Derivation and external validation of the SIMPLICITY Score as a simple immune-based risk score to predict infection in kidney transplant recipients.

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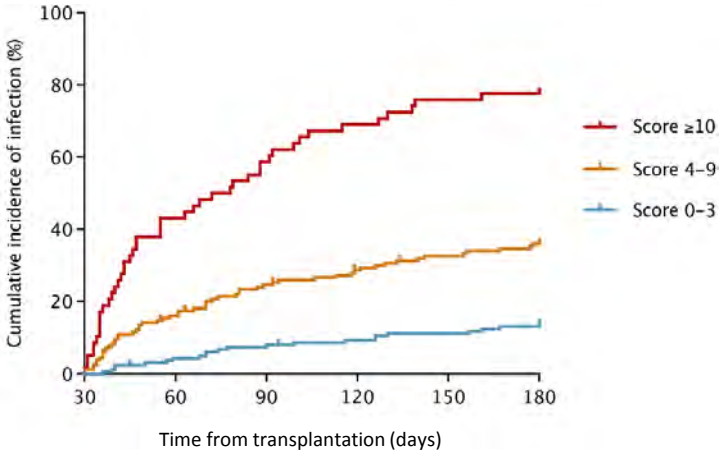
Derivation and external validation of the SIMPLICITY Score as a simple immune-based risk score to predict infection in kidney transplant recipients.

Construction of the risk score → Derivation Cohort (n = 410) → Validation Cohort (n = 522)

- Retrospective analysis of prospectively assembled cohort
- Single center in Madrid (Spain)
- November 2008 and March 2013
- Study outcome: post-transplant infection between months 1 and 6

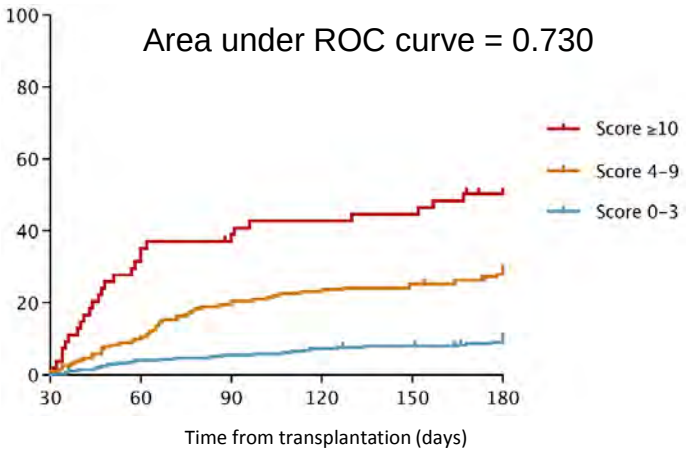
Variable	Score
Recipient age ≥62 years	3
eGFR at month 1 <37 mL/min	3
Infection within the first post-transplant month	3
$CD4^+$ T-cell count (at month 1) <40 cells/μL	3
$CD8^+$ T-cell count (at month 1) <155 cells/μL	2
Serum IgG levels (at month 1) <500 mg/dL	3
Serum C3 levels (at month 1) <78 mg/dL	4

Area under ROC curve = 0.774



- Prospective cohort
- 16 centers across Spain
- July 2014 to November 2015

Area under ROC curve = 0.730



CONCLUSION:

The SIMPLICITY score, based on easily available immune and clinical parameters, allows for stratification of KT recipients according to their expected risk of post-transplant infection.

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Original Article

Title page

Derivation and external validation of the SIMPLICITY Score as a simple immune-based risk score to predict infection in kidney transplant recipients.

Running title: Risk score to predict post-transplant infection.

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Abstract (250 words)

Existing approaches for infection risk stratification in kidney transplant recipients are suboptimal. Here, we aimed to develop and validate a weighted score integrating non-pathogen-specific immune parameters and clinical variables to predict the occurrence of post-transplant infectious complications. To this end, we retrospectively analyzed a single-center derivation cohort of 410 patients undergoing kidney transplantation in 2008-2013 in Madrid. Peripheral blood lymphocyte subpopulations, serum immunoglobulin and complement levels were measured at one-month post-transplant. The primary and secondary outcomes were overall and bacterial infection through month six. A point score was derived from a logistic regression model and prospectively applied on a validation cohort of 522 patients undergoing kidney transplantation at 16 centers throughout Spain in 2014-2015. The SIMPLICITY score consisted of the following variables measured at month one after transplantation: C3 level, $CD4^+$ T-cell count, $CD8^+$ T-cell count, IgG level, glomerular filtration rate, recipient age, and infection within the first month. The discrimination capacity in the derivation and validation cohorts was good for overall (areas under the receiver operating curve of 0.774 and 0.730) and bacterial infection (0.767 and 0.734, respectively). The cumulative incidence of overall infection significantly increased across risk categories in the derivation (low-risk 13.7%; intermediate-risk, 35.9%; high-risk77.6%) and validation datasets (10.2%, 28.9% and 50.4%, respectively). Thus, the SIMPLICITY score, based on easily available immune parameters, allows for stratification of kidney transplant recipients at month one according to their expected risk of subsequent infection.

Keywords: immune biomarkers; infection; kidney transplantation; prediction; outcomes; SIMPLICITY score.

Introduction

The occurrence of infectious complications is one of the major drawbacks with current clinical practices in solid organ transplantation (SOT).[1] Despite its restrictive pharmacokinetic nature, therapeutic drug monitoring of immunosuppressive drugs constitutes the only approach widely used to investigate the state of post-transplant immunosuppression.[2,3] An increasing number of immunological monitoring strategies have been proposed over the last years, ranging from the enumeration of non-pathogen-specific parameters (e.g. peripheral blood lymphocyte subpopulations [PBLSs]) to labor-consuming functional assays for pathogen-specific (e.g. cytomegalovirus [CMV]) cell-mediated immunity.[4,5] Our group has previously demonstrated the value of post-transplant hypogammaglobulinemia (HGG), C3 hypocomplementemia (HCC) and low PBLS counts to identify kidney transplant (KT) recipients at increased risk of post-transplant infection.[6-8]

Clinicians still face challenges in translating such biomarkers, which usually explore non-overlapping effector immune mechanisms in a compartmentalized fashion, into daily practice. The integration of various easily available parameters covering innate and adaptive responses into a single risk score might provide a valuable support to the clinical decision-making process. Nevertheless, previous attempts to develop an immune-based score aimed at assessing the risk of infection after SOT have been of limited applicability due to the single-center design or small sizes of studies.[9-16] Moreover, some of them incorporate complex assay procedures (such as T-cell proliferative responses) not always accessible to clinical laboratories.[10,14] More importantly, none of these scores have been externally validated and, since the same datasets were usually used to derive prediction rules and to calculate diagnostic proprieties, overestimation of predictive capacity cannot be ruled out.[17]

With these gaps in mind, our study was aimed at developing a weighted risk score based on simple non-pathogen-specific immune parameters and clinical variables to predict infection among KT recipients recruited in a single-center derivation cohort. We next assembled a large cohort at 16 Spanish centers to externally validate the predictive accuracy of the score.

Results

Characteristics of derivation and validation cohorts

Overall, 489 and 570 KT recipients were potentially eligible for inclusion in the derivation and validation cohorts. After screening assessment, 410 and 522 patients were finally included in each cohort, respectively (**Figure S1** in Supporting Material). As expected in view of their different designs (single-center versus multicenter) and recruitment periods (2008-2013 and 2014-2015, respectively), both cohorts differed in a number of characteristics, such as the proportion of participants with previous KT, age and type of donor, induction therapy, immunosuppressive regimen (mainly in the use of mammalian target of rapamycin [mTOR] inhibitors), and duration of anti-CMV prophylaxis (**Table 1**).

One-year patient and death-censored graft survival in the derivation cohort were 91.4% and 93.6%. The corresponding rates for the validation cohort were 97.6% and 97.1%, respectively. Three (0.7%) and 4 (0.8%) patients were lost to follow-up in each cohort before completing the 6-month post-transplant period.

Post-transplant infection in derivation and validation cohorts

In the derivation cohort, 133 (32.4%) patients developed a total of 235 episodes of post-transplant infection (primary study outcome [see definitions below]) between post-transplant months 1 and 6 (0.39 [95% confidence interval [CI]: 0.35 – 0.45] episodes per 100 patient-years). Regarding the secondary outcome, 87 (21.2%) patients experienced 127 episodes of bacterial infection (0.24 [95% CI: 0.20 – 0.28] episodes per 100 patient-years) (**Figure 1a**). A detailed description of causative agents and clinical syndromes is provided as Supporting Material (**Table S1**).

On the other hand, 105 (20.1%) patients in the validation cohort were diagnosed with 161 separate episodes of infection between months 1 and 6 (0.21 [95% CI: 0.18 – 0.24] episodes per 100 patient-years), whereas 78 (14.9%) patients experienced 107 episodes of bacterial infection (0.14 [95% CI: 0.12 – 0.17] episodes per 100 patient-years) (**Figure 1b**). Causative agents and clinical syndromes are also detailed in Supporting Material (**Table S2**).

