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.


KEYWORDS: biomarker; gene expression; graft survival; operational tolerance; renal transplantation

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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.4–6 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 maintenance6 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, 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, 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 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, 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).

We identified a combination of 6 genes \( (AKR1C3 \) [aldol-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 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 \( (P < 0.0001, \text{Figure 1c}) \) 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. However, removing either the 2 age parameters or the 6 genes decreases the AUC values compared with the cSoT \( (AUC = 0.947 \text{ and } 0.828, P = 0.10 \text{ and } 0.00031, \text{respectively}) \), and gene expression contributes more significantly than demographic parameters \( (P = 0.011, \text{Supplementary Figure S3}) \). A final cross-validation was then performed on a recent microarray dataset composed of 16 TOL patients and 9 patients with chronic allograft nephropathy. The combination of the 6 genes allowed a significant discrimination of the TOL patients from the others \( (AUC = 0.825, 95\% \text{ CI } 0.636–1.014; P = 0.0061) \).

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, the cSoT is not influenced or associated with patient origin \( (P = 0.13; \text{Figure 2a}) \). Our analysis failed to reveal an association with a history of a posttransplantation lymphoproliferative disorder, the main intentional reason for cessation of immunosuppression \( (N = 4, P = 0.19, \text{Figure 2b}) \). 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 \( (P = 0.58; \text{Figure 2c}) \). With nonliving donors only, the cSoT is still able to differentiate TOL patients from STA patients with a very good AUC \( (AUC = 0.977, 95\% \text{ CI } 0.9559–0.9975, 10 \text{ TOL patients, 189 STA patients}) \). 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 \( (P = 0.74, 0.81, \text{and } 0.61, \text{respectively}; 29 \text{ TOL patients, Figure 2d}) \). 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: 1 cohort...
of patients under CsA (N = 14) or rapamycin (N = 23) monotherapy and a second set of patients after a conversion from azathioprine to mycophenolic acid (N = 5 paired before and 3 months after mycophenolic acid conversion). Neither the combination of the 6 genes (P = 0.99 and 0.77, respectively; Figure 2f) nor the 6 genes independently (Supplementary Figure S2) were modified according to immunosuppression regimen.

cSoT is associated with de novo antibody and immune tolerance breakdown

We previously reported that loss of graft function may be observed in the long-term survey of our TOL cohort. Among the 15 TOL patients from the Nantes cohort, for which most clinical data were available, 10 showed a decline in function during follow-up (17.15 ± 3.27 years post-transplantation; Supplementary Figure S3). We measured the cSoT at a time when all patients still exhibited a good graft function (creatinemia, <150 μmol/l; proteinuria, <1 g/24 hr) and found that the cSoT was not predictive of isolated progressive long-term degradation of graft function (P = 0.14; data not shown). In contrast, of these 10 patients, the 7 patients who had impaired function and in whom de novo anti-HLA antibody (Ab) developed after immunosuppression withdrawal had a lower cSoT (N = 7, mean cSoT = 2.73 ± 1.24) than the 3 patients who only showed degraded graft function, without associated anti-HLA Ab appearance (mean cSoT = 8.34 ± 1.37; P = 0.026; Figure 3a), although these patients presented similar function at the time of testing (P = 0.81, mean = 120.7 ± 8.78 and 125.0 ± 14.36). Regarding initial pathology, of the 3 patients who had impaired function and no donor-specific Ab (DSA), 2 had pyelonephritis and 1 had glomerulonephritis/sclerosis, whereas of the 7 who had impaired function and in whom DSA developed, 4 had glomerulonephritis/sclerosis, 1 pyelonephritis, and 2 an unclassified etiology. Biopsy samples were available for 3 of the patients with anti-HLA Ab, highlighting lesions of chronic Ab-mediated rejection for 2 of them (cases 7 and 10) and for 1 patient without anti-HLA Ab that showed only isolated and nonspecific lesions (case 5).
Finally, we examined the association of the cSoT with de novo anti-HLA only, independent of graft loss. Of the 15 TOL patients, de novo anti-HLA Ab developed in 8 (14.67 ± 1.13 years posttransplantation, Supplementary Figure S3) and DSA developed in 4 patients as assessed by LABScreen single Antigen assay (Labscreen Single Antigen; One Lambda, Canoga Park, CA) (13.41 ± 0.21 years posttransplantation). We found that the cSoT is significantly associated with TOL patients who developed de novo anti-HLA Ab after immunosuppression withdrawal ($P = 0.016$ and 0.015; collection time = 1.59 ± 2.20 years and 0.29 ± 1.24 years before Ab detection, for anti-HLA Ab and DSA, respectively; Figure 3b). Although the number of patients was limited, these data suggest that the cSoT is associated with immune tolerance breakdown with humoral immunologic signs of rejection and not with an isolated decrease of graft function alone.
cSoT is specific for the spontaneous operational tolerance state

