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A simple score can identify kidney transplant recipients at high risk of severe infection over the following 2 years

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None of the authors have a commercial or other association that poses a conflict of interest.

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AUTHORSHIP

Claire Dendle, William R. Mulley and Stephen Holdsworth performed the clinical research.

Kevan R. Polkinghorne performed the data analysis.

Claire Dendle, Rhonda L Stuart, William R. Mulley, Kevan R. Polkinghorne, Poh-Yi Gan, John Kanellis, Karin Thursky, and Stephen Holdsworth participated in research design and manuscript preparation

ABSTRACT

Background: The aim of this study was to determine whether a composite score of simple immune biomarkers and clinical characteristics could predict severe infections in kidney transplant recipients.

Methods: We conducted a prospective study of 168 stable kidney transplant recipients who underwent measurement of lymphocyte subsets, immunoglobulins and renal function at baseline and were followed for two years for the development of any severe infections, defined as infection requiring hospitalization. A point score was developed to predict severe infection based on logistic regression analysis of factors in baseline testing.

Results: 59 (35%) patients developed severe infection, 36 (21%) had 2 or more severe infections and 3 (2%) died of infection. A group of 19 (11%) patients had the highest predicted infectious risk (>60%), as predicted by the score. Predictive variables were mycophenolate use, graft function, CD4+ and natural killer cell number. The level of immunosuppression score had an area under the receiver operating curve of 0.75 (95% CI 0.67-0.83).

Conclusion: Our level of immunosuppression score for predicting the development of severe infection over 2 years has sufficient prognostic accuracy for identification of high risk patients. This data can inform research that examines strategies to reduce the risks of infection.

KEYWORDS

Infection; kidney transplant; score

MAIN TEXT

INTRODUCTION

Improvements in graft outcomes following solid organ transplantation have meant that the prevention of infection and its associated morbidity and mortality, has become a key focus in the care transplant recipients. [1,2]

Severe infection can indicate that the degree of immunosuppression is too high for a particular patient, but to date clinical factors alone cannot identify which patients are at an increased risk of infection. While most transplant units offer standardized immunosuppressive regimens, these can have markedly heterogeneous immunological effects on patients and biomarkers are needed to stratify patients into those at the highest risk of infection.

The level of immunosuppression study which commenced in 2015, was a prospective study whereby we tested immune biomarkers and their association with different infectious outcomes in kidney transplant recipients. We found that natural killer cell (NK) cytotoxic function was predictive of infection. [3] However, NK cell cytotoxic function is a specialised test and is expensive, thus limited its implementation in to routine clinical practice. We therefore wanted to determine whether other cheaper and more readily available tests of immune function could also predict severe infection.

The aim of this current study was to determine whether a composite score of simple immune biomarkers along with specific clinically important characteristics could predict severe infections in kidney transplant recipients. We hypothesised reduced lymphocyte subsets (T cell, B cell and NK cell counts) combined with kidney transplant function would identify patients at high risk of infection.

METHODS

We conducted a prospective, single center cohort study to evaluate the performance of a composite immune score to identify transplant recipients at risk of severe infection during 2 years of follow-up.

Ethical statement

The study was approved by the human research ethics committee of Monash Health (Number 13085). Written informed consent was obtained from all participants.

Setting

This prospective cohort study was performed at Monash Health, a 1500 bed academic health service in Melbourne, Australia. Monash Health performs approximately 90 kidney transplants per year and has 850 kidney transplant recipients who receive ongoing care.

Patients

In total 168 patients were enrolled in the study between April 2015 and August 2015. All kidney transplant recipients who were greater than 3 months post-transplant were invited to participate. Patients were excluded from the study if they were ≤18 years of age if they had

received augmented immunosuppression to treat rejection within three months or had an infectious illness just prior to or at the time of the study enrolment. All patients underwent measurement of immune biomarkers at study entry and were followed for two years for the development of any severe infections. Baseline clinical characteristics were determined. The estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI formula. [4]

Baseline laboratory testing

The biomarkers selected for inclusion in this study were based on outcome data from the level of immunosuppression cohort (3) as well as published literature demonstrating an association between the biomarkers and infection. [5] The biomarkers included for analysis in this study were CD45+, CD4+, CD8+, CD4+:CD8+ ratio, CD19+, CD56±16± (NK) cell numbers and immunoglobulin concentrations.