Model derivation

As expected from previous studies,[6-8] patients in the derivation cohort that subsequently developed infection had lower PBLS counts and IgG and complement levels at month 1

compared to those who remained free from infection. Significant differences were found for $CD3^+$ (*P*-value = 0.007), $CD4^+$ (*P*-value = 0.025) and $CD8^+$ T-cell counts (*P*-value = 0.004), and serum C3 levels (*P*-value = 0.0026) (**Figure S2**). Although IgG levels measured as a continuous variable did not significantly differ between patients with or without infection, a dose-response gradient in the cumulative incidence at month 6 was found across increasing degrees of IgG HGG (**Figure S3**).

Since strong collinearity was found between the $CD3^+$ T-cell count and the other PBLSs (variance inflation factor [VIF] values >3.5), this variable was not further considered for score construction. The following optimal cut-off values for predicting post-transplant infection were set according to the Youden's index[18] for the remaining parameters: 40 cells/µL for $CD4^+$ T-cell count, 155 cells/µL for $CD8^+$ T-cell count, 500 mg/dL for serum IgG levels, and 78 mg/dL for serum C3 levels.

We next explored in the derivation cohort the clinical (i.e. non-immune) variables available at post-transplant month 1 that predicted the development of infection (**Table S3**). Recipient age, underlying glomerulonephritis, donor age, donation after circulatory death donor, cold ischemia time, and infection and biopsy-proven acute graft rejection (BPAR) within the first month were identified as risk factors in the univariate analysis, whereas living donation and estimated glomerular filtration rate (eGFR) at month 1 revealed as protective factors.

Construction of the risk score

The logistic regression model of immune and clinical factors associated with the primary outcome is detailed in **Table 2**. Recipient and donor age showed strong multicollinearity (Pearson's r: 0.841, *P*-value <0.0001; VIF value: 3.417) and, therefore, only the former was kept into the model. Recipient age, cold ischemia time and eGFR were dichotomized at the optimal cut-off value for inclusion. The model showed good discriminative capacity (area under receiver operating characteristics curve [auROC]: 0.787; 95% CI: 0.737 – 0.838) and goodness-of-fit (Hosmer-Lemeshow test $\chi^2 = 3.44$; *P*-value = 0.904).

As we aimed at constructing a user-friendly score based on the most parsimonious model that provided the best fit to the data, we explored a set of alternative simplified models. When cold ischemia time was removed to maintain only those clinical variables that are routinely available at month 1, the discriminative capacity was not meaningfully compromised (auROC: 0.781; 95%

CI: 0.731 – 0.831; Hosmer-Lemeshow test χ^2 = 3.65; *P*-value = 0.888).

Finally, we developed the SIMPLICITY (Seeking for Immune Status on Peripheral Blood Lymphocytes, Immunoglobulins and Complement Activity) score on the basis of three clinical variables (recipient age ≥62 years, eGFR <37 mL/min and previous infection) and four immune parameters ($CD4^+$ T-cell count <40 cells/μL, $CD8^+$ T-cell count <155 cells/μL, IgG levels <500 mg/dL and C3 levels <78 mg/dL) measured at month 1. No relevant multicollinearity was found (all VIF values <1.5) (**Table S4**). Point scores assigned to the corresponding β regression coefficients are shown in **Table 3**.

Performance of the risk score in the derivation cohort

The SIMPLICITY score showed auROCs of 0.774 (95% CI: 0.718 – 0.823) and 0.767 (95% CI: 0.706 – 0.827) for predicting overall (primary outcome) and bacterial infection (secondary outcome), respectively, between post-transplant months 1 and 6 (**Figure 2**). Internal validation by bootstrap resampling (1,000 iterations with 325 samples with replacement) revealed a stable discriminative capacity with marginal shrinkage (auROC for the primary outcome: 0.744 [95% CI: 0.689 – 0.793]). The donor/recipient (D/R) CMV serostatus had no meaningful impact on the score performance (auROC for D+/R- group: 0.821 [95% CI: 0.643 – 0.999]; auROC for R+ group: 0.775 [95% CI: 0.721 – 0.829]).

The median score value was 5 (interquartile range [IQR]: 2 - 8). The diagnostic accuracy in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive values (NPV), and likelihood ratios is shown in **Table 4**. For instance, a 70-year-old recipient with 30 $CD4^+$ T-cells/μL and a serum C3 level of 50 mg/dL at month 1 (score 10 [specificity of 94.9%]) would face a risk of developing infection through month 6 after transplantation (PPV) of at least 77.6%. On the contrary, a 40-year-old patient with no previous history of post-transplant infection, normal graft function (eGFR of 65 mL/min), 100 $CD4^+$ T-cells/μL, 80 $CD8^+$ T-cells/μL, and neither HGG (IgG level of 700 mg/dL) nor HCC (C3 levels of 110 mg/dL) at month 1 (score 2 [sensitivity of 91.8%]) would have a probability of remaining free from infection (NPV) of 88.4%, although the risk of developing this outcome (PPV) would still be relatively high (38.8%). Overall, 177 (43.2%), 169 (41.2%) and 64 (15.6%) recipients were stratified into low-risk (score 0-3), intermediate-risk (score 4-9) and high-risk (score ≥10) strata, respectively. The cumulative incidence of study outcomes through month 6 was significantly higher across increasing risk

strata, both for overall (low-risk [score 0-3]: 13.7%; intermediate-risk [score 4-9]: 35.9%; high-risk [score ≥10]: 77.6%; *P*-value <0.0001) and bacterial infection (low-risk [score 0-3]: 7.5%; intermediate-risk [score 4-9]: 22.9%; high-risk [score ≥10]: 59.4%; *P*-value <0.0001) (**Figure 3**).

The excess risk of overall infection associated with higher scores was confirmed after adjusting for the occurrence of BPAR through post-transplant month 6 as a time-dependent covariate (hazard ratio [HR] per one-point increment: 1.18; 95% CI: 1.50 – 1.23; *P*-value <0.0001), as well as in a set of sensitivity analyses (**Figure 4**). These results also applied to post-transplant bacterial infection (secondary outcome) (**Figure S4**).

Performance of the risk score in the validation cohort

Similarly to that observed in the derivation cohort, PBLS counts ($CD3^+$, $CD4^+$ and $CD8^+$ T-cells) and serum C3 levels at post-transplant month 1 were significantly lower among patients with overall infection through month 6 compared to those without (**Figure S5**).

The auROC of the SIMPLICITY score for predicting overall infection between post-transplant months 1 and 6 in the validation cohort was 0.730 (95% CI: 0.666 – 0.794), which was similar to that from the derivation dataset. The corresponding figure for bacterial infection was 0.734 (95% CI: 0.673 – 0.794) (**Figure S6**). The median score value was 3 (IQR: 0 - 6). The diagnostic accuracy (sensitivity, specificity, PPV, NPV and likelihood ratios) in the validation cohort is show in **Table S5**. Overall, 277 (53.1%), 190 (36.4%) and 55 (10.5%) patients were stratified into low-risk (score 0-3), intermediate-risk (score 4-9) and high-risk (score ≥10) strata, respectively. The cumulative incidence of overall infection through post-transplant month 6 significantly increased across risk strata (low-risk [score 0-3]: 10.2%; intermediate-risk [score 4-9]: 28.9%; high-risk [score ≥10]: 50.4%; *P*-value <0.0001), with a comparable trend also evident for bacterial infection (low-risk [score 0-3]: 6.5%; intermediate-risk [score 4-9]: 23.2%; high-risk [score ≥10]: 35.4%; *P*-value <0.0001) (**Figure 5**). The Hosmer-Lemeshow goodness-of-fit test confirmed that the score was well calibrated in the validation cohort ($\chi^2 = 2.26$; *P*-value = 0.689).

The risk of developing infection between months 1 and 6 increased by 19% with each one-point increase in the SIMPLICTY score after adjusting for the occurrence of BPAR (HR [per one-point increment]: 1.19; 95% CI: 1.13 – 1.23; *P*-value <0.0001). Such a risk increase was similar for bacterial infection and across sensitivity analyses, although the association did not reach statistical significance for some specific causes of end-stage renal disease (ESRD) or CMV risk

categories (D+/R- serostatus) (**Figure S7**).

Calibration of the risk score

Hazard ratios for study outcomes across increasing categories of the SIMPLICITY score were in the same order of magnitude in both the derivation and validation cohorts (for example, 8.188 and 6.922, respectively, for high-risk [score ≥10] versus low-risk [score 0-3] strata), indicating a robust capacity for risk stratification (**Table S6**).

