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Diagnosis of Acute Humoral Rejection by C4d and Donor Specific Antibody Testing

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DIAGNOSIS OF ACUTE HUMORAL REJECTION

BY C4d AND DONOR SPECIFIC ANTIBODY TESTING

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BACKGROUND

In transplanted kidneys the ischemia-reperfusion inflammatory response is paramount to setting the stage for acute rejection. Reperfusion of the renal allograft produces an inflammatory response in the graft. The production of cytokines and recruitment of inflammatory cells into the graft introduces recipient T cells to the foreign donor human leukocyte antigens (HLA) expressed on the vascular endothelium of the graft. Normal resting endothelium expresses Class I HLA; however the activated endothelium of a transplanted kidney also expresses Class II HLA.[2] The allograft also carries its own passenger leukocytes into the recipient. After reperfusion these cells migrate out of the graft, enter the venous circulation and are transported to regional lymphoid tissues. In the paracortex of the lymph nodes the passenger leukocytes contact naïve T cells.[5] Both areas of contact between donor antigens and recipient T cells allow for direct and indirect presentation of donor HLA to the recipients' T cells.

While T cells are the main mediators of graft rejection, the production of antibodies by B cells plays an important role in the effector phase of graft rejection, hyperacute rejection or accelerated acute rejection, acute humoral rejection, and chronic rejection.[2] The production of cytokines by activated CD4 T cells stimulates B cells to proliferate and differentiate into plasma cells. The antibodies produced by the plasma cells bind to the specific alloantigen, and either activate the complement system or mediate antibody-dependant cellular toxicity. [2]

Hyperacute rejection and accelerated acute rejection are the direct result of pre-formed HLA antibodies. These antibodies are formed by prior exposure to foreign HLA, either through prior transplant, transfusion, or pregnancy. The graft is lost either immediately (for hyperacute) or within the first few days (for accelerated acute). The circulating antibodies cause rapid and widespread vascular thrombosis which affects arteries, arterioles, and glomeruli; frequently incorporating polymorphonuclear leukocytes (PMNs) into the thrombi. Accelerated acute rejection causes anuria or oliguria, fever, and graft tenderness. There is little to no renal perfusion of the graft on renal scan and there may be evidence of intravascular coagulation. The incorporation of PMNs into the thrombi differentiates hyperacute and accelerated acute rejection from other early graft insults such as perfusion injury alone (due to arterial or venous thrombosis), or cold-reacting IgM red blood cell antibody damage. Due to highly sensitive crossmatch techniques this form of rejection has become increasingly rare. [2]

Acute humoral rejection (AHR) generally occurs in the first few weeks post-transplant, and the patient often has a history of prior sensitizing events. The patient may show nothing more than an asymptomatic rise in serum creatinine. There are two histologically recognized forms of AHR: the vascular type and the C4d positive type. Vascular humoral rejection is characterized by necrotizing arteritis, with mural fibrinoid necrosis and luminal thrombosis. In addition to the damage described for hyperacute rejection this form of rejection has

inflammatory infiltrates of lymphocytes, monocytes and PMNs. The end result is cortical infarction with focal interstitial hemorrhage.

The protein C4 is a factor in the classical complement pathway. After a complement binding IgG antibody binds to an antigen on the cell the C1qrs complex splits C4 into C4a and C4b. C4b is covalently bound to the cell surface and is used in the conversion of C3. After C3 has been converted C4b is converted to a catalytically inert C4d. The current criteria for diagnosis of C4d positive AHR requires a positive C4d staining in the peritubular capillary endothelium or basement membrane collagen. Histologically the kidney also shows scattered PMNs in the glomeruli, peritubular capillaries, and in the tubulointerstitium. It may be very difficult to distinguish between C4d positive humoral rejection and acute tubular necrosis or acute cellular rejection, and these conditions may also co-exist with the C4d positive AHR. In earlier studies C4d positive staining was associated with steroid resistant rejection, without histological evidence of classic vascular humoral rejection, 95% of the time. Other studies indicated that the presence of C4d staining was both sensitive (95%) and specific (96%) for the presence of DSA. [3]

Accurate and timely diagnosis of humoral rejection is crucial, as the standard treatment for acute graft dysfunction is high-dose steroids while humoral rejection does not typically respond to this treatment. [1] AHR does respond to treatment with intravenous immune globulin with or without plasmapheresis. Due to the potentially serious side-effects of IVIG, it is important to know with relative certainty that this costly treatment is warranted. [2]

AIM

Acute humoral rejection (AHR) is difficult to diagnose and portends a poor renal transplant outcome. Donor specific antibody titers (DSA) and staining of allograft biopsies for the complement degradation byproduct C4d have been utilized to establish the presence of AHR. We hypothesize that utilizing both tests would give a higher specificity for AHR, than C4d alone.

