SUPPLEMENTAL TABLES AND FIGURES

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Supplemental Table 2. Clinical and histologic characteristics according to the preformed/*de novo* status of the post-transplant immunodominant donor-specific anti-HLA antibody

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Supplemental Figure 1. Kaplan-Meier curves for death-censored kidney allograft survival according to Day-0 IgG3-positive iDSA status (A), Day-0 C1q-positive iDSA status (B), post-transplant IgG3-positive iDSA status (C), and post-transplant C1q-positive iDSA status (D)

Supplemental Table 1. Characteristics of the post-transplant donor-specific anti-HLA antibodies according to their preformed/*de novo* status

	Preformed DSA N=81	***De novo* DSA N=105**	**P**
Characteristics of all anti-HLA DSAs			
Number - mean±SD	2.0±1.4	1.7±1.0	0.5894
HLA class specificity - n (%)			0.045
I	20 (24.7)	24 (22.9)	
II	28 (34.6)	54 (51.4)	
I + II	33 (40.7)	27 (25.7)	
Characteristics of immunodominant DSA			
HLA class specificity - n (%)			0.382
I	36 (44.4)	40 (38.1)	
II	45 (55.6)	65 (61.9)	
MFI - mean±SD	7778.6±5278.7	4179.1±3311.2	<0.001
C1q-binding - n (%)	36 (44.4)	21 (20.0)	<0.001
IgG subclasses - n (%)			
IgG1	68 (84.0)	69 (65.7)	0.005
IgG2	37 (45.7)	43 (41.0)	0.519
IgG3	25 (30.9)	17 (16.2)	0.018
IgG4	29 (35.8)	17 (16.2)	0.002

DSA, donor-specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity

Supplemental Table 2. Clinical and histologic characteristics according to the preformed/*de novo* status of the post-transplant immunodominant donor-specific anti-HLA antibody

	Preformed iDSA N=81	*De novo* iDSA N=105	P
Clinical characteristics			
eGFR at biopsy (mL/min/1.73 m^2) - mean±SD	41.9±20.6	40.7±19.3	0.7920
Proteinuria (g/g) - mean±SD	0.5±0.7	0.5±0.8	0.1657
Histologic characteristics			
Acute/active ABMR - n (%)	50 (61.7)	52 (49.5)	0.136
Chronic/active ABMR - n (%)	19 (23.5)	11 (10.5)	0.017
TCMR - n (%)	4 (4.9)	13 (12.4)	0.081
g + ptc score - mean±SD	2.9±1.6	2.1±1.8	0.0011
i + t score - mean±SD	0.9±1.5	1.3±2.1	0.3532
v score - mean±SD	0.2±0.5	0.2±0.7	0.8945
cg score - mean±SD	0.4±0.9	0.2±0.7	0.0186
IF/TA score - mean±SD	1.1±1.0	1.2±1.0	0.6320
cv score - mean±SD	1.5±1.1	1.3±1.0	0.3156
ah score - mean±SD	0.8±0.9	0.8±0.8	0.7527
C4d deposition - n (%)	34 (42.0)	25 (23.8)	0.008