We applied the cSoT in a trial of tolerance induction in which 15 renal recipients of HLA-identical living donor siblings were followed for 5 years after transplantation.9,10 The protocol consisted of infusions of donor hematopoietic CD34+ stem cells with a conditioning regimen, and immunosuppression was withdrawn 2 years after transplantation. Both the cSoT and the 6 genes score failed to classify the 5 tolerant patients as TOL, before and after cessation of immunosuppression, independent of the time posttransplantation (Supplementary Figure 4A). These data suggest that the cSoT is specific to the operational tolerance state and reinforce the fact that the cSoT is not influenced by immunosuppression treatment.

A quantitative polymerase chain reaction cSoT applicable in clinical settings

Quantitative PCR (qPCR) was performed in samples from 5 independent TOL patients and 5 other TOL patients from the meta-dataset at different time points compared with 12 independent STA patients. The cSoT was able to discriminate TOL and STA patients (P = 0.0072, N = 10 and 12, mean = 2.48 ± 5.26 and −2.58 ± 2.37) with an AUC of 0.842 (95% CI 0.65–1.00; Figure 4). Furthermore, a bootstrap procedure (1000 times) confirmed the robustness of this validation with a mean AUC of 0.840 (95% CI 0.833–0.846). These data demonstrated that the cSoT microarray-derived score can be reproduced using qPCR technology and thereby supporting its use in the clinical setting.

DISCUSSION

Finding the right—or at least the best—balance in drug dosage to avoid both rejection and adverse events is a great challenge for clinicians.4 Major objectives are to find new immunosuppressive drugs to prevent rejection and to identify biomarkers that would allow predicting rejection and assessing the level of immunosuppression “necessary and sufficient” for a selected patient. Ideally, such a biomarker, or most likely a panel of biomarkers, can be performed rapidly, is sensitive and inexpensive, and should reveal changes in the level or nature of the alloresponse, indicating the need for an appropriate modification of treatment to prevent under- or overimmunosuppression.

Operational tolerance, observed in patients with prolonged graft survival in the absence of immunosuppression and without evidence of destruction of the graft,13–15 offers a
unique situation for the discovery of biomarkers of low immunologic risk. The identification of a common blood B-cell signature in several independent studies\textsuperscript{17,27,28} and its recent confirmation through a meta-analysis\textsuperscript{21} have raised the interest that this signature could provide a useful monitoring tool to improve clinical management of recipients.\textsuperscript{15} To create a minimally invasive and clinically scalable tool, we adjusted a stable and robust model of nonredundant information composed of 6 genes and 2 clinical parameters. The cSoT allowed differentiation of 42 TOL patients from 189 STA patients more accurately than any parameter alone or creatininemia, the most commonly used biomarker to date. This composite score was tested on 231 recipients (42 TOL, 189 STA), cross-validated in an independent dataset,\textsuperscript{38} and validated with qPCR on independent samples with excellent AUCs.