METHODS

We performed a retrospective analysis of all post-transplant biopsies at the University of New Mexico between January 2001 and June 2007. Biopsies were performed either for immunosuppression surveillance, usually at 3 and 6 months post-transplant, to direct treatment modification, or to rule out rejection in the clinical setting of a rising creatinine. C4d staining of biopsies was performed by immunohistochemistry. The development of post-transplant DSA was assessed at the time of biopsy either by flow cytometric or Luminex methodologies.

Biopsy diagnosis of acute humoral rejection was based on: PMN peritubular infiltrates, vasculitis, vascular necrosis, mononuclear intracapillary infiltrate, intracapillary thrombosis, arterial thrombosis, or the presence of significant C4d staining in the peritubular capillaries.

Other data collected to determine any significant interference included: patient sex, ABO blood group, creatinine at 1 month post transplant, creatinine at biopsy, creatinine at 1 and 6 months post biopsy, pre-transplant antibody status, post-transplant development of new non-DSA antibodies, number of mismatched antigens, outcome of allograft, and cross-match results.

RESULTS

We reviewed a total of 96 biopsies. Of these, the majority (56) were negative for both C4d and DSA. Fifteen were positive for both C4d and DSA. Eleven were positive for C4d only while ten were positive for DSA only. Finally, two were equivocal for C4d; one of which was positive for DSA while the other was negative for DSA. These findings are represented graphically in figure 1.1.

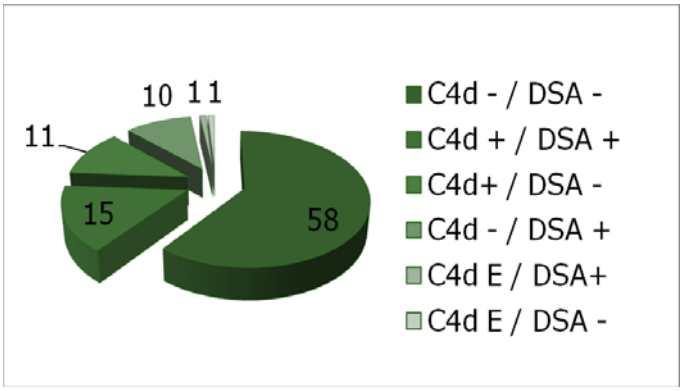

Fig. 1.1, C4d and DSA results for all biopsies reviewed

Of these 96 biopsies, 15 were suggestive of acute humoral rejection (AHR) based on the criteria outlined above in the methods section. Seven of the 15 were positive for both C4d and DSA, while eight were positive for C4d only. None of the 15 biopsies with AHR were negative for both C4d and DSA, positive for DSA only, or equivocal for C4d. These findings are represented graphically in figure 1.2.

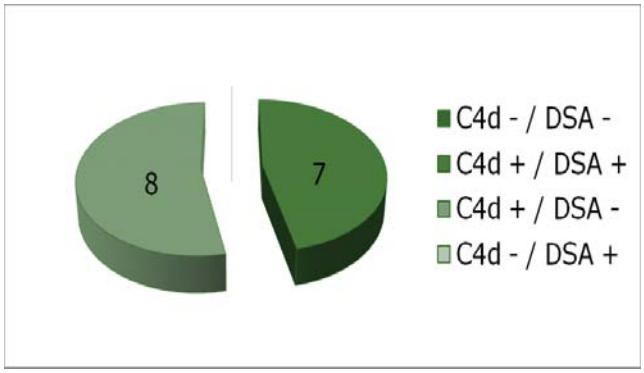

Fig 1.2, C4d and DSA results for the 15 biopsies that showed evidence of acute humoral rejection.

This means that all of the biopsies that demonstrated evidence of AHR were positive for C4d, indicating that C4d had a sensitivity of 100% in our study. Approximately half (53%) of these biopsies were also positive for DSA, giving DSA a much lower sensitivity. The sensitivities and specificities of both tests are shown below in table 1.1.

	C4d alone	DSA alone	C4d with DSA
Sensitivity	100	47	47
Specificity	84	74	90

Table 1.1 Sensitivity and Specificity of C4d, DSA, and combined testing

Interestingly, although the independent tests did not show impressive specificities, when combined they demonstrated an improved specificity of 90%. Based on this finding, our center has proposed following both tests for all patients following transplant.

Another value that we followed with interest was the creatinine level of each patient at four different points in time: one month after transplant, at the time of the biopsy (whether surveillance or for suspicion of rejection), and one and six months after biopsy. Although the numbers were not statistically significant, we found a trend in patients with a positive DSA result to have higher creatinine levels at one and six months post biopsy than patients with a negative DSA result, and the former were more likely to lose graft function. This is further support of the proposal to continue to follow DSA results post-transplant.

Other data collected was analyzed for significance as follows in table 1.2.

