Reappraisal of HLA Antibody Analysis and Crossmatching in Kidney Transplantation

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ABSTRACT

Enzyme-linked immunosorbent assay (*ELISA*) and flow cytometric techniques have been introduced to overcome the limited sensitivity and specificity of the CDC assay. This retrospective study used lambda antigen tray-mixed screening and Luminex HLA class I and II specificity assays to re-examine: (1) the accuracy with which detection of HLA antibody and specificity by ELISA predicts pretransplantation National Institutes of Health (NIH)/Centers for Disease Control and Prevention (CDC) crossmatch; and (2) a comparison of Luminex and ELISA methods to detect HLA antibodies. Sera from 481 patients awaiting kidney transplantation were tested using the ELISA method lambda antigen tray-mixed and using NIH-CDC to determine how well HLA antibodies detected using ELISA predicted crossmatches using CDC. Pretransplantation sera from 48 patients with follow-up data were retested using both ELISA lambda antigen tray-mixed and Luminex to compare the efficacy of the 2 methods.

RECENTLY enzyme-linked immunosorbent assay (ELISA) and flow cytometric techniques have been introduced to overcome the limited sensitivity and specificity of the CDC assay.[1] These technological advances—combined with a better understanding of the epitopes of HLA antigens—have provided a more efficient, structurally based strategy to determine HLA compatibility. These emerging approaches may be reliably used to predict crossmatches among highly sensitized patients and also to monitor their development of clinically relevant anti-HLA antibodies after transplantation. This retrospective study used the lambda antigen tray-mixed (LAT-M) screen and Luminex HLA class I and II specificity assays to re-examine: (1) the accuracy with which detection of HLA antibody and specificity by ELISA predicted pretransplantation National Institutes of Health (NIH)/Centers for Disease Control and Prevention (CDC) crossmatches and (2) a comparison of Luminex and ELISA methods to detect HLA antibodies.

PATIENTS AND METHODS

We tested sera from 2 groups of patients. First, sera from 481 patients awaiting kidney transplantation were tested to determine how well ELISA detection of HLA antibody predicted crossmatches revealed by NIH-CDC. Second, 48 pretransplantation sera

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were tested by both ELISA and Luminex to compare their relative ability to detect HLA antibody.

Screening by ELISA of HLA Antibodies and Analysis of Specificity

Sera were screened for the presence of HLA-I and HLA-II immunoglobulin (Ig)G antibodies (Abs) using ELISA following the manufacturer's instructions (LAT-M, One Lambda Inc., Los Angeles, Calif, United States). The HLA specificity of positive sera was analyzed using an additional ELISA test (LAT Class I & II, One Lambda Inc.). The ELISA plates were read on an ELX-800 plate reader (Bio-Tek Instruments Inc., Winooski, VT, United States). The obtained optical densities (OD) were analyzed using LAT software for Windows (One Lambda Inc.)[2] Sera with a correlation coefficient (r) of >0.65 were considered specific; when the donor had the corresponding HLA antigen, the HLA Ab was considered to be donor-specific.[3] Results were invalid if the ratio of the average positive serum control OD value over the average negative serum control OD value was ≤ 10 or the average positive serum OD was ≤ 1000. To facilitate comparison of the strength of reactions, results were expressed as ratios of the positive cutoff value, with >1 considered positive. Specific antibodies identified in a given test sample were categorized as "2," "4," "6," and "8" reactions, as in the cytotoxicity assay. Those called "2" reactions were considered weakly positive or negative, whereas "4," "6," or "8" reactions were usually considered positive: "4" (21%–50%) being moderately positive; "6" (51%–80%) strongly positive; and "8" very strongly positive.