Whereas the TOL state is probably the combination of some intrinsic factors inherent in the donor and the recipients and external influences such as the environment, in accordance with previous reports,\textsuperscript{17,21,27,28} the 6-gene signature is mainly related to a unique B-cell population. TCL1A was reported to be overexpressed in TOL patients in 3 analyses\textsuperscript{17,21,28} and to be decreased during acute rejection, stressing the potential of TCL1A as a marker of immune regulation.\textsuperscript{43,44} Similarly, AKR1C3 was also reported to be overexpressed in TOL patients in 3 analyses.\textsuperscript{17,21,40} This dominant B-cell signature fits with the immunoregulatory properties of B cells reported in TOL patients\textsuperscript{25,27,45} and with the fact that STA patients with superior graft function have a larger number of B cell subsets.\textsuperscript{46} Other gene expression–derived scores for tolerance have been recently reported,\textsuperscript{17,28,30,38} but because of the absence of these genes in the meta-dataset\textsuperscript{21} and the small number of samples in each separate study, patient variability and technical heterogeneity make comparison impossible. In addition to the 6 genes, 2 clinical parameters have been incorporated in the cSoT. Although the 6-gene signature is related to B cells, no association between age at transplantation and B-cell frequency was reported,\textsuperscript{27} as also confirmed by the absence of a correlation between CD20 gene expression (MS4A1) and age at transplantation in both TOL and STA patients ($r = 0.010$ and $-0.012$, $P = 0.95$ and 0.87, $N = 42$ and 190, respectively). Younger recipients at transplantation were associated with a higher tolerance prediction in accordance with previous observations.\textsuperscript{13–15} This association may reflect the lower percentage of experienced antigen memory cells in younger recipients that accumulate with age and prevent graft tolerance.\textsuperscript{47} In liver transplantation, Sánchez-Fueyo and colleagues reported that immunosuppression withdrawal success was directly correlated with the time posttransplantation.\textsuperscript{48} This is likely due to the fact that older recipients exhibit fewer acute rejection episodes\textsuperscript{49} and are less prone to the development of de novo DSA.\textsuperscript{50}

As previously reported,\textsuperscript{15,17,21,40} we chose to devise the score based on a comparison of TOL and STA patients. The “stable” population appears to be the most clinically relevant as future potential candidates for minimization protocols. Nevertheless, because immunosuppression has been shown to alter gene expression,\textsuperscript{41,42,51,52} and particularly circulating B cells,\textsuperscript{53} immunosuppression may affect the cSoT. Unfortunately, samples before immunosuppression withdrawal are not available, notably because TOL patients are mainly noncompliant and intentional immunosuppression withdrawal trials have failed.\textsuperscript{18,20} However, several elements argue against that. First, we tested this score among different cohorts with different immunosuppression regimens\textsuperscript{11,42} and found that the cSoT was not influenced by current or previous immunosuppression treatments. None of the 6 genes was differentially expressed in association with calcineurin inhibitor, mycophenolate mofetil, azathioprine, or steroids in a recent report demonstrating the association of immunosuppression with genes from the Indices of Tolerance signature.\textsuperscript{30} Finally, the fact that the cSoT was neither modulated after immunosuppression withdrawal in a tolerance induction protocol nor decreased in TOL patients who lost tolerance despite the absence of treatment reinforces this conclusion and the robustness of the score.

There is accumulating evidence that tolerance may break down even years after transplantation in TOL patients.\textsuperscript{13–15} However, to what extent the loss of graft function is due to an active shift of tolerance driven by immunologic phenomena and not a physiologic degradation of the kidney has not yet been demonstrated as few biopsy specimens have been examined.\textsuperscript{15} The cSoT was associated with de novo anti-HLA Ab, including DSA, with or without graft dysfunction, strongly suggesting that the cSoT is specific for immunologically driven mechanisms, as confirmed by the results of 2 available biopsy specimens. This fits with an cSoT largely based on B cell–related genes fitting with a breakdown of B-cell tolerance during chronic Ab-mediated rejection\textsuperscript{54} or pretransplantation sensitization and retransplantation that may be associated with a loss of tolerance.\textsuperscript{15,55} In this study, 7 TOL patients had a panel reactive Ab test before transplantation and 5 had decreased function in the presence of de novo anti-HLA Ab 15.12 ± 2.43 years after transplantation. Thus, the cSoT may not only be useful to discriminate patients with a profile of operational tolerance among STA patients but would also be helpful to follow-up TOL patients themselves. This finding also stresses that anti-HLA Ab seems to be inherently incompatible with the intrinsic definition of tolerance in clinical settings. If this highlights the limitation of the definition of “operational tolerance”\textsuperscript{54} and provides clear evidence of its metastable status, it also validates the importance of this score that is able to discriminate anti-HLA Ab+ and anti-HLA Ab− TOL patients. Indeed, current clinical trials would preclude drug minimization in any patient who would have been selected on the basis of a signature observed in sensitized TOL patients.