C4d/DSA		Total	N/N	P/P	P/N	N/P	E/P	E/N
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total		96 (100%)	58 (60%)	15 (16%)	11 (11%)	10 (10%)	1 (1%)	1 (1%)
ABO	A	28 (29%)	15 (26%)	4 (27%)	5 (45%)	4 (40%)	0 (0%)	0 (0%)
	B	5 (5%)	5 (9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	O	50 (52%)	35 (60%)	3 (20%)	4 (36%)	6 (60%)	1 (100%)	1 (100%)
	AB	4 (4%)	1 (2%)	3 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	p		0.44	**0.001**	0.436	0.745	0.864	0.864
Pre-txp Ab	Neg	63 (66%)	40 (69%)	10 (67%)	8 (73%)	4 (40%)	0 (0%)	1 (100%)
	CI	14 (15%)	8 (14%)	1 (7%)	0 (0%)	4 (40%)	1 (100%)	0 (0%)
	CII	5 (5%)	4 (7%)	0 (0%)	1 (9%)	0 (0%)	0 (0%)	0 (0%)
	CI&CII	9 (9%)	5 (9%)	2 (13%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)
	Unk	5 (5%)	1 (2%)	2 (13%)	2 (18%)	0 (0%)	0 (0%)	0 (0%)
	p		0.767	0.466	0.159	0.104	0.21	0.971
B cell XM	Neg	55 (57%)	44 (76%)	4 (27%)	4 (36%)	3 (30%)	0 (0%)	0 (0%)
	Pos	13 (14%)	5 (9%)	2 (13%)	3 (27%)	2 (20%)	0 (0%)	1 (100%)
	Historic Pos	3 (3%)	1 (2%)	1 (7%)	0 (0%)	1 (10%)	0 (0%)	0 (0%)
	Unkown	28 (29%)	9 (16%)	9 (60%)	4 (36%)	5 (50%)	1 (100%)	0 (0%)
	P		**0.033**	0.053	0.393	0.233	0.469	0.085
DSA	None	70 (73%)	58 (100%)	0 (0%)	11 (100%)	0 (0%)	0 (0%)	1 (100 %)
	CI only	7 (7%)	0 (0%)	3 (20%)	0 (0%)	4 (40%)	0 (0%)	0 (0%)
	CII only	16 (17%)	0 (0%)	9 (60%)	0 (0%)	6 (60%)	1 (100%)	0 (0%)
	Both	2 (2%)	0 (0%)	2 (13%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	P		0	0	0.269	0	0.176	0.949
Non DSA	None	68 (71%)	54 (93%)	1 (7%)	10 (91%)	2 (20%)	0 (0%)	1 100%)
	CI related	7 (7%)	4 (7%)	1 (7%)	1 (9%)	1 (10%)	0 (0%)	0 (0%)
	CII related	12 (13%)	0 (0%)	8 (53%)	0 (0%)	3 (3%)	1 (100%)	0 (0%)
	CI & CII related	3 (3%)	0 (0%)	3 (20%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Other	15 (16%)	0 (0%)	8 (53%)	1 (9%)	6 (60%)	0 (0%)	0 (0%)
	p		0	0	0.609	**0.002**	0.101	0.969

Table 1.2, results of AB blood group, pre-transplant antibodies (pre-txp Ab), B cell crossmatch (B cell XM), CI and CII related antibodies for all 96 biopsies.

CONCLUSION

In this retrospective analysis of 96 biopsies performed at the University of New Mexico Hospital between January 2001 and June 2007, C4d testing showed a higher sensitivity than donor specific antibody testing (DSA) for acute humoral rejection (100% vs. 47% for DSA). C4d and DSA each alone had similar specificities of 84% and 77% respectively, but together the two tests demonstrated a sensitivity and specificity for AHR of 47% and 90% respectively. While the sensitivity of the combined tests is diminished, the clinical utility of combining C4d and DSA is supported by the significant increase in the specificity.

As noted above, this increase in specificity, as well as the correlation with persistently raised creatinine levels and ultimate loss of graft function (although the latter were not statistically significant), has prompted these investigators to propose that DSA results be routinely monitored in renal transplant patients with the hope that AHR can be definitively diagnosed at an early time, allowing for more efficacious treatment.

This retrospective review also revealed a number of other interesting observations. To begin with, patients with blood group AB were more likely to demonstrate both C4d and DSA positivity than other blood groups. In addition, development of DSA was more likely to occur in patients with an unknown B cell crossmatch result. These crossmatch results were unknown because these transplants occurred prior to flow crossmatch methodology availability. Another unexpected finding was that patients with a weak positive B cell crossmatch and no identifiable CII DSA were more likely to have either C4d or DSA positivity. In addition, patients who developed only CI DSA were less likely to have a positive C4d stain. Interestingly, all 15 patients with AHR developed CI CREG related antibodies regardless of their C4d or DSA status. Also, patients with DSA positivity were more likely to also express CII related antibodies than DSA negative patients. Finally, unrelated antibodies were detected in all categories regardless of C4d or DSA status.

Other data that was collected but did not reveal statistical significance included: patient gender, T cell crossmatch results, number of mismatched antigens, and clinical indication for biopsy.

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