The calibration plot for predicting the primary outcome (**Figure S8a**) revealed a calibration intercept (β_0) of -0.669 (95% bootstrap CI: -0.985 – -0.363), whereas the calibration slope was 0.752 (95% bootstrap CI: 0.539 – 1.018) (**Table S7**). The intercept relates to calibration-in-the-large, which compares the mean of all predicted risks with the mean observed risk.[19] Since the 95% CI of this estimate was negative, it could be deduced that the predicted probabilities were systematically too high. On the other hand, the calibration slope –which is related to the average strength of the predictor effects– did not significantly differ from 1.00, indicating that there was no evidence of average stronger or weaker effects in the derivation model.

To investigate the cause of this suboptimal calibration, we calculated the mean and the SD of the linear predictor (LP) of the original model in both datasets. The LP results from the logit transformation of the predicted risks in logistic regression, with an increased (or decreased) variability of LP indicating more (or less) case-mix heterogeneity between derivation and validation cohorts. Conversely, the comparison of means of the LP reveals differences in outcome frequencies.[20] Mean LP was higher in the derivation (-0.897) than in the validation dataset (-1.141), with similar SDs (1.177 and 1.114, respectively), which suggests that the calibration-in-the-large of the model in the external validation dataset would be mostly affected by differences in case mix-severity rather than by other sources of heterogeneity.[20]

To overcome this circumstance, we explored to which extent an “updated” score would adjust better to differences in outcome incidence observed in the validation cohort. It has been shown that a prediction model may be updated (i.e. adjusted) to the new dataset obtained from a setting different from that in which it was originally developed.[21,22] The updated model is simultaneously constructed on both the derivation and the validation data, yielding better risk estimates. After adjusting the intercept of the model, we obtained an alternative point assignment (**Table S8**). Predictive performance and calibration of this updated score are

available as Supporting Material (**Figure S8b** and **Table S7**).

Imputation of missing data

Finally, imputation of missing values for the continuous parameters included in the score (eGFR, $CD4^+$ and $CD8^+$ T-cell counts, and IgG and C3 levels) was performed in the derivation cohort, obtaining similar β regression coefficients (**Table S9**). The resulting model was then validated in four different imputed datasets, with auROC values for predicting the primary study outcome very close to that obtained in the original analysis with no missing data imputation (**Table S10**).

Discussion

Given the deleterious impact on graft and patient outcomes attributable to post-transplant infection, the development and validation of prediction rules able to effectively stratify the KT population according to individual susceptibility constitutes a crucial unmet clinical need. Herein, we propose the SIMPLICITY score, which integrates four non-pathogen-specific quantitative immune biomarkers ($CD4^+$ and $CD8^+$ T-cell counts and serum IgG and C3 levels) and three simple clinical variables (recipient age, prior infection and graft function). In contrast to previous efforts,[9-16] our score has two major advantages. Firstly, the immune parameters included are broadly available in routine practice with a short turnaround time and no specialized laboratory equipment. Secondly, we have been able to validate the discriminative capacity and diagnostic accuracy of the score in an independent cohort recruited in 16 Spanish centers.

From a practical point of view, a prediction rule for estimating the risk of infection after KT may be clinically implemented through two non-mutually exclusive approaches. On one hand, those recipients at low risk would benefit from the earlier discontinuation of anti-infective prophylaxis (such as valganciclovir or valacyclovir) or less close clinical monitoring, resulting in cost saving and reduction of associated adverse events (e.g. leukopenia). The safety of such strategy would be supported by the relatively high NPVs observed for patients with low scores. On the other hand, a multifaceted intervention based on the extension of prophylaxis, closer follow-up care or immune-targeted actions (such as intravenous or subcutaneous immunoglobulin replacement therapy in case of HGG) should be applied when the predicted infection risk is elevated. An alternative approach may consist of corticosteroid or tacrolimus tapering in recipients with evidence of over-immunosuppression. Furthermore, mTOR conversion may be considered due to the demonstrated decreaed risk of viral infection with these agents.[23] By collapsing the SIMPLICITY score into three strata, we can define a low-risk population (score 0-3) in which the expected cumulative incidence of overall infection through month 6 would be below 15% (13.7% and 10.2% in the derivation and validation cohorts). Alternatively, KT recipients in the high-risk segment (score ≥10) face a cumulative risk of infection that exceeds 50%. The decision to categorize the score according to these thresholds was based on the low number of patients with very high values. In accordance with this observation, the score did not exhibit a normal distribution, with a median of 5 points and 3 points for the derivation and validation cohorts,

respectively. Future studies should estimate the equivalent to the "number necessary to treat", or how many patients above the different score thresholds should receive extended prophylaxis or immunoglobulin therapy to prevent one additional episode of infection.

The observed rates of infection during the post-transplant period were lower in the validation than in the derivation cohort and, therefore, the PPV for scores ≥10 differed across datasets. In view of the different recruitment periods (2008-2013 versus 2014-2015), such a discrepancy may be explained by long-term improvements in surgical procedures, immunosuppression and prophylaxis regimens. Even in the more contemporary period, in which a secular trend towards a sustained reduction in the incidence of infectious complications after KT has been suggested,[24] the score was still able to identify a subgroup of recipients exposed to an unacceptable risk of severe, potentially life-threatening infection. By simply updating the intercept of the original model for differences in outcome frequency (a methodological approach increasingly used in clinical research[21,22]), we also propose an alternative "updated" score with improved calibration that could be applicable in settings with low *a priori* infection rates.

Different prediction rules have been previously developed for the KT population. Blazik et al. created a "leukocyte phenotype and function (LPF) score" composed of $CD4^+$ T-cell count, immunoglobulin levels, lymphoproliferative response to phytohaemagglutinin A, reactive oxygen species generation and neutrophil phagocytic function.[10] In addition to the complex nature of the latter parameters, the derivation cohort only comprised 70 patients and no validation effort was made. Crepin et al. applied markers of immunosenescence (R+ CMV serostatus, $CD4^+/CD8^+$ ratio <1 and/or $CD8^+$ T-cell count >700 cells/µL) to predict opportunistic and bacterial infection in a multicenter French cohort.[13] However, no information on score performance was provided, and we were not able to externally validate the discriminative capacity of this immune risk phenotype.[25] Dendle et al. have recently proposed a "level of immunosuppression score" (based on $CD4^+$ T-cell and NK cell counts, graft function and use of mycophenolate mofetil) for predicting the occurrence of severe infection over the next 2 years. The resulting auROC was 0.750, and the cumulative incidence of infection in patients classified within the highest risk category reached 84%.[16] Nevertheless, this score still lacks external validation, and the inclusion criteria was restricted to patients at least at their third post-transplant month, which implies that the earlier period (with the highest incidence of infection) was not accounted for.

Regarding other SOT populations, Sarmiento et al. constructed an infection risk score for heart transplant recipients that included serum IgG levels <600 mg/dL at baseline or post-transplant day 7, serum C3 levels <80 mg/dL at day 7 and D/R CMV mismatch.[14] Again, the authors did not supply data on the diagnostic accuracy.

The SIMPLICITY score integrates into a single rule a number of immune parameters that have been already shown to predict infection after KT.[6-8] Similarly to the well-established approach in human immunodeficiency virus (HIV) patients, post-transplant kinetics of $CD4^+$ and $CD8^+$ T-cell counts have been consistently correlated with the occurrence of opportunistic infection, including CMV disease and *P. jirovecii* pneumonia.[26,27] Post-transplant HGG is a common and often neglected complication, with mild-to-severe forms occurring in as many as 39% and 15% of SOT recipients during the first year.[28] Since the humoral response is responsible for the clearance of encapsulated bacteria through opsonization and complement activation, post-transplant IgG HGG serves as a good predictor for bacterial infection.[29] Finally, the C3 component plays a pivotal role in the complement activation cascade to form the C5 convertase and to assemble the membrane attack complex.[30] Therefore, the assessment of C3 HCC by nephelometry may advantageously replace more complex *in vitro* haemolytic assays to explore its functionality.[11,31] It may be hypothesized that reductions in post-transplant immunosuppression would translate into subsequent normalization of these parameters and, eventually, decreasing score values.

A number of limitations should be acknowledged. Our score was designed to predict exclusively the occurrence of infection beyond the first post-transplant month, despite the fact that earlier events (i.e. surgical site infection) can be also life-threating and increase hospital stay and costs. We have attempted at developing a comprehensive tool to predict any type of infection by combining a set of immune biomarkers with different pathophysiological significance, since they explore disparate compartments of the host response. The lack of an apparent impact on score performance of the use of antithymocyte globulin as induction therapy—a cause of profound lymphocytopenia— could be explained by this non-mechanistic approach. By excluding the explanatory variable “cold ischemia time” we gained in model simplicity at expense of losing some discriminative capacity. As previously noted, both cohorts differed in various clinical features (such as the frequency of previous KT, dialysis vintage, type of donor or duration of

anti-CMV prophylaxis). While such imbalances should have no relevance in the external validation process, which is aimed at demonstrating the discrimination and calibration of the score in an independent dataset not previously used to develop the model, the lower incidence of study outcomes in the validation cohort necessarily resulted in lower PPVs compared to the derivation cohort. Moreover, NPVs dot not exceed 90% even among low-risk categories, questioning the ability to effectively rule out the possibility of subsequent infection. Differences in clinical practices across participating centers might have led to some heterogeneity in event reporting, although this would have reinforced the external validity of the results. Finally, information on tacrolimus or mTOR inhibitor levels was not collected in the derivation dataset.