Some inducing therapies, such as alemtuzumab, are associated with rapid and long-term expansion of different B-cell subsets,\textsuperscript{51,56,57} likely favoring a state of tolerance. We took advantage of a transcriptomic analysis of nonchimeric tolerance induction using lymphodepleting alemtuzumab...
In agreement with no evidence of B cell–related signature in these patients,9,10 the cSoT was not discriminative in these patients. Whether the dissimilarity of these results reflects the deep B-cell depletion following induction or that the set of genes in the cSoT may need more time after transplantation to be expressed cannot be totally excluded.57 This suggests different immunologic statuses of the 2 types of tolerance, with an immune quiescence on the one hand9 and a full immunocompetence on the other.16 Contrasting results were reported by Newell’s group with patients harboring the B cell tolerance signature of Immune Tolerance Network,17,21 suggesting that different mechanisms may be involved in tolerance induction, according to the protocol and likely due to the chimerism levels. Tolerance is not an “all or none” phenomenon, as reported by Newell et al.58

In summary, we proposed a simple score that is easily applicable in routine clinical settings, using standard qPCR practices, and not influenced by immunosuppression, donor type or posttransplantation lymphoproliferative disorder experience. The cSoT is specific to spontaneous operational tolerance and associated with de novo anti-HLA Ab linked to tolerance loss.

This score would not only allow the detection of patients with a tolerance profile but may also be used for monitoring patients and prevent under- or overimmunosuppression, in the general kidney recipient population. Indeed, in addition to being markers of tolerance, several reports showed that the expression of some of these B-cell genes could be used to diagnose patients without acute rejection,43,44 suggesting that this score may also be useful for identifying immune activation or a lack of B-cell regulation. Based on sample size and selected nature of the population, tolerance signature may not be universally applicable yet and needs further validation in a large cohort of patients. Nevertheless, we think that such a score may allow proposing high- or low-risk individual patient therapies with the best drug combinations and appropriate dosages in the future.

METHODS
Gene expression dataset
The gene expression meta-dataset was obtained from the Gene Expression Omnibus (GEO) database (GSE28456) as previously described.51 These data are the results of a meta-analysis of 5 independent studies gathering 596 samples17,24,28,39,40 and composed of 1846 merged genes.51 Demographic description of available clinical parameters from the 312 identified patients (46 TOL and 266 STA) are given in Supplementary Table S1. Three publically available microarray datasets were collected from the GEO database: GSE14630,41 GSE22224,42 and GSE45593 and normalized with the robust multiarray average method (RMA, affy package59 with R software, R Foundation, Vienna, Austria). Normalized collected expression values from dataset GSE4521857 were used for in silico cross-validation.

Validation cohort
Twenty-two kidney recipients from Nantes Hospital were enrolled to perform qPCR validation, including 10 TOL and 12 STA patients (Supplementary Table S3). The local ethics committee approved all aspects of this study, and all patients gave their written informed consent.

qPCR validation
Venous blood samples were collected in ethylenediamine tetraacetic acid vacutainers and peripheral blood mononuclear cells were separated on a Ficoll layer (Eurobio, Les Ulis, France) and frozen in TRIzol reagent (Thermo Fisher Scientific, Waltham, MA) at −80°C. qPCR was performed on a StepOnePlus instrument (Thermo Fisher Scientific) using commercially available primer and probe sets (Taqman, Thermo Fisher Scientific) for the 6 tested genes and 4 reference genes (ACTB, B2M, GAPDH, and HPRT1).