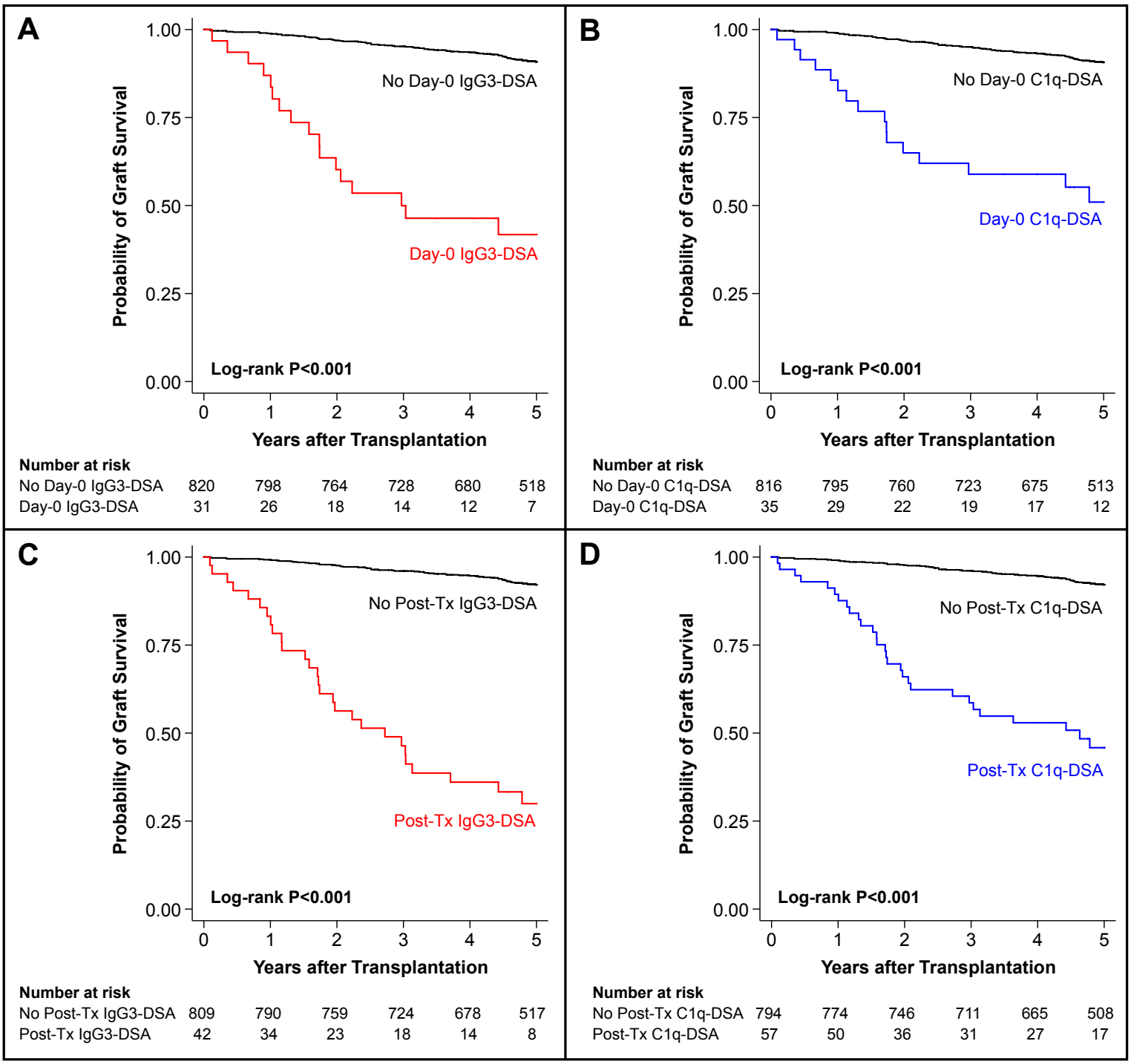
ABMR, antibody-mediated rejection; ah, arteriolar hyaline thickening; cg, allograft glomerulopathy; cv, vascular fibrous intimal thickening; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; g, glomerulitis; i, mononuclear cell interstitial inflammation; IF/TA, interstitial fibrosis/tubular atrophy; ptc, peritubular capillaritis; t, tubulitis; TCMR, T cell-mediated rejection; v, intimal arteritis

Supplemental Table 3. Predictive value for allograft loss of a strategy based on a systematic monitoring of anti-HLA DSAs and integration of anti-HLA DSA characteristics after excluding patients with preformed anti-HLA DSA

	N of patients	N of events	C-statistic [95%CI]	1000 bootstrap mean difference [95%CI]
Overall population				
Day-0 Reference Model	741	65	0.670 [0.614-0.726]	-
Post-Tx DSA Model	741	65	0.730 [0.678-0.782]	0.060 [0.059-0.063]
Post-Tx DSA Model + C1q	741	65	0.761 [0.708-0.814]	0.033 [0.032-0.034]
Post-Tx DSA Model + IgG3	741	65	0.771 [0.717-0.826]	0.040 [0.039-0.041]
Patients with *de novo* DSA				
Post-Tx MFI	105	19	0.751 [0.633-0.869]	-
Post-Tx MFI + C1q	105	19	0.779 [0.655-0.904]	0.028 [0.025-0.032]
Post-Tx MFI + IgG3	105	19	0.879 [0.770-0.988]	0.118 [0.115-0.122]