In conclusion, we have developed and validated a prediction rule for post-transplant infection between months 1 and 6 after KT —the SIMPLICITY score— based of easily available immune ($CD4^+$ and $CD8^+$ T-cell counts and IgG and C3 levels) and clinical variables (recipient age, prior infection and graft function). This score offers the possibility to identify, as early as month 1, a subgroup of KT recipients at increased risk of subsequent infection that would benefit from individualized prevention strategies and tailoring of immunosuppression. Future intervention studies should be aimed at confirming the feasibility of this approach.

Methods

Study population and setting

The present observational derivation-validation study was performed in two non-overlapping cohorts. The first one (derivation cohort) comprised adult patients (≥18 years) with ESRD who consecutively underwent KT between November 2008 and March 2013 at the Hospital Universitario "12 de Octubre" (Madrid, Spain). The second, independent sequential cohort (validation cohort) comprised adult patients undergoing KT between July 2014 and November 2015 at 16 Spanish transplant centers. Double organ recipients and those with HIV infection were excluded.

All participants provided written informed consent at study entry, which was carried out in accordance with the ethical standards laid down in the Declarations of Helsinki and Istanbul. The study protocol (ClinicalTrials.gov identifier: NCT03083756) was approved by the Clinical Research Ethics Committee of the coordinating center (Hospital Universitario "12 de Octubre" [ref: 09/176]) and by local committees at other sites as required. This paper is compliant with the STROBE guidelines for observational studies.

Study design

All participants were enrolled at the time of KT and followed-up for 12 months, unless graft loss (retransplantation or permanent return to dialysis) or death occurred earlier. Patients were subjected to an immune status evaluation based on the enumeration of total lymphocyte and selected PBLS counts and the assessment of serum immunoglobulins and complement levels at pre-established time points (months 1 and 6 after transplantation). Pre-transplant, peri-operative and post-transplant variables were prospectively collected by local investigators in an anonymized manner using a standardized case report form and entered into a secure electronic database. In addition to the usual follow-up at each center, study visits were scheduled at baseline and post-transplant months 1, 3, 6 and 12.

We focused on infectious complications occurring between months 1 and 6 since the overall amount of immunosuppression usually peaks during this intermediate post-transplant period, as does the risk of overall and, particularly, opportunistic infection. On the other hand, surgical factors and donor-derived infections rather than host's response play a predominant role during the earlier period (first post-transplant month).[32] The *primary study outcome* was the occurrence

of overall infection throughout the intermediate post-transplant period (months 1 to 6 after transplantation). Bacterial infection during such period was analyzed as a *secondary outcome*.

Immune status evaluation

Whole blood samples were collected into EDTA-containing Vacutainer tubes and analyzed within 18-24 hours in the laboratories of the participating centers. PBLS counts ($CD3^+$, $CD4^+$ and $CD8^+$ T-cells) were assessed by means of an automated multicolor flow cytometry system. The 6-color BD Multitest system was used at the coordinating center, with acquisition performed on the BD FACSCanto II instrument using BD FACSCanto clinical software (all from BD Biosciences, San Jose, CA). Comparable methods were used in the remaining study sites. Serum immunoglobulin (IgG, IgA and IgM) and complement (C3 and C4) levels were measured by nephelometry (Image-System, Beckman Coulter GmbH, Krefeld, Germany, or similar).

Study definitions

Post-transplant infection was defined by any of the following: (a) isolation of an unequivocally pathogenic microorganism from any sample; (b) isolation of any microorganism from a clinically relevant sample obtained under sterile conditions; (c) isolation of a potentially pathogenic microorganism from any sample accompanied by signs and/or symptoms of local or systemic infection; and/or (d) clinical data suggestive of infection without microbiological isolation and complete resolution on antimicrobial therapy. To be analyzed as study outcome the episode should have required hospitalization and/or administration of intravenous therapy. Opportunistic infections typically indicative of excessive immunosuppression (such as mucosal herpes simplex virus reactivation or shingles) were also counted as study outcome regardless of the requirement for hospitalization.

Specific infectious syndromes were diagnosed on the basis of commonly accepted criteria.[33-36] *CMV disease* included viral syndrome and end-organ disease defined as per the American Society of Transplantation criteria.[37] Proven or probable *invasive fungal infection* was defined according to the European Organization on Research and Treatment in Cancer and the Mycoses Study Group criteria.[38] The eGFR was assessed by the 4-variable Modification of Diet in Renal Disease equation.[39] The occurrence of BPAR was suspected in case of an otherwise unexplained rise in serum creatinine and diagnosed by histological examination.[40]

Statistical analysis

Quantitative data were shown as the mean ± standard deviation or the median with IQR. Qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the χ^2 test. Student's t-test or Mann-Whitney U test were applied for continuous variables, as appropriate. Pearson's correlation coefficient or Spearman's rank correlation coefficient were used to investigate the correlation between continuous variables.

A weighted score was created to predict the occurrence, in the derivation cohort, of the primary outcome between post-transplant months 1 to 6 on the basis of the immune status evaluation performed at month 1 and on the clinical parameters available at that point. To be included in either the derivation or validation cohort, the patient had to be alive and with functioning graft at month 1. Observation was censored at the time of diagnosis of the first episode of infection, post-transplant month 6 or lost to follow-up. No imputation for missing data was performed.

Multivariate analysis was used to identify immunological and clinical variables associated with the occurrence of the study outcome in the derivation cohort. Variables with univariate *P*-values ≤0.1 were entered into a logistic regression model in a backward stepwise selection fashion. Continuous variables were dichotomized according to the optimal cut-off value, as determined by the Youden's index.[18] The Hosmer-Lemeshow test was used to assess the goodness-of-fit of the models. Discriminative capacity was estimated by the auROC. Multicollinearity among explanatory variables was analyzed using the VIF, with values <3 being considered acceptable. The most parsimonious model (i.e. the highest outcome variability explained with the lowest number of variables) was selected for the construction of the score.

The weighted risk score for predicting overall infection between post-transplant months 1 to 6 (primary outcome) was derived from the point estimate for each variable using the β coefficients of the final model. Relative point values were assigned by dividing each regression coefficient by one-half of the smallest coefficient and rounding to the nearest integer. The resulting score was first internally validated by means of the bootstrap method in 1,000 iterations with 325 samples drawn with replacement from the original derivation dataset. Diagnostic accuracy was assessed by sensitivity, specificity, PPV, NPV and likelihood ratios using different risk cutoff scores.

Time-to-event curves were plotted by the Kaplan-Meier method and inter-group differences were compared with the log-rank test to analyze the ability of the score for stratification across increasing risk categories. Univariate and multivariate Cox regression models were constructed to investigate whether the associations found between score risk categories and outcomes remained significant after adjusting for clinical covariates, with associations expressed HRs and 95% CIs. To show robustness of the score, a set of sensitivity analyses restricted to specific causes of ESRD, D/R CMV serostatus, or immunosuppression or prophylaxis regimens was also performed. All these validation analyses were also carried out for post-transplant bacterial infection (secondary outcome).

The resulting score was next applied to the independent external validation cohort to assess its discriminative capacity (auROC), diagnostic accuracy and calibration. Calibration indicates how closely predicted probabilities match observed frequencies of occurrence, and was graphically assessed by means of calibration plots.

Finally, we performed a sensitivity analysis by imputing missing data with a chained equation approach. The number of imputations to add was four. For the process of model construction, continuous variables with missing values were imputed and then dichotomized using original cut-off values. The regression coefficients were re-estimated for each of the four datasets. The same approach was applied for the validation process. In each of these imputed datasets, the discrimination capacity of the original model was estimated by means of auROC values.

All the significance tests were two-tailed. The threshold for significance was set at a *P*-value <0.05. Statistical analysis was performed with SPSS version 20.0 (IBM Corp., Armonk, NY), STATA version 16.0 (StataCorp, College Station, TX), and R software version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria).[41] Graphs were generated with Prism version 6.0 (GraphPad Software Inc., La Jolla, CA).

Disclosure

Daniel Serón has received lecture fees from speaking on behalf of Astellas Pharma and Novartis. He has held research grants from Diaverum and Chiesi. Ángel Alonso has received lecture fees from speaking on behalf of Astellas Pharma. Gonzalo Gómez has received lecture fees from speaking on behalf of Novartis. He has been paid advisory boards by Alexion. José María Portolés has held research grants from Baxter Healthcare. The remaining authors declare no conflicts of interest to disclose.

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Tables

Table 1. Demographics and main clinical characteristics of derivation and validation cohorts.