cSoT construction
Parameters associated with TOL patients compared with STA patients in a logistic univariate analysis (glm package) were used for cSoT construction with $P < 0.20$ in order not to bias parameter selection.60 To identify the most discriminative combination among the 24 parameters associated with tolerance in univariate analysis, we used the Bolasso method,54 which involves bootstrap resampling (10,000 times) combined with a least absolute shrinkage and selection operator regression analysis followed by multiple testing to select the significant variables associated with the model (false discovery rate <0.05) using the mht package (version 3.2.2).35 This cSoT was centered on using the best threshold of the receiver-operating characteristic curve (Youden index) to associate positive and negative scores with TOL and STA diagnosis, respectively. An inconclusive zone ("gray zone") was defined by values with specificity and sensitivity <90% (predictive tolerance of 10%) (Figure 1d, Supplementary Figure S1).61

From other microarray datasets or qPCR experiments, expression and demographic values are centered/scaled, as performed by the Bolasso algorithm, and the following equation is then applied: $cSoT = 1.231 \times \text{Expr}_{\text{AKR1C3}} + 1.038 \times \text{Expr}_{\text{CD40}} + 0.937 \times \text{Expr}_{\text{CTLA4}} + 1.386 \times \text{Expr}_{\text{D3}} + 1.163 \times \text{Expr}_{\text{MZB1}} + 1.223 \times \text{Expr}_{\text{TCL1A}} + 2.908 \times \text{age}$. 

Statistical analysis
Statistical analyses were performed using R software version 3.2.2 or GraphPrism version 4 software (GraphPad Software, La Jolla, CA). Parametric analysis of variance with a Tukey post hoc test, Student t test, or $\chi^2$ test were used for group comparisons. Differences were defined as statistically significant when $P < 0.05$ or as indicated. Additional details can be found in the Supplementary Material.

DISCLOSURE
All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL
Supplementary Materials and Methods
Table S1. Demographic and clinical parameters of operationally tolerant (TOL) patients (N = 46) and renal transplanted patients with stable graft function (STA) under an immunosuppressive regimen (N = 199).

Table S2. Parameters associated with tolerance status according to univariate logistic regression (operationally tolerant [TOL] patients, N = 46); only intrinsic and nonvariant patient-related demographic parameters and known for at least half of the TOL patients were tested.

Table S3. Demographic parameters of patients used in the quantitative polymerase chain reaction validation step (operationally tolerant [TOL] patients, N = 10; renal transplanted patients with stable graft function under immunosuppressive regimen [STA], N = 12).

Figure S1. (A) Sensitivity (green line) and specificity (blue line) according composite score of operational tolerance (cSoT) values. The red line represents the centered best threshold of the receiver-operating characteristic curve (Youden index). The gray zone represents the inconclusive zone defined by values with specificity and specificity < 90% (predictive tolerance of 10%). (B) Removing either the 2 age parameters (green line) or the 6 genes (black line) decreases the area under the curve (AUC) values compared with the cSoT values (red line) (AUC = 0.947 and 0.828, P = 0.10 and 0.00031, respectively).

Figure S2. Individual expression for the 6 genes of the score calculated using 2 available microarray datasets of renal recipients with different immunosuppression regimens: cyclosporine A (CSA) versus rapamycin (Rapa) (A, N = 14 and 23) and conversion from azathioprine (Aza) to mycophenolic acid (MPA) (B, N = 5). Unpaired (A) and paired (B) P values from t tests are displayed.

Figure S3. Anti-human leukocyte antigen antibody (HLA Ab) status and outcome for the 15 operationally tolerant patients for whom clinical data were available. The t test P values of comparison of composite score of operational tolerance (cSoT) values between indicated groups are indicated. Histologic diagnostic biopsies were performed in a previous study, and corresponding cases are displayed in brackets (cases 5, 7, 10, and 13). CAMR, chronic antibody-mediated rejection; CCR, chronic cellular rejection; DSA, donor-specific antibody; IF/TA, interstitial fibrosis with tubular atrophy.

Figure S4. The composite score of operational tolerance (cSoT) does not diagnose induction of tolerance after alemtuzumab treatment and multiple hematopoietic stem cell infusions. Individual cSoT values as a function of time posttransplantation at testing for patients with successful tolerance induction during the posttransplantation period. The gray zone represents the inconclusive zone defined by values with specificity and sensitivity < 90%.

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

REFERENCES


