A composite score associated with spontaneous operational tolerance in kidney transplant recipients

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New challenges in renal transplantation include using biological information to devise a useful clinical test for discerning high- and low-risk patients for individual therapy and ascertaining the best combination and appropriate dosages of drugs. Based on a 20-gene signature from a microarray meta-analysis performed on 46 operationally tolerant patients and 266 renal transplant recipients with stable function, we applied the sparse Bolasso methodology to identify a minimal and robust combination of six genes and two demographic parameters associated with operational tolerance. This composite score of operational tolerance discriminated operationally tolerant patients with an area under the curve of 0.97 (95% confidence interval 0.94–1.00). The score was not influenced by immunosuppressive treatment, center of origin, donor type, or post-transplant lymphoproliferative disorder history of the patients. This composite score of operational tolerance was significantly associated with both *de novo* anti-HLA antibodies and tolerance loss. It was validated by quantitative polymerase chain reaction using independent samples and demonstrated specificity toward a model of tolerance induction. Thus, our score would allow clinicians to improve follow-up of patients, paving the way for individual therapy.

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Because of immunosuppression side effects,[1,2] physicians are encouraged to reduce immunosuppression while still protecting the graft from immune aggression.[3] No clinical biomarker that allows the safe personalization of immunosuppression has been validated.[4,5] Achieving allograft tolerance in solid organ transplantation—allograft acceptance in the absence of immunosuppression—would be a tremendous stride forward by avoiding these side effects but also by decreasing the cost of transplantation maintenance[6] while improving recipient quality of life. Several protocols of tolerance induction have been, attempted but these approaches remain experimental.[7–12] Spontaneous tolerance has also been observed as a result of immunosuppression interruption for noncompliance or medical decisions (especially posttransplantation lymphoproliferative disorders).[13–15] These operationally tolerant (TOL) recipients display stable and good graft function for years, respond to immunologic challenge,[13,16] and do not experience more opportunistic infections than healthy volunteers.[13,14] From a clinical point of view, these patients are comparable to renal recipients with stable graft function under standard immunosuppression (STA) with only a few differences, including a higher proportion of grafts from living donors and lower levels of human antileukocyte antigen (HLA) mismatch.[13,17]

To date, no parameter has been identified that will safely permit weaning off immunosuppression, even in trials based on a stringent selection of nonsensitized recipients.[18–20] Thus, the intentional replication of immunosuppression withdrawal in renal transplantation requires the integration of appropriate clinical parameters and new laboratory tests. Our group and others highlighted gene signatures associated with operational tolerance, but none have yet been evaluated in clinical trials.[17,21–30] Recently, an integrative meta-analysis further highlighted 20 genes, mainly B cell related, specific to operational tolerance.[21] Collectively, these reports suggest that B cells of tolerant patients may offer potential biomarkers of low immune risk in transplantation and may actively regulate the immune response to a transplanted kidney, with their induction and expansion likely being favored by induction therapies.[31] Although the utility of such signatures

has been established,[32,33] we need to demonstrate their safety and reliability for immunosuppression minimization and follow-up of transplant recipients.

Herein, we identified and validated a composite score that allows TOL patients to be identified with excellent accuracy, representing a potential predictive score of tolerance applicable in clinical practice in order to improve follow-up of renal transplant recipients.

RESULTS

Clinical parameters associated with operational tolerance

From the meta-dataset that we previously described,[21] clinical data from Nantes, Indices of Tolerance (IOT), and Immune Tolerance Network (ITN) databases were used to identify 312 nonredundant patients: 46 individual TOL patients of 96 TOL samples and 266 STA patients of 311 STA samples (Supplementary Table S1). To construct a predictor score of operational tolerance easily applicable and reproducible in various centers, we selected intrinsic and nonvariant patient-related clinical parameters, excluding parameters that may depend on technique and transplant center. Due to missing data, likely reflecting patient noncompliance, only parameters available for at least half of the TOL patients were used. Four of these parameters associated with operational tolerance status ($P < 0.20$) were selected: age at transplantation ($P < 0.0001$), age at testing ($P = 0.176$), number of HLA mismatches ($P < 0.0001$), and donor sex ($P = 0.154$) (Supplementary Table S2).