Variable	Derivation cohort (n = 410)	Validation cohort (n = 522)	*P*-value
Age of recipient, years [mean ± SD]	54.9 ± 14.9	55.6 ± 13.4	0.483
Gender of recipient (male) [n (%)]	254 (62.0)	343 (65.7)	0.263
Previous kidney transplantation [n (%)]	82 (20.0)	59 (11.3)	0.0003
Underlying end-stage renal disease [n (%)]			
Glomerulonephritis	90 (22.0)	129 (24.7)	0.363
Diabetic nephropathy	72 (17.6)	74 (14.2)	0.187
Policystosis	54 (13.2)	90 (17.2)	0.106
Nephroangiosclerosis	52 (12.7)	41 (7.9)	0.019
Chronic interstitial nephropathy	36 (8.8)	35 (6.7)	0.289
Congenital nephropathy	18 (4.4)	12 (2.3)	0.108
Reflux nephropathy	15 (3.7)	6 (1.1)	0.019
Obstructive nephropathy	3 (0.7)	11 (2.1)	0.149
Unknown	35 (8.5)	101 (19.3)	0.0001
Other	35 (8.5)	28 (5.4)	0.075
CMV serostatus [n (%)]			
D+/R+	274 (76.3)	360 (69.0)	0.533
D-/R+	54 (15.0)	74 (14.2)	0.729
D+/R-	27 (6.6)	49 (9.4)	0.153
D-/R-	4 (1.0)	16 (3.1)	0.051
D unknown/R+	51 (12.4)	23 (4.4)	0.0001
Positive HCV serostatus [n (%)]	33 (8.0)	21 (4.0)	0.014
Positive HBsAg status [n (%)]	1 (0.7)	13 (2.5)	0.005
Pre-transplant renal replacement therapy [n (%)]	386 (94.1)	472 (90.4)	0.049
Hemodialysis	332 (80.9)	366 (70.1)	0.0002
Continuous ambulatory peritoneal dialysis	54 (13.2)	106 (22.5)	0.005
Time on dialysis, months [median (IQR)]	21.3 (10.8 – 43.1)	25.0 (13.0 – 43.8)	0.050
Age of donor, years [mean ± SD]	53.4 ± 16.5	55.5 ± 14.9	0.036
Gender of donor (male) [n (%)]	267 (65.1)	279 (53.4)	0.0004
Type of donor [n (%)]			
DBD donor	263 (64.1)	413 (79.1)	0.0001
DCD donor	135 (32.9)	51 (9.8)	0.0001
Living donor	12 (2.9)	58 (11.1)	0.0001
Cold ischemia time, hours [median (IQR)]	17.9 (11.5 – 22.0)	15.0 (9.3 – 20.0)	<0.0001
Induction therapy [n (%)][a]			
ATG	206 (50.2)	186 (35.6)	0.0001
Basiliximab	137 (33.4)	241 (46.2)	0.0001
Eculizumab	0 (0.0)	5 (1.0)	0.071
None	67 (16.3)	95 (18.2)	0.512
Primary immunosuppression regimen [n (%)][a]			
Steroids	404 (98.5)	519 (99.4)	0.193

Tacrolimus	406 (99.0)	520 (99.6)	0.414
Cyclosporine	0 (0.0)	2 (0.4)	0.507
Mycophenolate mofetil/mycophenolic acid	364 (88.8)	415 (79.5)	0.0002
Azathioprine	42 (10.2)	8 (1.5)	0.0001
mTOR inhibitor	2 (0.5)	99 (18.9)	0.0001
Anti-CMV prophylaxis [n (%)]	206 (50.2)	335 (64.2)	0.0001
Prophylaxis for 3 months	178 (43.4)	136 (26.1)	0.0001
Prophylaxis for ≥3 months	28 (6.8)	178 (34.0)	0.0001
Anti-*Pneumocystis* prophylaxis [n (%)]	393 (95.6)	407 (78.0)	0.0001
Non-infectious complications [n (%)]			
Delayed graft function	249 (60.7)	160 (30.7)	0.0001
New-onset diabetes	57 (13.9)	28 (5.4)	0.0001
BPAR within the first post-transplant year	75 (18.3)	70 (13.4)	0.051
≥2 episodes	7 (1.7)	6 (1.1)	0.577
Time from transplantation to the first episode, days [median (IQR)]	30.0 (14.0 – 115.0)	19.5 (11.0 – 135.0)	0.458
Infection (≥1 episode) within the first post-transplant month [n (%)][b]	76 (18.5)	86 (16.5)	0.461
eGFR at month 1, mL/min [mean ± SD]	41.2 ± 20.6	43.9 ± 21.7	0.057
eGFR at month 6, mL/min [mean ± SD]	49.6 ± 20.2	48.7 ± 19.4	0.509

ATG: antithymocyte globulin; BPAR: biopsy-proven acute graft rejection; CMV: cytomegalovirus; D: donor; DBD: donation after brain death; DCD: donation after circulatory death; eGFR: estimated glomerular filtration rate; HBsAg: hepatitis B virus surface antigen; HCV: hepatitis C virus; IQR: interquartile range; KT: kidney transplantation; mTOR: mammalian target of rapamycin; R: recipient; SD: standard deviation.

[a] The sum of percentages exceeds 100% since some patients received more than one drug.

[b] Includes 81 episodes in the derivation cohort (incisional surgical site infection [n = 24], acute graft pyelonephritis [n = 21], gastrointestinal infection [n = 10], skin and soft-tissue infection [n = 6], catheter-related bloodstream infection [n = 5], pneumonia [n = 4], intraabdominal infection [n = 3], primary bloodstream infection [n = 3], CMV viral syndrome [n = 2], and other [n = 3]) and 86 episodes in the validation cohort (acute graft pyelonephritis [n = 51], incisional surgical site infection [n = 11], gastrointestinal infection [n = 7], catheter-related bloodstream infection [n = 6], pneumonia [n = 4], skin and soft-tissue infection [n = 3], CMV viral syndrome [n = 2], intraabdominal infection [n = 1] and primary bloodstream infection [n = 1]).

Table 2. *Derivation cohort:* Multivariate logistic regression analysis of variables predicting overall infection between post-transplant months 1 and 6.

	Univariate			Multivariate		
	OR	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Recipient age ≥62 years	3.08	2.00 – 4.75	<0.0001	2.67	1.53 – 4.65	0.010
Glomerulonephritis as ESRD	0.48	0.28 – 0.84	0.009	–	–	–
DCD donor	0.59	0.38 – 0.95	0.028	–	–	–
Living donor	0.96	0.93 – 0.98	0.011	–	–	–
Cold ischemia time >19.5 hours	2.28	1.49 – 3.48	0.0001	1.99	1.13 – 3.53	0.018
eGFR (at month 1) <37 mL/min	3.42	2.21 – 5.27	<0.0001	1.99	1.18 – 3.34	0.010
Infection within the first month	2.69	1.62 – 4.48	<0.0001	2.38	1.31 – 4.35	0.005
BPAR within the first month	2.55	1.29 – 4.99	0.007	–	–	–
$CD4^+$ T-cell count (at month 1) <40 cells/μL	2.46	1.41 – 4.27	0.001	2.47	1.15 – 5.33	0.021
$CD8^+$ T-cell count (at month 1) <155 cells/μL	2.38	1.54 – 3.68	0.0001	1.83	0.99 – 3.39	0.053
Serum IgG levels (at month 1) <500 mg/dL	2.18	1.18 – 4.03	0.013	1.97	0.96 – 4.05	0.066
Serum C3 levels (at month 1) <78 mg/dL	4.55	2.47 – 8.38	<0.0001	3.38	1.62 – 7.06	0.001

BPAR: biopsy-proven acute graft rejection; CI: confidence interval; DCD: donation after circulatory death; eGFR: estimated glomerular filtration rate; ESRD: end-stage renal disease; IgG: immunoglobulin G; OR: odds ratio.

Table 3. SIMPLICITY score: point assignment based on the regression coefficients obtained for the variables selected in the final multivariable logistic regression model in the derivation cohort.

Variable	β regression coefficient (95% CI)	Score
Recipient age ≥62 years	0.94 (0.36 – 1.50)	3
eGFR at month 1 <37 mL/min	0.69 (0.18 – 1.23)	3
Infection within the first post-transplant month[a]	0.85 (0.25 – 1.46)	3
$CD4^+$ T-cell count (at month 1) <40 cells/μL	0.94 (0.17 – 1.70)	3
$CD8^+$ T-cell count (at month 1) <155 cells/μL	0.56 (-0.07 – 1.18)	2
Serum IgG levels (at month 1) <500 mg/dL	0.71 (-0.03 – 1.44)	3
Serum C3 levels (at month 1) <78 mg/dL	1.14 (0.39 – 1.88)	4
Intercept (β_0)	-2.08 (-2.13 – -2.03)	–

CI: confidence interval; eGFR: estimated glomerular filtration rate; IgG: immunoglobulin G.

Table 4. *Derivation cohort:* Diagnostic accuracy of the SIMPLICITY score at month 1 for predicting overall infection between post-transplant months 1 and 6 (primary study outcome).