Lymphocyte subsets

Lymphocyte subset testing was performed on freshly collected peripheral blood. 100µL aliquots of heparinized blood were labelled with appropriately titred monoclonal antibodies. Fluorescently labelled antibodies used in this study were obtained from Beckman Coulter (Brea, California, USA) and included combinations of CD3-APC, CD4-PE, CD8-FITC, CD14-PE, CD16-FITC, CD19-FITC, CD56-PE and CD45-PC7. Following incubation, red blood cells were lysed using the Beckman-Coulter Q-Prep system and acquired on a FC500 flow cytometer (Beckman Coulter). Total white cells were identified by the immunophenotyped CD45+, T-cells were identified by the immunophenotype CD3+/CD4+ and CD3+/CD8+, B-cells were identified by the immunophenotype CD3-/CD19+ and NK cells were identified by the immunophenotype CD56±16± after gating on lymphocytes by forward and side scatter characteristics. Absolute numbers were calculated using the lymphocyte count provided by full blood examination.

Immunoglobulins

Immunoglobulin isotype (IgA, IgG, IgM) concentrations in serum were measured by Southern Cross Pathology (Dade Behring Dimension Clinical Chemistry System).

Infectious outcomes

The primary outcome was severe infection, defined as any infection requiring admission to hospital after the date of study enrolment. The secondary outcomes were opportunistic infection and recurrent infection. Opportunistic infection was defined as those due to intracellular bacteria (mycobacteria *spp., Nocardia spp*., *Legionella spp*. and *Listeria monocytogenes*), herpesviruses (cytomegalovirus (CMV), herpes simplex virus (HSV) and varicella- zoster virus (VZV) and Epstein-Barr virus-related post- transplant lymphoproliferative disease), yeasts (Candida spp. and Cryptococcus spp.), moulds, *Pneumocystis jiroveci* (PJP), and parasites (*Toxoplasma gondii* and *Leishmania spp*.). [6]

Polyoma virus was only included under the definition of opportunistic infection, and not included as a severe infection. Probable polyoma virus disease was defined as viral replication >10^4 copies per mL or together with compatible symptoms and signs of viral syndrome or organ disease, but without histological confirmation. Proven polyoma virus disease was defined as evidence of virus replication plus corresponding specific histopathology. [7,8] Patients underwent polyoma virus screening through polyoma virus plasma PCR testing monthly for the first six months, then every three months until two years post-transplant. Recurrent infection was defined as two or more episodes of severe infections requiring admission after the date of enrolment. All recurrent infections were new infections, rather than relapse of previously diagnosed infections.

Details of all severe infections were collected. All infections reported in this study (including viral and urinary tract infections) were associated with hospitalization

Study Investigators were notified when patients were hospitalized, at which time they underwent clinical review by a specialist infectious diseases physician to determine the site of infection and to review the microbiological investigations

Infectious episodes were classified as: microbiologically defined (whereby a microorganism related to the clinical presentation was isolated) or clinically defined (whereby no microorganism related to the clinical presentation was isolated but a clinical diagnosis of the site of infection could be determined by the study investigators). Fever without focus was defined as a febrile patient, with no microorganism related to the clinical presentation isolated and no site of infection able to be determined.

CMV infection was defined as virus isolation, or detection of viral nucleic acid in any body fluid or tissue specimen. [9] CMV disease was defined as the presence of appropriate clinical symptoms and/or signs are required together with documentation of CMV in tissue from the relevant organ by histopathology, virus isolation, rapid culture, immunohistochemistry, or DNA hybridization. Only CMV disease was included as an infectious episode. Patients underwent CMV screening through plasma CMV PCR testing fortnightly for the first 3 to 6 months, then every 3 months until 2 years post-transplant. Fungal infections and blood stream infections and sepsis were defined by internationally recognized criteria. [10-12]

Each episode of infection was classified according to source.

Statistical analysis

We assumed an expected rate of severe infection of 20% based on previous studies from our center and other reported rates. [13-15] The minimum required sample size for an area under the receiver operating characteristic (ROC) curve of 0.80 was 64 patients assuming a 90% power and an alpha at 0.05.

Variables with a normal distribution were presented as mean ± standard deviation; variables

with a skewed distribution were presented as medians and interquartile range. Chi-square or Fischer's exact test was used to determine whether or not an association existed between severe infection and categorical variables. Mann-Whitney U test was used to assess differences between non-parametric continuous variables in patients with and without severe infection.