CI, confidence interval; DSA, donor-specific antibody; MFI, mean fluorescence intensity

Supplemental Figure 1. Kaplan-Meier curves for death-censored kidney allograft survival according to Day-0 IgG3-positive iDSA status (A), Day-0 C1q-positive iDSA status (B), post-transplant IgG3-positive iDSA status (C), and post-transplant C1q-positive iDSA status (D)
DSA, donor-specific antibody



SUPPLEMENTAL APPENDIX

Supplemental methods

Immunosuppression protocols

All of the patients received induction immunosuppressive therapy consisting of rabbit antithymocyte globulin (1.5 mg/kg/day for 10 days) or basiliximab (20 mg at day 0 and day 4). Maintenance immunosuppressive therapy consisted of prednisone, mycophenolate mofetil (1000 mg twice daily), and tacrolimus administered to maintain a trough level of 8 to 10 ng/mL for the first 3 months and 6 to 8 ng/mL thereafter or cyclosporine administered to maintain a two-hour post-dose level of 800 to 1200 ng/mL for the first 3 months and 600 to 800 ng/mL thereafter. In addition, the patients considered at the highest immunological risk (preformed DSA by Luminex assay with MFI >3000) received intravenous immunoglobulin (2 g/kg body weight on day 0, day 20, and day 40).

Treatment of allograft rejections

All of the patients who had episodes of acute antibody-mediated rejection were equally treated with methylprednisolone pulses (500 mg/day for 3 days), intravenous immune globulin (2 g/kg, repeated every three weeks for 4 rounds), four plasmaphereses and two weekly doses of rituximab (375 mg/m^2 body-surface area). Patients with T cell-mediated rejection were treated with methylprednisolone pulses (500 mg/day for 3 days).

Statistical analysis interpretation

Discrimination

The C-statistic is a generalization of the area under the ROC curve (AUC). The C-statistic estimates the proportion of all pairwise patient combinations from the sample data for which the model assigned higher probability to the person who would experience the event than the person who would not. The C-statistic ($0 \leq C \leq 1$) is the probability of concordance between predicted and observed survival, with C-statistic = 0.5 for random predictions and C-statistic = 1 for a perfectly discriminating model. The C-statistic, used to assess the discriminative ability of the models proposed in this study, is the gold standard method in the context of survival-censored data.[1]

Risk reclassification

Once improvement in model discrimination was demonstrated, we used category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to assess risk reclassification. These statistics were specifically developed to assess the risk reclassification provided by a new biomarker when added to a reference model, given the shortcomings of standard methods (significance of P-values and C-statistics, which do not measure clinically meaningful quantities).[2,3]

Category-free net reclassification improvement

Category-free NRI is the sum of the net percentages of persons with and without the event of interest (allograft loss) correctly assigned to a different predicted risk when adding the new biomarker to the reference model. The change in individual

calculated risk is in the correct direction if it is higher for patients with allograft loss and lower for those without allograft loss (Figure 5). It can be interpreted as a measurement of event rate increase among those reclassified upwards and event rate decrease among those reclassified downwards. The theoretical range is -2 to 2.[2,3]

Integrated discrimination improvement

The IDI considers the magnitude of the change in individual calculated risk of allograft loss in patients with allograft loss and in those without allograft loss when adding the new biomarker to the reference model and not the direction of the change, as with the NRI. The change in the predicted risk of allograft loss is adequate if it is positive for patients with allograft loss (increased calculated risk) and negative for those without allograft loss (decreased calculated risk).

Thus, the IDI allows for the examination of the magnitude of the change in the predicted risk of allograft loss (Figure 4), while NRI allows for the examination of the direction of the change in the predicted risk (Figure 5).[2,3]

Finally, C-statistic increase, NRI and IDI provide three different and complementary approaches to evaluating the additive value of a new biomarker to a reference model for risk stratification. In the present study, these 3 statistics converged to indicate an incremental value of anti-HLA DSA monitoring and characterization for the risk stratification of allograft loss beyond conventional determinants.

Supplemental references

1. Pencina MJ, D'Agostino RB: Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. *Stat Med* 23: 2109-2123, 2004

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