Significance of pre-transplant anti-HLA antibodies detected on an ELISA mixed antigen tray platform

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ABSTRACT

This study aims at examining the clinical impact, of antibodies detected on an ELISA mixed antigen tray format (LATM, One Lambda) in the absence of complement dependent cytotoxicity (CDC) positivity. All patients who underwent renal transplantation in 2007 and 2008 had their final pre-transplant sera retrospectively analyzed by the LATM assay. These patients were then followed-up with clinical, biochemical, and histopathological end points defined by elevation of serum creatinine and/or histopathological criteria. Among 164 patients who were studied, 149 received grafts from live related donors and 15, from deceased donors. 31 (19%) of the transplanted patients demonstrated pre-transplant anti-HLA IgG antibodies on the assay. Totally, 15 were positive for class I antibodies, 4 for class II antibodies, and 12 for both class I and class II antibodies. 44 patients (36%) experienced rejection. 8 out of 31 (26%) ELISA positive patients and 36 out of 133 (27%) ELISA negative patients experienced rejection. Among 15 patients who received deceased donor transplants, 4 were positive for ELISA, and 11 were negative. All 4 (100%) of the ELISA positive patients experienced rejection as compared to 3 out of 11 (27%) ELISA negative patients (P = 0.01). The ELISA LATM assay did not show any predictive value for rejection in our overall patient population; however, results in the specific setting of deceased donor transplants merit further exploration.

Key words: Anti-HLA antibodies, ELISA, renal transplant

Introduction

The complement dependent cytotoxicity (CDC) test was among the earliest established method for detection of anti-HLA antibodies and it has remained the gold standard until today, on account of its high correlation with early rejection. Solid phase platforms with increased sensitivity and specificity are presently available. However, the clinical significance of antibodies detected on these platforms, yet undetected on CDC, remains unclear, with available studies giving conflicting reports. This study aims at examining the clinical impact of results of the ELISA LATM assay on patients who underwent renal transplants in our hospital.

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Background

Live related transplants comprise the overwhelming majority of transplants performed at our center as opposed to deceased donor transplants. Pre-transplant screening is performed by CDC with extended incubation to enhance sensitivity, and Dithiothreitol to differentiate IgM from IgG. Patients with two consecutive negative CDC cross matches, or who show consistent IgM with no detectable IgG are cleared for transplant.

The LATM assay was incorporated as a first step towards developing a more sensitive screening algorithm. It specifically detects anti-HLA antibodies of the IgG class, using pooled bound class I and class II HLA antigens. It does not determine the antigenic specificity of the detected antibodies and hence does not specify the presence of donor specific antibodies (DSA).

Materials and Methods

All patients who underwent renal transplantation in

2007 and 2008 had their final pre-transplant sera retrospectively analyzed by the LATM assay (OneLambda Inc.).

Tests were performed according to the manufacturer's instructions. Patients' sera in 1:2 dilutions were dotted on Terasaki microplates pre-coated with a mixture of class I and class II antigens. Following a period of incubation, the microplates were washed and any bound anti-HLA IgG molecules were detected by adding a conjugate of antihuman IgG antibody with alkaline phosphatase, followed by a colorigenic substrate for alkaline phosphatase. The optical density (od) was quantified on an ELISA reader and LAT software with positive or negative results assigned by comparing the od reading of the test samples with that of a threshold calculated using od of control wells.

Results were categorized as negative, positive for class I, positive for class II, or positive for class I and II. These patients were then followed-up for rejection with clinical, biochemical, and histopoathological end points defined by elevation of serum creatinine and/or histopathological criteria amounting to Banff 1a or more. A one tailed P (Mid P exact) value was calculated by using the Epi-info software.

Results

A total of 169 patients were transplanted in 2007 and 2008, Pre-transplant serum samples were insufficient or unavailable for the LATM assay in 5 patients. Among the remaining 164 patients, 149 received grafts from live related donors and 15, from deceased donors. 31 (19%) of the transplanted patients demonstrated pre-transplant anti-HLA IgG antibodies on the assay. Totally, 15 were positive for class I antibodies, 4 for class II antibodies, and 12 for both class I and class II antibodies.

A total of 44 patients (36%) experienced rejection. The results with respect to the incidence of rejection are shown in Table 1. Out of 31 (26%), 8 ELISA positive patients and 36 out of 133 (27%) ELISA negative patients experienced rejection. Among 15 patients who received deceased donor transplants, 4 were positive for ELISA and 11 were negative. All 4 (100%) of the ELISA positive patients experienced rejection as compared to 3 out of 11 (27%) ELISA negative patients ($P = 0.01$). The results with respect to the incidence of rejection in deceased donor transplants are shown in Table 2.