Logistic regression was used to evaluate immune biomarkers as potential predictors for the first episode of severe infection. Clinical and immune biomarker variables were entered into univariable models. Variables that demonstrated a significant association ($p \leq 0.05$) with the development of severe infection were included in the multivariable model. Age, time from transplant, mycophenolate use and eGFR were included in all multivariable models due to their known influence on infection risk. [16,17] There was no evidence of multicollinearity between covariates in the logistic regression models (variance inflation factor <1.4 for all independent variables). Calibration was assessed using the Hosmer-Lemeshow goodness-of-fit statistic.

A simple point score tool was derived from the multivariable logistic regression modelling. Variables that demonstrated a significant association ($p \leq 0.05$) with a coefficient with an absolute value equal or greater than 0.1 were input into the level of immunosuppression score. The level of immunosuppression score were created by multiplying each estimate by 4 and rounding the values to one decimal place for continuous variables and to the nearest integer for the categorical variables. [18] In the case of eGFR, CD4+ cell number and NK cell number a decreasing score was associated with an increasing risk of infection the resulting score was negative (i.e. subtracted). A constant of 18 was added to each individual derived score in order to scale the final total level of immunosuppression score in the range of 0–20 where an increasing score associated with a higher risk of infection. Level of immunosuppression scores were categorized to identify subgroups at higher risks and proportion of patients with severe infection was summarized for these subgroups. The calculated risk score was then entered in a separate logistic regression model with bootstrapping (1,000 replications) to derive standard errors and hence 95% confidence intervals. Receiver operating characteristic curve (ROC) and diagnostic accuracy statistics were estimated from the model. All tests were two-sided with a p-value <0.05 considered significant. All statistical analyses were conducted using Stata (version 15, College Station, Texas, USA) and Graph Pad Prism (Version 7 La Jolla, California, USA).

RESULTS

Characteristics of kidney transplant recipients

One hundred and sixty-eight of a possible 850 (20%) kidney transplant recipients accepted the offer to participate in this study, 114 (13%) declined to participate, 50 (6%) met exclusion

criteria and 519 (61%) did not respond to the invitation. Two patients did not undertake blood testing, leaving 166 (20%) included in the analysis.

The median age was 56.1(47.1-63.9) years and 106 (63%) were male. The median time from transplant was 4.1(1.6-7.8) years. Most patients (81%) were taking tacrolimus, mycophenolate and prednisolone maintenance immunosuppression. All patients were followed for two years. The clinical characteristics and immune biomarkers are presented in table 1. The patients in this study were similar in age and other demographic details to the cohort of kidney transplant recipients at our institution. [19]

Description of severe infections

Fifty-nine (35%) of the 168 patients had at least one severe infective episode, with a total of 141 episodes, during the 2 year follow-up. Of the patients with severe infections, 36 (21%) had recurrent infection (2 or more infections) and 23 (14%) had 3 or more severe infections. Of the 141 episodes of severe infection, 68 (48.2%) were microbiologically proven, 72 (51.1%) were clinically defined and 1 (0.7%) was fever without focus.

The microbiology proven infections included 33 (48%) bacterial, 29 (43%) viral, 5 (7%) fungal and 1 (2%) parasitic. *Escherichia coli* was the most frequently detected bacteria causing severe infection.

The most common source of infection was respiratory, accounting for 53 (38%) severe infections. There were 8 (6%) episodes of blood stream infection and 10 (7%) episodes of sepsis.

The median length of stay for severe infection was 5.0 days (IQR 2.0 to 215.5), 17 (12%) admissions required intensive care. At the end of the follow-up period, 115 (82%) participants were cured of their infections, 23 (16%) were not cured.

Seven (9%) participants died during the follow-up period, four (3%) from malignancy and three (2%) directly attributable to infection.

69 (48.9%) of the 141 infections were defined as opportunistic infection, occurring in 28 (17%) patients. There was one *Nocardia spp*. pulmonary infection. Viral infections were the predominant microbiologically proven opportunistic infection with one VZV, three EBV, seven CMV, one HSV encephalitis, one disseminated adenovirus infections and 51 cases of polyoma virus (42 polyoma virus replication, 4 probable polyoma virus disease and 5 proven polyoma virus disease. There were 5 fungal infections (3 cases of proven invasive pulmonary aspergillosis and one case of disseminated cryptococcosis) and one probable fungal infection (*Pneumocystis jirovecii* pneumonia (PJP). There was one case of disseminated microsporidiosis.