SIMPLICITY score	Proportion of patients (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	PLR (95% CI)	NLR (95% CI)
Score ≥0	100.0	100.0 (97.0 – 100.0)	0.0 (0.0 – 1.5)	32.5 (32.5 – 32.5)	NA	1.00 (1.00 – 1.00)	NA
Score ≥2	77.1	91.8 (85.4 – 96.0)	30.0 (24.5 – 36.1)	38.8 (36.5 – 41.1)	88.4 (80.3 – 93.4)	1.31 (1.19 – 1.45)	0.27 (0.15 – 0.51)
Score ≥4	56.8	81.9 (73.9 – 88.3)	55.3 (48.9 – 61.6)	46.9 (42.9 – 50.9)	86.4 (81.1 – 90.4)	1.84 (1.56 – 2.15)	0.33 (0.22 – 0.48)
Score ≥6	44.5	72.9 (64.2 – 80.6)	69.2 (63.1 – 74.8)	53.3 (47.9 – 58.6)	84.1 (79.7 – 87.8)	2.37 (1.91 – 2.93)	0.39 (0.29 – 0.53)
Score ≥8	27.5	52.5 (43.2 – 61.6)	84.6 (79.5 – 88.8)	62.1 (54.0 – 69.6)	78.7 (75.3 – 81.8)	3.40 (2.44 – 4.75)	0.56 (0.46 – 0.68)
Score ≥10	15.5	36.9 (28.3 – 46.1)	94.9 (91.4 – 97.2)	77.6 (66.0 – 86.1)	75.7 (73.1 – 78.2)	7.18 (4.03 – 12.80)	0.67 (0.58 – 0.76)
Score ≥12	7.2	19.7 (13.0 – 27.8)	98.8 (96.6 – 99.8)	88.9 (71.1 – 96.3)	71.8 (70.0 – 73.6)	16.59 (5.09 – 54.03)	0.81 (0.74 – 0.89)
Score ≥14	3.7	9.8 (5.2 – 16.6)	99.2 (97.2 – 99.9)	85.7 (57.7 – 96.4)	69.5 (68.3 – 70.8)	12.44 (2.83 – 54.73)	0.91 (0.86 – 0.96)

CI: confidence interval; NA: not applicable; NLR: negative likelihood ratio; NPV: negative predictive value; PLR: positive likelihood ratio; PPV: positive predictive value.

Figure legends

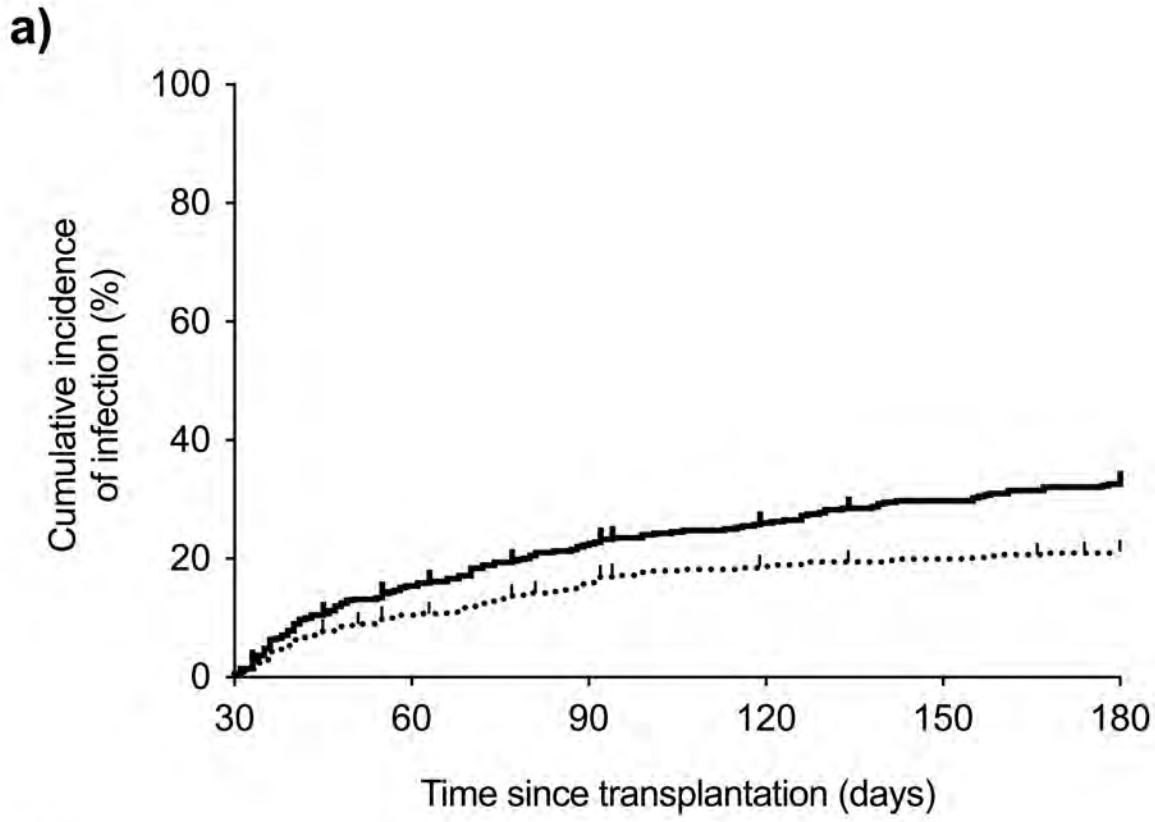
- **Figure 1.** Kaplan-Meier curves of cumulative incidence of overall and bacterial infection (continuous and dotted lines, respectively) between post-transplant months 1 and 6 in the derivation **(a)** and validation cohorts **(b)**.
- **Figure 2.** *Derivation cohort:* Discriminative capacity, as assessed by the area under receiver operating characteristics curves (auROCs) and 95% confidence intervals (CIs), of the SIMPLICITY score at month 1 to predict the occurrence of overall **(a)** and bacterial infection **(b)** between post-transplant months 1 and 6.
- **Figure 3.** *Derivation cohort:* Kaplan-Meier cumulative incidence curves of overall **(a)** and bacterial infection **(b)** between post-transplant months 1 and 6 according to increasing risk categories of the SIMPLICITY score.
- **Figure 4.** *Derivation cohort:* Hazard ratios (HRs) with 95% confidence intervals (CIs) for the occurrence of overall infection between post-transplant months 1 and 6 according to the SIMPLICITY score at month 1. ATG: antithymocyte globulin; BPAR: biopsy-proven acute graft rejection; CMV: cytomegalovirus; DBD: donation after brain death; DCD: donation after circulatory death; ESRD: end-stage renal disease.
- **Figure 5.** *Validation cohort:* Kaplan-Meier cumulative incidence curves of overall **(a)** and bacterial infection **(b)** between post-transplant months 1 and 6 according to increasing risk categories of the SIMPLICITY score.