Discussion

ELISA assays for anti-HLA antibody detection have several reported advantages over cell based assays. These include greater sensitivity as compared to CDC, specificity for clinically significant anti-HLA IgG antibodies, greater objectivity, and improved turnaround time. However, whereas, cell based assays carry antigens in their natural state, the processing of HLA antigens required for ELISA and other solid phase assays, carries the potential of changing the configuration of HLA molecules, exposing cryptic epitopes, which may give false positive reactions with patient's sera, or leading to loss of epitopes producing false negative reactions, which may affect their sensitivity.[1,2] Another limitation of solid phase assays is that though they can detect antibodies against the most prevalent antigens, they do not cover the entire gamut of HLA phenotypes, which attains more relevance when testing in non-Caucasian populations and ethnically distinct groups.[2]

It is apparent, from our study, that the presence of pre-transplant anti-HLA antibodies detected on the ELISA LATM platform has not significantly predicted rejection in living donor organ recipients in our center on up to 3 years follow-up after transplant. It is known that DSA are deleterious to a graft while non-DSA are not so.[3]

Table 1: Overall results of ELISA LATM assay with respect to rejection

Clinical outcome	ELISA LATM class I positive	ELISA LATM class II positive	ELISA LATM class I and II positive	ELISA LATM negative	Totals
Rejection	3	2	3	36	44
No rejection	12	2	9	97	120
Totals	15	4	12	133	*N*=164

Table 2: Results of ELISA LATM in recipients of deceased donor grafts with respect to incidence of rejection

Clinical outcome	ELISA LATM class I positive	ELISA LATM class II positive	ELISA LATM class I and II positive	ELISA LATM negative	Totals
Rejection	1	2	1	3	7
No rejection	0	0	0	8	8
Totals	1	2	1	11	*N*=15

The ELISA LATM non-specifically detects antibodies irrespective of they being donor specific or not. Since, only patients who were CDC cross match negative were transplanted, many patients who carried DSA would automatically be weeded from our study, unless their titers were too low to be detected by CDC. An added factor is that the donors in our study population predominantly consisted of first degree relatives, most of whom would at least be haplomatched for the patient, further reducing the chance of DSA. It is possible that a few patients with low titer DSA who were positive on the LATM assay were transplanted, which can only be shown with a sensitive single antigen assay or cross match. However, in effect, our study shows, the LATM test when used in a pre-transplant setting does not seem to effectively identify CDC negative patients at risk of rejection following transplant with live related donors. Some studies have shown a value for ELISA in prediction of rejection. Christiaans *et al*., found an association between ELISA in final pre-transplant serum and rejection, notably in deceased donors.[4] Lee and Ozawa found a significant difference in graft survival at 5, 10 and 15 years between patients who were positive on the LATM assay and those who were negative.[5] Wu *et al*., found a relation between persistent class I positivity using the LATM on pre-transplant serum and rejection whereas transient positivity did not bear the same association.[6] Whether the latter apply to deceased or live related donation scenarios is unclear.

Yet, though, present numbers are too small for any definite conclusion, our study suggests an association with rejection in a deceased donor setting. Deceased donor transplants are well-known to have worse outcomes as compared to living donor transplants. This could be due to the higher degree of HLA mismatch in deceased donor transplant settings as compared to living related donors. Degree of HLA matching has a highly significant effect on graft survival and this effect has been shown to be potentiated in patients with preformed antibodies.[7,8] Yet, it has been shown that transplants from living unrelated donors have superior outcomes as compared to similarly matched cadaveric grafts, implying that histoincompatibility is not the only factor.[9]

Another reason postulated for poor outcome in cadaveric transplants is that traumatic brain death is associated with cytokine production, adhesion molecule expression by renal endothelium and renal inflammation, which facilitate rejection. A greater degree of injury is associated with deceased donor transplantation due to hypoperfusion in the donor, and greater cold ischemia.[10] It is possible that these factors prime the immune response, raising the clinical impact of anti-HLA antibodies in this particular setting, even when they are not manifest on a CDC cross match.

Conclusion

The ELISA LATM assay has not shown any predictive value for rejection in our overall patient population; however, results in the specific setting of deceased donor transplants merit further exploration.

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