Composite score of operational tolerance

Expression levels of the 20 genes that were previously reported as the differential between TOL and STA patients[21] were confirmed in this set of 312 patients (46 TOL and 266 STA patients; $P < 0.0001$). To identify the most discriminative combination in the 20 genes and the 4 clinical parameters, we used the Bolasso method,[34] which is a least absolute shrinkage and selection operator regression analysis combined with bootstrap resampling (10,000 times) followed by multiple testing (false discovery rate <0.05).[35]

We identified a combination of 6 genes (*AKR1C3* [aldo-keto reductase family 1], *CD40*, *CTLA4* [cytotoxic T-lymphocyte-associated protein 4], *ID3* [inhibitor of DNA binding 3], *MZB1* [marginal zone B and B1 cell–specific protein], *TCL1A* [T-cell leukemia/lymphoma protein 1A], and 2 clinical parameters (age at testing and age at transplantation) (Figure 1a and b) that enabled us to establish a composite score, the composite score of operational tolerance (cSoT), discriminating TOL and STA patients (42 TOL patients, 189 STA patients, $P < 0.0001$) with an area under the curve (AUC) of 0.973 (95% confidence interval [CI] 0.939–1.00), with negative and positive predictive values of 0.989 and 0.800, respectively (Figure 1c, d). The consistency of the 8 selected parameters was validated by a 10-fold cross-validation repeated 100 times. The 8 selected parameters were present in at least 80% of the 1000 generated models (Figure 1a). The cSoT robustness was validated by a 10-fold cross-validation repeated 100 times with a mean AUC for test sets of 0.967 (95% CI 0.966–0.968). The cSoT discriminated TOL patients from STA patients significantly better than each parameter alone ($P < 0.0001$, Figure 1c) and better than graft function (AUC = 0.615). The cSoT represents the best combination of parameters compared with the combination of the 6 genes only as observed by the goodness of fit of these scores ($P < 0.0001$ in a Fisher test based on the residual sum of squares). Inherent in this composite score and due to the Lasso method, cSoT equation coefficients provide biased and limited information to interpret parameters contribution.[36,37] However, removing either the 2 age parameters or the 6 genes decreases the AUC values compared with the cSoT (AUC = 0.947 and 0.828, $P = 0.10$ and 0.00031, respectively), and gene expression contributes more significantly than demographic parameters ($P = 0.011$, Supplementary Figure S1). A final cross-validation was then performed on a recent microarray dataset composed of 16 TOL patients and 9 patients with chronic allograft nephropathy.[38] The combination of the 6 genes allowed a significant discrimination of the TOL patients from the others (AUC = 0.825, 95% CI 0.636–1.014; $P = 0.0061$).

Center of origin, posttransplantation lymphoproliferative disorder, donor type, and immunosuppressive regimen do not influence the cSoT

Despite the heterogeneity of TOL samples obtained from multiple sites (Nantes, IOT, and ITN) and different blood collection methods,[17,24,28,39,40] the cSoT is not influenced or associated with patient origin ($P = 0.13$; Figure 2a). Our analysis failed to reveal an association with a history of a posttransplantation lymphoproliferative disorder, the main intentional reason for cessation of immunosuppression ($N = 4$, $P = 0.19$, Figure 2b). Despite an imbalance of donor type (living vs. nonliving donor) in our meta-dataset (Supplementary Table S1), scores were not different between TOL samples receiving organs from living donors or nonliving donors ($P = 0.58$; Figure 2c). With nonliving donors only, the cSoT is still able to differentiate TOL patients from STA patients with a very good AUC (AUC = 0.977, 95% CI 0.9559–0.9975, 15 TOL patients, 189 STA patients). Because the 2 patient groups used to create the cSoT differed in immunosuppression status (STA patients are under immunosuppression and TOL patients received no more immunosuppression), we assessed whether immunosuppression could affect the cSoT values. Regarding the TOL patients, the previous immunosuppression regimen before its withdrawal, including cyclosporine A (CsA), mycophenolic acid, and azathioprine did not influence cSoT values ($P = 0.74$, 0.81, and 0.61, respectively; 29 TOL patients, Figure 2d). Similarly, in the STA population ($N = 189$), cSoT was not influenced by current calcineurin-based immunosuppression regimen (i.e., CsA or tacrolimus [$P = 0.64$], corticosteroids [$P = 0.42$], and antimetabolite agents [$P = 0.66$]) (Figure 2e). Finally, we tested the effect of immunosuppression on the cSoT in 2 independent cohorts of STA patients[41,42]: 1 cohort