Specificity and Sensitivity of Screening for Anti-HLA Antibodies in Kidney Allograft Dysfunction

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ABSTRACT

Background. Prospective testing for posttransplant circulating anti-HLA antibodies seems to be a critical noninvasive tool, but confirmatory data are lacking.

Materials and Methods. Over the last 3 years, peritubular capillary (PTC) C4d deposition was prospectively sought by an immunofluorescence technique applied to frozen tissue in biopsies obtained for allograft dysfunction. Screening for circulating anti-HLA class I/II alloantibodies (AlloAb) by the flow cytometric test was performed simultaneously.

Results. We evaluated 132 sets of biopsies and simultaneous serum samples. PTC C4d deposition was demonstrated in 15.9% (21/132) of biopsies. Circulating anti-HLA I/II AlloAb were detected in 25% (33/132) of serum samples. Employing receiver-operator characteristic (ROC) curves for all C4d-positive biopsies, screening for AlloAb showed a global specificity of 82% and sensitivity of 61.9%. When this analysis was restricted to biopsies obtained in the first month posttransplantation, the sensitivity increased to 81.8%, but the specificity decreased to 76.9%. After the first month posttransplantation, we observed sensitivity of 40.0% and a specificity of 86.4%. In the first month posttransplantation, all patients with a diagnosis of acute antibody-mediated rejection displayed circulating anti-HLA class I/II, but not always at the same time as the C4d-positive biopsy.

Conclusions. In the first month posttransplantation, prospective monitoring of anti-HLA antibodies may be useful. The high sensitivity allows the identification of patients at risk, affording an earlier diagnosis of antibody-mediated rejection. After the first month, the test can be used to evaluate allograft dysfunction episodes, since positivity is highly suggestive of an antibody-mediated process.

PRESENTLY, anti-HLA antibodies are recognized as important mediators of acute and chronic rejection, differing in pathogenesis from T-cell-mediated rejection. Alloantibodies (AlloAb) preferentially attach to peritubular and glomerular capillaries, in contrast to T cells, which typically infiltrate tubules and arterial endothelium. The important role of these anti-HLA AlloAb in mediating rejection had been emphasized first by Terasaki.¹ The antibodies found in the peripheral circulation were not necessarily donor-specific, and their association with failure was consistent with a causality hypothesis.² Several studies observed that patients who had rejected transplants displayed anti-HLA antibodies more frequently than those with functioning grafts. Hence it was concluded that screening for HLA antibodies posttransplantation could be an

important tool in patient follow-up.^{3,4} Deposition of complement fragment C4d in peritubular capillaries (PTC) is a marker of alloantibody-dependent graft injury. Currently, several questions need to be answered regarding the monitoring of circulating HLA antibodies.⁵ What is the more useful method? What is the optimal timing and frequency of monitoring? The purpose of our study was to determine the sensitivity and specificity of screening for circulating

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anti-HLA antibodies compared to the present gold standard test (C4d deposition in renal tissue).

MATERIALS AND METHODS

Over the last 3 years, we prospectively evaluated C4d deposition by an immunofluorescence technique applied to frozen allograft biopsy tissue (monoclonal antibody, Quidel) which had been obtained due to renal dysfunction or delayed graft function. A serum sample was obtained at the time of the biopsy to detect the presence of circulating anti-HLA class I/II AlloAb by the flow cytometric assay (FlowPRA screening test, Lambda one). The clinical parameters were registered at biopsy time. Data analysis was performed using the MedCalc software 9.0.1.1. Descriptive data were expressed as mean values $\pm$ standard deviations. Fisher test and independent sample Student *t* test were used for comparisons of groups. Receiver-operator characteristic (ROC) curves were used to calculate the sensitivity and specificity of the test (serum anti-HLA antibodies) for the disease (PTC C4d in renal tissue). Antibody-mediated rejection was diagnosed according to the 2007 Banff classification.⁶ *P* < .05 was considered to be significant.