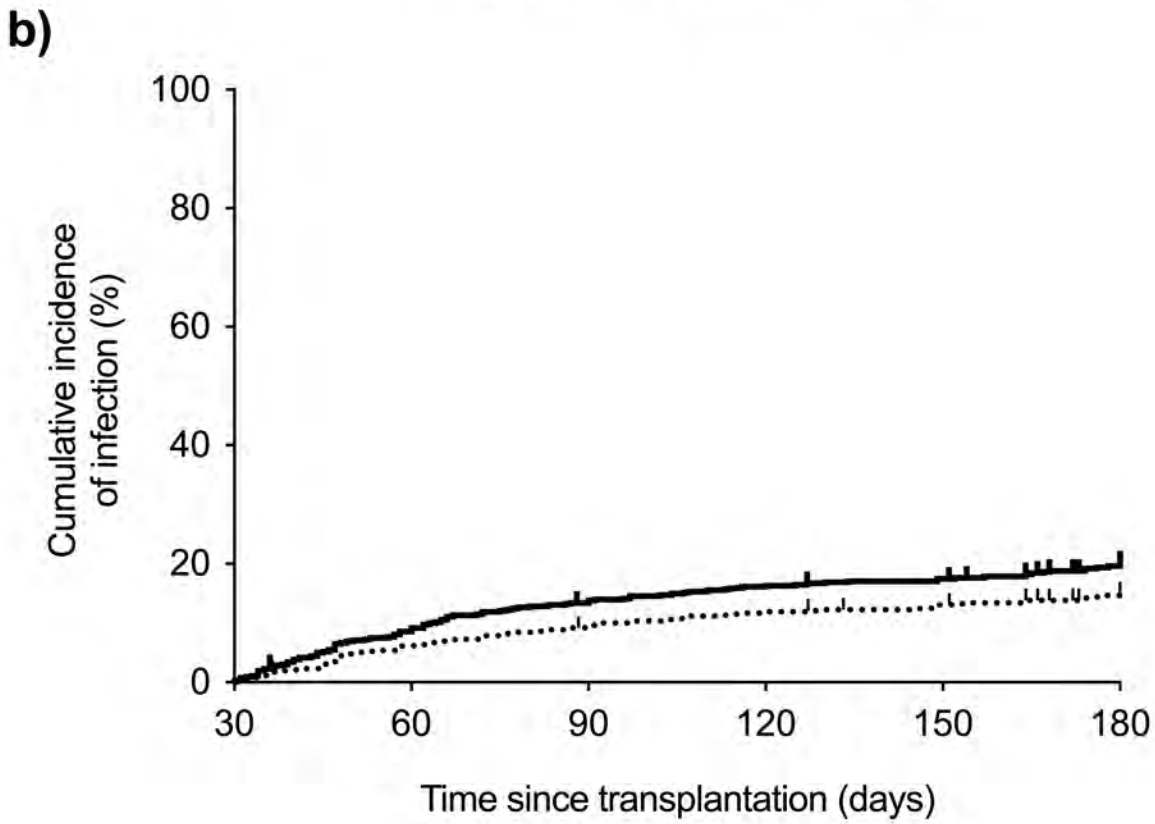
Supporting Material

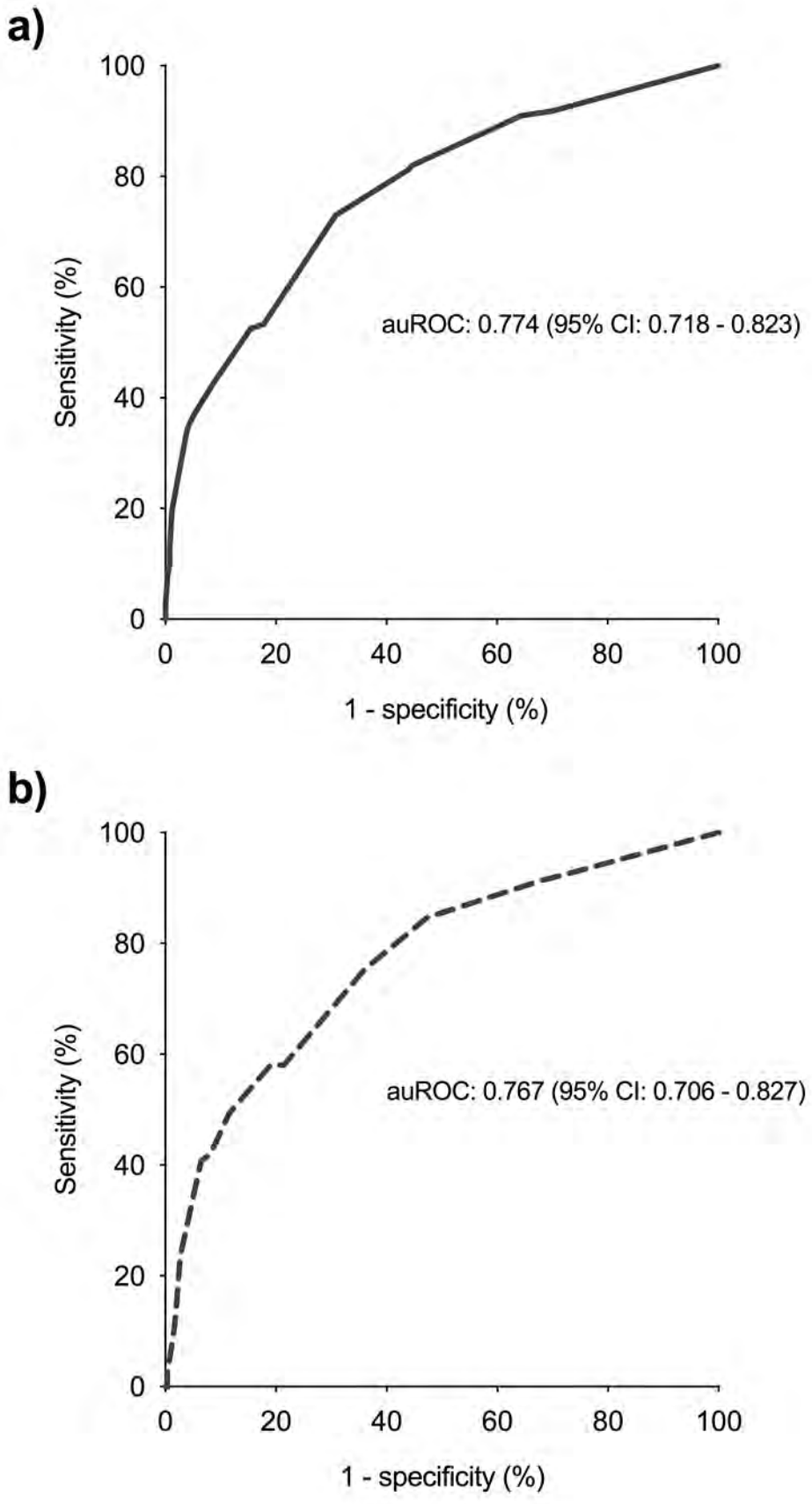
- *Supplementary Results:*
  - **Table S1.** *Derivation cohort:* Clinical syndromes and causative agents involved in 235 episodes of infection between post-transplant months 1 and 6.
  - **Table S2.** *Validation cohort:* Clinical syndromes and causative agents involved in 161 episodes of infection between post-transplant months 1 and 6.
  - **Table S3.** *Derivation cohort:* Univariate analysis of clinical (i.e. non-immune) variables predicting the development of post-transplant infection between months 1 and 6.
  - **Table S4.** *Derivation cohort:* Variance inflation factors assessed to control for multicollinearity among the explanatory variables included in the predictive model.
  - **Table S5.** *Validation cohort:* Diagnostic accuracy of the SIMPLICITY score at month 1 for predicting overall infection between post-transplant months 1 and 6 (primary outcome).
  - **Table S6.** Hazard ratios across SIMPLICITY score risk categories for overall and bacterial infection between post-transplant months 1 and 6 in the derivation and validation cohorts.
  - **Table S7.** Predictive performance and calibration parameters of the SIMPLICITY score.
  - **Table S8.** Updated SIMPLICITY score: the intercept (β_0) of the original model has been updated according to the dataset derived from the validation cohort, resulting in alternative point assignment.
  - **Table S9.** *Missing data imputation for model construction:* Regression coefficients obtained for the variables selected in the final logistic regression model after imputation of missing values in the derivation cohort.
  - **Table S10.** *Missing data imputation for model validation:* Diagnostic accuracy for predicting overall infection between post-transplant months 1 and 6 (primary outcome) in each of the four imputed datasets.
  - **Figure S1.** Patient flow diagram.
  - **Figure S2.** *Derivation cohort:* Peripheral blood lymphocyte subpopulations and serum IgG and complement levels measured at post-transplant month 1 according to the occurrence of infection between months 1 and 6.
  - **Figure S3.** *Derivation cohort:* Cumulative incidence of post-transplant infection at month 6 according to the degree of IgG hypogammaglobulinemia at month 1.

- **Figure S4.** *Derivation cohort:* Hazard ratios for the occurrence of bacterial infection between post-transplant months 1 and 6 according to the SIMPLICITY score at month 1.
- **Figure S5.** *Validation cohort:* Peripheral blood lymphocyte subpopulations and serum IgG and complement levels measured at post-transplant month 1 according to the occurrence of infection between months 1 and 6.
- **Figure S6.** *Validation cohort:* Discriminative capacity assessed by the area under receiver operating characteristics curves (auROCs) of the SIMPLICITY score at month 1 to predict overall **(a)** and bacterial infection **(b)** between post-transplant months 1 and 6.
- **Figure S7.** *Validation cohort:* Hazard ratios for the occurrence of overall **(a)** and bacterial infection **(b)** between post-transplant months 1 and 6 according to the SIMPLICITY score at month 1.
- **Figure S8.** Calibration plot comparing observed and predicted probabilities of overall infection between post-transplant months 1 and 6 in the validation dataset for the original **(a)** and updated models **(b)** of the SIMPLICITY score.