Logistic regression for first severe infection

Results of the logistic regression models for first severe infection are presented in table 2.

On univariable logistic regression, a decreasing CD4+ number was associated with an increased risk of infection (per each decrease of 100 cells/mL, the odds ratio was 0.85; 95% CI, 0.77-0.94; p = 0.002) and a decreasing NK number was also associated with an increased risk of infection (per each decrease of 100 cells/mL, the odd ratio was 0.71; 95% CI, 0.51 to 0.99; p = 0.044)

Multivariable logistic regression demonstrated that only clinical variables that was statistically associated with development of infection was mycophenolate use. The biomarker variables that were statistically associated with development of infection were decreasing eGFR (per 10 ml/min/m^2 decrease) (OR 0.70; 95% CI 0.58-0.84, $p < 0.0001$), decreasing numbers of CD4+ cells (OR 0.86 95% CI 0.77-0.96 p<0.0001) and decreasing NK cells (OR 0.64 95%CI 0.43-0.93 p=0.019). They all remained predictive of an increased likelihood of severe infection in the multivariable model. Immunoglobulin concentrations were not significantly associated with severe infections. Model fit was satisfactory (Hosmer-Lemeshow test statistic 4.56, p=0.80).

Calculation of the level of immunosuppression score

The level of immunosuppression score was calculated by converting coefficients in table 2. Decreasing CD4+ cell number, decreasing NK cell number, decreasing eGFR and MMF use increased the risk of severe infection, whereas age and time from transplant were not sufficiently strongly predictive to warrant a point score. The individual risk factors that received points were eGFR= -1.2 points, mycophenolate use = 6 points, CD4+ = -0.5 points, NK number = -1.7 points. A constant of 18 was added to each score with maximal score of 20, suggesting global immune impairment. Table 3 describes predictions for the level of immunosuppression score and theoretical examples.

The baseline level of immunosuppression score demonstrated a linear increase in the probability of severe infection, such that when the score was above 15, there was an 0.6 probability of hospitalization with infection in the next two years. For every 1 point increase in the level of immunosuppression score, the likelihood for severe infection increased by 27% (Odds ratio (OR) 1.27 95%CI 1.16-1.40 p<0.001), figure 1. Model fit was again satisfactory (Hosmer-Lemeshow test statistic 9.18, p=0.33).

The median level of immunosuppression score was 9.9 (IQR 6.5 -13.5). The level of immunosuppression score categorized according to ranges and the proportions of patients with infection is presented in table 4. The level of immunosuppression score had a threshold effect for the first severe infection. Severe infections occurred in 3 of 25 (12%) of patients with a score of 0-5, 13 of 61 (21%) of patients with a score of 5.1-10, 27 of 61 (44%) of patients with a score of 10.1-15 and 16 of 19 (84%) of patients with a score of 16.1-20.

The baseline increasing level of immunosuppression score was also significantly predictive of opportunistic infection (OR 1.25 95%CI 1.12-1.40 p<0.0001) and recurrent infections (OR 1.39 95%CI 1.23-1.58 p<0.0001).

Table 5 presents describes the diagnostic accuracy (sensitivity, specificity, positive and negative predictive values) at selected calculated risk score with corresponding probability of severe infection of 0.25, 0.5 and 0.75. The optimal infection score cut point that maximises sensitivity and specificity was 10.5, with a sensitivity of 71.1% and a specificity of 68. The area under the ROC for first severe infection by level of immunosuppression score was 0.75 (95% CI 0.67-0.83).

Description of rejection episodes

Thirteen patients (8%) experienced a rejection episode after study entry. Of the 13 rejection episodes, 12 were chronic antibody-mediated rejection and one combined chronic antibody medicated plus acute cellular rejection. Of the 168 patients, 104 (62%) had neither severe infection nor rejection, 51 (30%) developed severe infection alone, five (3%) developed rejection alone and eight (5%) developed severe infection and rejection.

DISCUSSION

The key finding of this study was that a baseline score of simple biomarkers and clinical characteristics could predict hospitalization with severe infection in the next two years in kidney transplant recipients. This data can be used to help clinicians identify patients at high risk of infection and it can help inform trials examining strategies to reduce infections in transplant recipients.