RESULTS

We evaluated 132 biopsies and simultaneous serum samples in 92 patients (Table 1). PTC C4d deposition was demonstrated in 15.9% (21/132) of biopsies. We observed no significant difference between the incidence of C4d in PTC before or after the first month posttransplantation (17.5% vs 15.5%). Anti-HLA I/II AlloAb were detected in 25% (33/132) of serum samples at 31.6 ± 54.6 months posttransplantation. Among the positive serum samples observed in the first month after transplantation, 90.4% (19/21) were obtained from patients with panel-reactive antibodies (PRA) $\geq 75\%$ before transplantation. All patients with acute antibody-mediated rejection (AMR; *n* = 11) in the first month posttransplantation showed a positive result for circulating anti-HLA class I/II AlloAb, but not always in the simultaneous sample obtained concomitant with the index C4d-positive biopsy. Employing ROC curves (Table 2), we observed that screening for AlloAb showed a global specificity of 82% and a sensitivity of 61.9%. We found 8 false negatives: 6 biopsies (obtained at 7.8 ± 2.2 years posttransplantation) had additional evidence of chronic antibody-mediated rejection (CMR) and 2 biopsies obtained during treatment of AMR (0.34 ± 0.15 years). When the analysis

was restricted to biopsies obtained in the first month posttransplantation, the sensitivity increased to 81.8%, with a specificity of 76.9%. After the first month posttransplantation, the sensitivity was 40.0% and the specificity was 86.4%.

DISCUSSION

First Month Posttransplantation

In August 2007, the transplant allocation scheme changed in our country, with extra points awarded to sensitized patients. These allocations changes explained the high number of highly sensitized patients transplanted in the study period. We observed a high sensitivity for the method in the first month after transplantation. Almost all the patients with a positive screening test for anti-HLA antibodies in the first month were highly presensitized (PRA $\geq 75\%$) before transplantation, supporting the conclusion that these antibodies were probably preformed and not de novo generated. The absence of an absolute correspondence between the existence of circulating antibodies and C4d deposition may be explained by the different dynamics of timing between anti-HLA antibody detection, complement activation, and renal tissue damage, which are still not well understood.

A novel interesting approach could be the evaluation of sequential antibody titers in addition to C4d deposition in protocol biopsies. This type of study could elucidate the dynamics and timing of antibody-mediated rejection to clarify whether antibody titers could dictate the appropriate timing of a renal biopsy, and their usefulness to monitor renal damage and therapeutic responses.

After 1 Month Posttransplantation

The high specificity of the test signified that a positive result was associated with an episode of allograft dysfunction mediated by antibody and therefore useful for diagnosis. The lower sensitivity in antibody-mediated processes after the first month can be explained by 2 hypotheses: anti-HLA antibodies are absorbed by the graft and not detected in serum and other non-anti-HLA antibodies may be involved. For example, other antibodies such as those against endothelial cells observed in renal transplant recipients have been associated with graft loss. The impaired allograft

Table 1. Patient Characteristics

	Total (N = 132)	Anti-HLA Antibody Positive (n = 33; 25%)	Anti-HLA Antibody Negative (n = 99; 75%)	<i>P</i>
Age (y)	48.0 $\pm$ 12.6	46.5 $\pm$ 11.7	48.5 $\pm$ 12.9	.434
Gender	63 F; 69 M	19 F; 14 M	44 F; 55 M	.2684
Race	118 C; 14 A	27 C; 6 A	91 C; 8 A	.1917
Creatinine (mg/dL)	3.3 $\pm$ 2.1	3.5 $\pm$ 2.7	3.2 $\pm$ 1.9	.471
Time posttransplantation (mos)	39.3 $\pm$ 55.5	31.6 $\pm$ 54.6	41.8 $\pm$ 55.8	.366
C4d positive	21 (15.9%)	13	8	.0001
Previous transplantation	25 (18.9%)	12	13	.0071
PRA $\geq 75\%$	33 (25%)	18	15	<.0001

F, female; M, male; C, Caucasian; A, African; PRA, panel-reactive antibodies.

Table 2. Sensitivity and Specificity of the Test

	Sensitivity (%)	95% CI	Specificity (%)	95% CI	Area Under Curve
Total	61.90	38.5–81.8	81.98	73.6–88.6	.719
≤1 month posttransplantation	81.82	48.2–97.2	76.92	63.2–87.5	.794
>1 month posttransplantation	40.0	12.4–73.6	86.44	75.0–93.9	.632

CI, confidence interval.

survival of patients with MICA antibody⁷ and the finding of MICA antibodies in eluates of kidney transplants suggest that they can be involved in the pathogenesis of kidney allograft rejection.⁸

In conclusion, in the first month after transplantation, the prospective sequential monitoring of anti-HLA antibodies using a screening technique is useful. The high sensitivity allows the identification of at-risk patients and affords earlier procedures for diagnosis of antibody-mediated rejection including appropriate timing for identification of specific anti-donor antibodies using other more expensive solid phase assays (Luminex). After the first month, the test can be used to evaluate an allograft dysfunction episode, since positivity highly suggests an antibody-mediated process.

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