Luminex Analysis

Identification of HLA Ab was also performed using LABScreen Single Antigen Beads (One Lambda Inc.).[4] In brief, HLA class I or II antigen-coated Luminex beads were incubated with patient serum in 96-well multiscreen filter plates (Millipore, Bedford, Mass, United States). Unbound excess serum was removed by washing the beads in the wells before addition of phycoerythrin-conjugated goat anti-human IgG. The plates were incubated on a rotating platform in the dark. Thereafter, the test samples were analyzed on a Luminex instrument, including both in-house as well as manufacturer's positive and negative controls.

Statistics

Observed and predicted crossmatch (XM) data were divided into 4 categories: observed and predicted positive (O+/P+); observed negative but predicted positive (O−/P+); observed positive but predicted negative (O+/P−); and observed and predicted negative (O−/P−). The predictive values of XM were calculated as follows: sensitivity = (O+/P+)/([O+/P+] + [O−/P+]); specificity = (O−/P−)/([O−/P−] + [O+/P−]); and efficiency = (O+/P+) + (O−/P−)/N.[5] Positive predictive value is the proportion of patients who tested positive and in whom graft dysfunction was present. Negative predictive value is the proportion of patients who tested negative and who did not have graft dysfunction.

RESULTS

Sera from 481 patients awaiting kidney transplantation were tested using the ELISA method LAT-M and using CDC to determine how well HLA Abs detected by ELISA predicted the crossmatches shown by CDC. HLA Abs found using ELISA ranged from 24% (115/481) weak reactivity (OD "2") to 17% (84/481) strongly reactive (OD "8"; Table 1). The positive predictive value (PPV) of ELISA-detected Abs for positive CDC crossmatches at the time of transplantation was 43%–54%. The negative predictive value (NPV)—negative by ELISA, negative CDC crossmatch—was 88%. The PPV was 55% for sera with HLA class I donor specific antibody and 67% with HLA class II sera. In contrast, NPV was 84% with sera negative for HLA class I donor specific antibody and 86% with sera negative for HLA class II DSA (Table 2).

Pretransplantation sera from 48 patients with follow-up data were retested using both ELISA LAT-M and Luminex to compare the efficacy of those 2 methods. ELISA detected pretransplantation HLA Abs in 8 of the 48 (17%), whereas Luminex found HLA Abs in 27 (56%; Table 3). Of the 8 "ELISA patients," 4 (50%) had DSA, whereas 17 (63%) of the 27 "Luminex patients" had DSA (Table 4).

DISCUSSION

In 1969, Patel and Terasaki published a landmark study in which they demonstrated that the presence of recipient antibodies to antigens expressed on donor white blood cells were a major risk factor for immediate graft loss.[6] During the past 40 years, our understanding has evolved considerably concerning processes by which patients become sensitized to allogeneic HLA antigens and the tests that are available to detect and identify antibodies.

We detected anti-HLA Abs and DSA using ELISA, a test that has been shown to be both sensitive and specific to detect Abs directed only against HLA antigens. It distinguishes IgG versus IgM, and offers the advantage over the CDC method of allowing tests of large numbers of samples in a time-effective and cost-effective manner. However, sera that have high reactivity (80%–100%) are difficult to determine specificities or donor reactivity by LAT class I or class II.[7] In this study, we used a low positive cutoff ($>10\%$ of the OD positive control) to increase the number of sera that could be identified to contain HLA Abs.

Most publications report higher rejection rates among patients with a positive flow cytometric crossmatch (FCXM). Although flow crossmatch is widely used at centers in the United States and United Kingdom, many European centers do not use the test because of concerns that patients may be excluded from transplantation due to false-positives.[8]

The FCXM has proven to be more sensitive than CDC assays to detect clinically relevant DSA.[9] However, FCXM has some limitations. First, nonspecific binding of antibodies irrelevant to the transplant may produce false-positive reactions. Second, the assay is not standardized; the definition of a cut-off for a positive FCXM is difficult. Furthermore, this procedure requires additional resources in the laboratory, prolonging cold ischemia time.[10] We have paper shown herein that data obtained using ELISA Ab analysis