortezomib-based desensitization represented a significant and sustained reduction in DSA, which may allow for increased transplantability.\(^{[6]}\) We achieved a negative C1q conversion in this highly sensitized patient by using bortezomib as an additional therapy. In addition, the patient did not suffer clinical rejection in the absence of postoperative PP and IVIG. The protocol biopsy also demonstrated no evidence of subclinical rejection.

Interestingly, the patient showed positive AHG-CDC-XM without C1q-binding DSA. Therapeutic agents used in desensitization protocols have been shown to cause interference in XM.\(^{[15,16]}\) In our case, however, the positive CDC XM is hard to be deemed a false positive considering the high MFI of DSA at the time of transplantation. Rather, epitope analysis can provide a possible explanation for the positive CDC XM reaction. Epitopes are parts of an antigen recognized by antibodies. As epitope reaction depends on the 3-dimensional structure, changes in this structure may lead to changes in the antibody reactivity.\(^{[18]}\) If there is an insufficient conformational area due to a structural change, even though some C1q epitope reactions were positive, but overall C1q DSA could be negative. After the desensitization, the HLA-B59 DSA itself turned into negative in the C1q assay, but multiple donor-specific epitope reactions remained positive in the IgG assay with several epitope reactions in the C1q assay. These complement-binding antibodies against donor-specific epitopes with high concentrations of noncomplement-binding antibodies might induce a positive AHG-CDC XM reaction even after desensitization.

In conclusion, we report a successful KT across a positive CDC XM at the time of transplantation, by using C1q assay-directed, bortezomib-assisted desensitization. The goal of desensitization is to maximize the opportunity for transplantation, with improved mortality and quality of life compared with that associated with dialysis. However, for over 40 years, transplantation decision making has been based on the CDC XM, regardless of the presence of more sensitive and more accurate immunologic tests. The cost of continuing the historical approach is that of eliminating some patients that could have undergone a successful transplant. Therefore, the results of XM tests and solid-phase assays must be interpreted in the context of the individual patient.

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