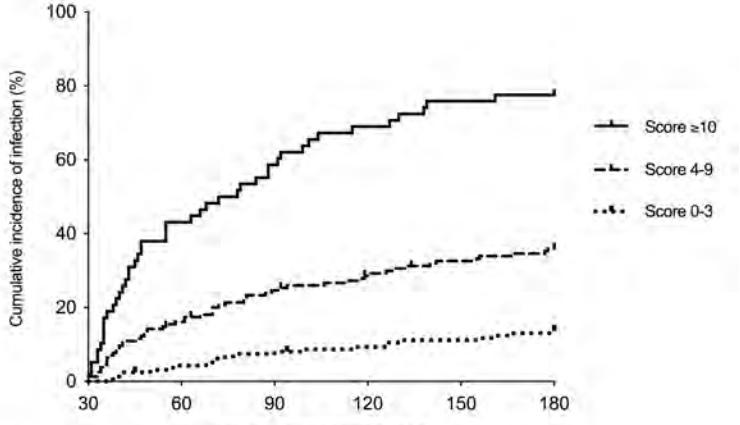
Supplementary information is available on Kidney International's web site.


a)
100
80
60
40
20
0
Cumulative incidence of infection (%)
30
60
90
120
150
180
Time since transplantation (days)


b)
100
80
60
40
20
0
Cumulative incidence of infection (%)
30
60
90
120
150
180
Time since transplantation (days)

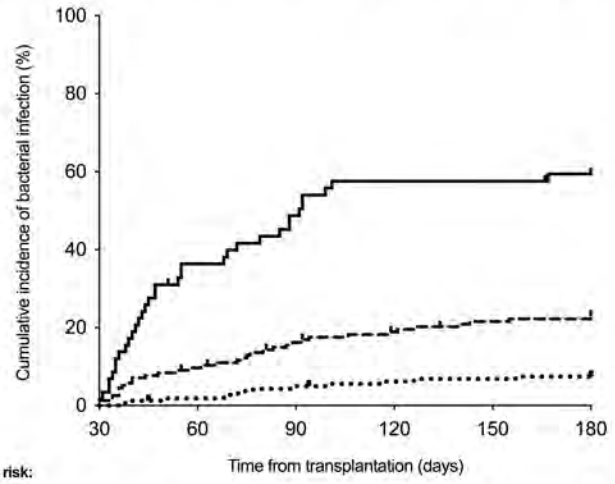

a)
100
80
60
40
20
0
Sensitivity (%)
auROC: 0.774 (95% CI: 0.718 - 0.823)
0
20
40
60
80
100
1 - specificity (%)
b)
100
80
60
40
20
0
Sensitivity (%)
auROC: 0.767 (95% CI: 0.706 - 0.827)
0
20
40
60
80
100
1 - specificity (%)

a)

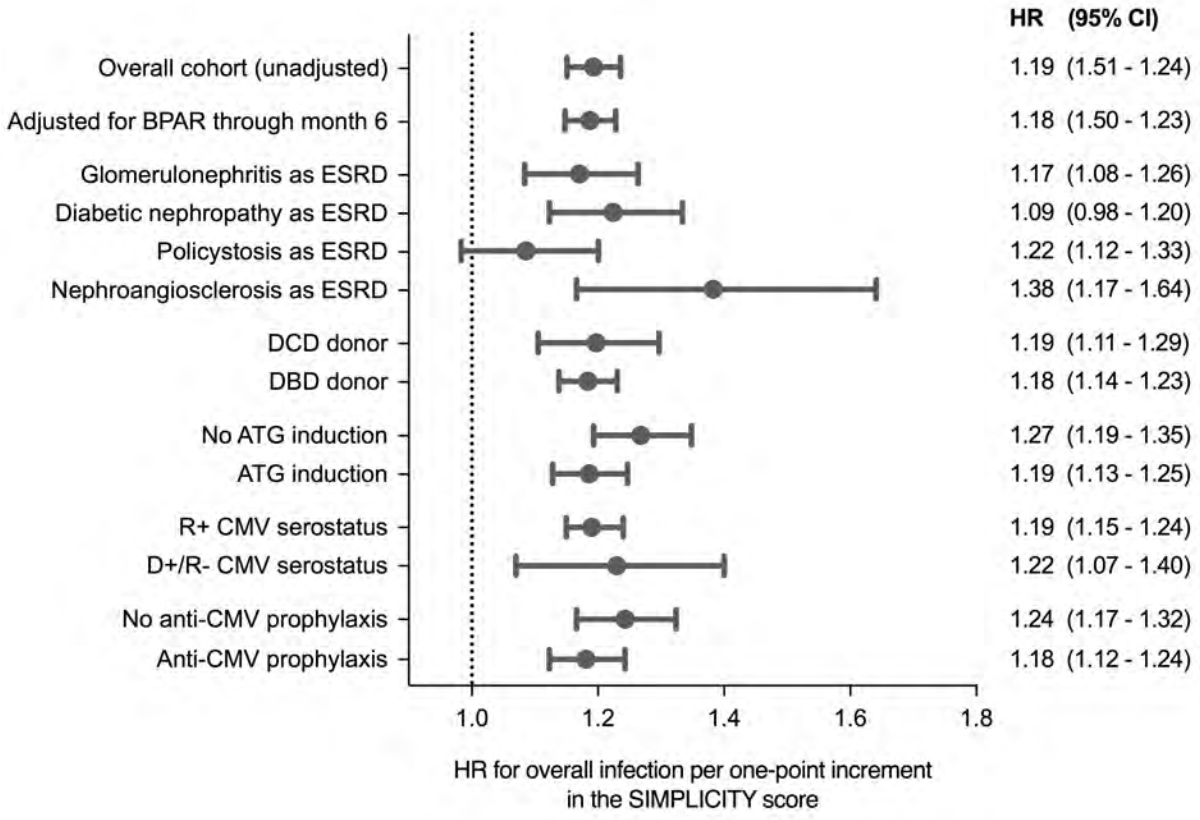


Number at risk:						
Score ≥10	64	42	31	23	17	15
Score 4-9	169	144	129	118	109	102
Score 0-3	177	170	165	153	146	141

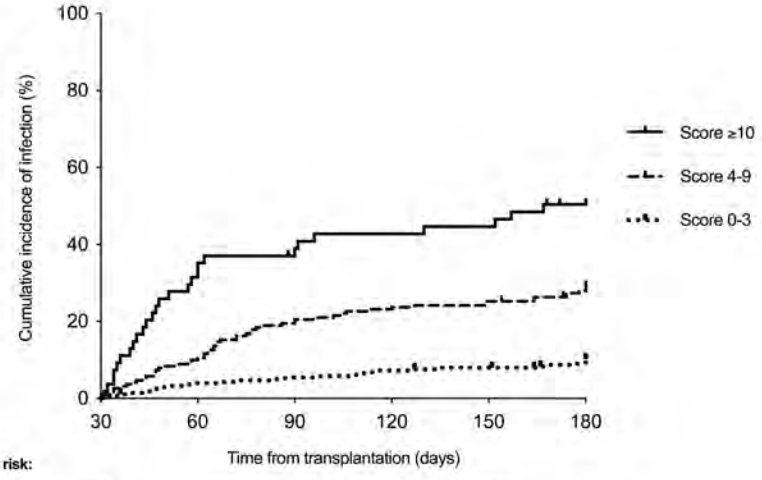
b)



Number at risk:						
Score ≥10	64	54	36	29	26	24
Score 4-9	169	141	138	128	123	121
Score 0-3	177	169	164	155	154	152

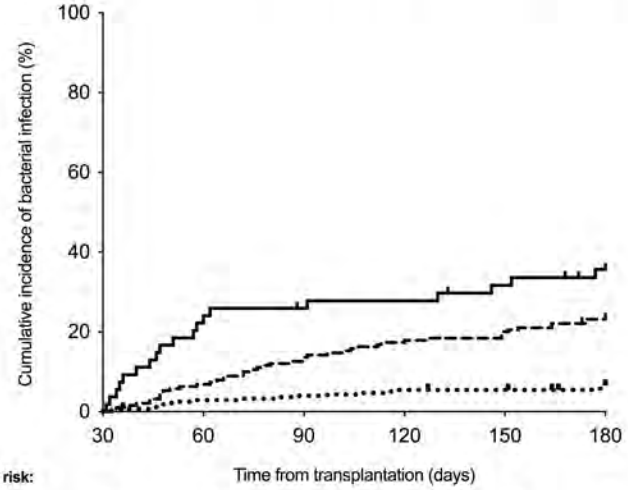

HR (95% CI)
Overall cohort (unadjusted)
1.19 (1.51 - 1.24)
Adjusted for BPAR through month 6
1.18 (1.50 - 1.23)
Glomerulonephritis as ESRD
1.17 (1.08 - 1.26)
Diabetic nephropathy as ESRD
1.09 (0.98 - 1.20)
Policystosis as ESRD
1.22 (1.12 - 1.33)
Nephroangiosclerosis as ESRD
1.38 (1.17 - 1.64)
DCD donor
1.19 (1.11 - 1.29)
DBD donor
1.18 (1.14 - 1.23)
No ATG induction
1.27 (1.19 - 1.35)
ATG induction
1.19 (1.13 - 1.25)
R+ CMV serostatus
1.19 (1.15 - 1.24)
D+/R- CMV serostatus
1.22 (1.07 - 1.40)
No anti-CMV prophylaxis
1.24 (1.17 - 1.32)
Anti-CMV prophylaxis
1.18 (1.12 - 1.24)
1.0
1.2
1.4
1.6
1.8
HR for overall infection per one-point increment
in the SIMPLICITY score

a)



Number at risk:						
Score ≥10	55	37	33	31	30	24
Score 4-9	190	171	153	146	144	135
Score 0-3	277	266	262	257	254	247

b)



Number at risk:						
Score ≥10	55	42	40	39	36	31
Score 4-9	190	178	166	157	155	145
Score 0-3	277	269	266	262	261	256