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Does flow cytometry crossmatch predict renal allograft outcome in patients with a negative antiglobulin crossmatch?

Original article Wen R *et al.* (2006) Biomarkers in transplantation: prospective, blinded measurement of predictive value for the flow cytometry crossmatch after negative antiglobulin crossmatch in kidney transplantation. *Kidney Int* **70:** 1474–1481

SYNOPSIS

KEYWORDS acute rejection, flow cytometry crossmatching, graft loss, kidney transplantation

BACKGROUND

Uncertainty surrounds the prognostic power of flow cytometry crossmatching for acute graft rejection after kidney transplantation.

OBJECTIVE

To ascertain whether a positive donor-T-cell-directed IgG flow cytometry crossmatch predicts acute rejection, allograft loss or death, in normal clinical practice.

DESIGN AND INTERVENTION

This prospective cohort study recruited individuals who received a kidney transplant from a living or deceased donor at the University of British Columbia, Vancouver, Canada, during the period January 1997 to December 2000. Each donor–recipient pair was ABO-compatible, and all recipients had a negative donor-T-cell-directed anti-human globulin crossmatch. Serum, for flow cytometry crossmatching, was obtained from prospective recipients before transplantation; clinical staff were blinded to the results. A channel shift of ≥10 in fluorescence intensity compared with the negative control indicated a positive flow cytometry crossmatch. Follow-up was censored at graft loss, at death, or at the final visit.

OUTCOME MEASURES

Biopsy-confirmed acute rejection, graft failure and death were the end points.

RESULTS

The 257 enrolled patients (39% female) were followed up for a mean of 2,046 days (range 0–3,112 days). There were 31 (12.1%) positive flow cytometry crossmatches, and 78 patients (30.4%) experienced acute rejection within a year of transplantation. There was no significant difference in the incidence of acute rejection between the patients with a positive crossmatch and those with a negative crossmatch (33.3% vs 30.7%; $P = 0.907$). This similarity persisted when patients were analyzed as separate subgroups according to whether or not they had previously undergone kidney transplantation, and according to whether they received a kidney from a living or deceased donor. There were 18 deaths (7.0%) and 41 graft losses (16.0%). The combined incidence of graft failure and death at 3,000 days did not differ significantly between patients with a positive flow cytometry crossmatch and those with a negative flow cytometry crossmatch (19.4% vs 17.8%; $P = 0.607$). Again, the lack of significance remained in each of the four subgroups when analyzed separately. There was no association between flow cytometry crossmatching and graft failure or death as single outcomes. Multivariate Cox regression analysis did not identify flow cytometry crossmatching as a significant independent risk factor for acute rejection, or for the combined end point of death or graft failure. A positive flow cytometry crossmatch had a sensitivity of 0.128 and a specificity of 0.883 for predicting acute rejection. The positive and negative post-test probabilities of acute rejection were 0.323 and 0.301, respectively, indicating that flow cytometry crossmatch had little predictive value.

CONCLUSION

The authors concluded that flow cytometry crossmatching in patients with a negative antiglobulin crossmatch did not identify individuals at high risk of acute rejection, graft failure or death following kidney transplantation.

COMMENTARY

J Michael Cecka

The risks associated with preformed antidonor human leukocyte antigen (HLA) antibodies in renal transplant candidates have been well documented.[1] By routinely performing simple crossmatch tests before transplantation, irreversible hyperacute rejection resulting from high levels of circulating cytotoxins has been almost completely eliminated. Nevertheless, the antibody response is dynamic and even when antibody levels have fallen, long after the immunizing event, they can rise again within days after transplantation as a result of engagement of immune memory. The consequences include early humoral rejection that is difficult to manage with current immunosuppression alone, and perhaps lasting damage that will ultimately shorten the graft survival time.

A number of modifications to the crossmatching process, including addition of anti-human globulin to enhance cytotoxicity, have been implemented to detect lower levels of anti-HLA antibodies and to reduce nonspecific reactions.[2] In order to improve sensitivity further, some centers also perform crossmatching using historical sera obtained when levels of antibody were higher. Flow cytometry, introduced in the 1980s, is even more sensitive because it measures immunoglobulin bound to donor cells without the requirement for complement-dependent cytotoxicity. These modifications and strategies have generally not been rigorously tested to demonstrate their utility in predicting graft survival or early rejections. More importantly, however, none of these commonly used crossmatch tests measures anti-HLA antibodies directly, but rather all use binding of immunoglobulin to donor cells as a surrogate. The more-sensitive tests are more prone to false-positive reactions that are not caused by anti-HLA antibodies.

Wen *et al.* report that the results of flow cytometry crossmatching did not predict graft survival or rejection when patients were selected for transplantation using an anti-human-globulin-enhanced crossmatch test. Another Canadian study of patients selected for transplantation based on a negative anti-human-globulin-enhanced crossmatch reported a significant association between a positive flow crossmatch (performed retrospectively using stored serum and donor cells obtained before transplantation) and early graft loss from antibody-mediated rejection.[3] A key difference between the two studies was that the latter showed that the antibodies detected by flow cytometry were directed against HLA antigens whereas the Wen *et al.* study did not. Interestingly, when the Vancouver group subsequently analyzed their data using a solid-phase assay for anti-HLA antibodies, they found that the presence of antidonor HLA antibodies correlated strongly with rejection and graft loss.[4]

The major issue then, seems to be the specificity rather than the sensitivity of the crossmatch tests. New solid-phase technologies that identify anti-HLA antibodies directly, using affinity-purified or recombinant HLA antigens,[5] should reduce the problem of false-positive crossmatches caused by non-HLA antibodies, and specific antibodies should be reported to support a positive crossmatch result. The results of Wen *et al.* should, however, encourage us to proceed with a critical eye. These newer solid-phase technologies are even more sensitive than flow cytometry crossmatching, and the challenge must be to determine the level of risk posed by antibodies that are detected by only the most sensitive tests. Now that we can more accurately identify anti-HLA antibodies with solid-phase tests, and recognize problematic humoral rejection by looking for deposition of the complement component C4d in peritubular capillaries, it should be possible to begin defining these risks without the surrogates of crossmatch results, clinical rejection or graft survival.

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Competing interests
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PRACTICE POINT
The capacity of sensitive crossmatch tests to predict outcomes of kidney transplantation remains controversial; however, new tools for identifying anti-HLA antibodies provide a means to resolve this issue