The score consisted of four components, CD4+ cell number, natural killer cell number, eGFR and mycophenolate use. Each of the predictors in the score have independently been associated with infection in other studies but ours is the first to combine them. CD4+ cell count is associated with opportunistic and all cause infections. [6,13,20-22] There is emerging evidence of an association between NK number and infections (such as fungal infections) in SOT recipients. [23-27] Interestingly, we found NK cell cytotoxic function as superior to the measurement of NK cell number. [3]

A poorly functioning graft, as evidenced by reduced eGFR, was a powerful risk factor for severe infection. It may be that patients with poor eGFR had been exposed to increased immunosuppression as treatment for chronic rejection. We controlled for this relationship by recruiting only patients with stable graft function who had not received increased immunosuppression in the past three months and including a past history of rejection and exposure to immunosuppressants in the univariable logistic regression. None of these factors were significantly associated with severe infections however this may represent a type 2 statistical error. Another possibility is that uraemia itself may predispose to infection above and beyond that from iatrogenic transplant immunosuppression. Infections are one of

the leading causes for the increased morbidity and mortality among patients with chronic kidney disease. [28] Uraemia directly or indirectly affects the function of a number of immune cells. [29] For example, defective phagocytosis can be caused by uremic toxins, iron overload and anaemia of renal disease. [30] Reduced eGFR in kidney transplant recipients has also been associated with poor vaccine responsiveness. [31] These factors may in part explain why reduced eGFR was an important risk factor for severe infection in our study. [16] Mycophenolate use had previously been shown to be associated with increased risk of infection. [32]

There are several studies that have examined the use of a composite immune score in SOT recipients but these are difficult to compare directly with ours. [13-15,23,26,33] The majority of these studies have been performed in the early post-transplant period whereas our study specifically examined patients who were stable on immunosuppression and some months or years post-transplant (median time from transplant 4.1 years). Quantifying the net state of immunosuppression with the level of immunosuppression score at this time is useful for clinicians in two circumstances. Firstly, if the patient is 'over immunosuppressed' with a high level of immunosuppression score and high infectious risk, it might be worthwhile considering whether the risk of infection outweighs the risk of rejection at an individual patient level. Secondly, when a patient with a high level of immunosuppression score is admitted with an active infection, an understanding of the degrees of immunosuppression may help clinicians decided upon the empirical antimicrobial management and diagnostic pathways.

A large proportion of patients (35%) were hospitalized with severe infection over the two years follow up period, which is substantially higher than the rate in the general population (34) and highlights the need to address infection prevention in our own institution. The rate of infections is similar to that reported elsewhere in the transplant literature suggesting infection is common and there is an unmet need to identify patients at highest risk. In this study we carefully followed the consequences of these infections. Three patients died as a direct result of infection but the impact was broader in terms of lengthy hospitalizations and intensive care admissions.

This study was performed at single transplant centre and although this study was prospective, the lack of internal and external validation represents a major limitation. Several factors hamper the interpretation of results. Only 20% of eligible patients were finally included, raising the potential for selection bias. The inclusion criteria was restricted to patients at least in their third post-transplant month, with the earlier post-transplant period (with the highest incidence of infection) not covered and although the median post-transplant period for included patients was 4.1 years, there was a large interquartile range (1.6 to 7.8). The immune assessment was cross-sectional with no prospective monitoring of the kinetics

of lymphocyte subpopulations. These factors are important because the amount of immunosuppression and the relative risk for infection decrease over time and such large heterogeneity in post-transplant follow-up may influence the risk prediction models. Importantly, we did not simultaneously examine rates of rejection which would be necessary in any study looking to reduce immunosuppression. [35] We showed that readily available clinical features and biomarkers at a single point in time can be used in a score to identify a subgroup of patient at very high risk of severe infection.

Further research could be directed to not only validating this data but also using these findings to inform clinical trials examining strategies to reduce the risk of infections, such as enhanced antimicrobial prophylaxis, personalised vaccination and careful reduction of immunosuppression.

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AUTHOR CONTRIBUTION STATEMENT

Claire Dendle, William R. Mulley, John Kanellis, Poh-Yi Gan, Rhonda L Stuart, Karin Thursky and Stephen Holdsworth designed the study and wrote the paper; and Kevan R Polkinghorne performed the analysis and wrote the paper.

TABLES

Table 1. Clinical, demographic immunological characteristics of kidney transplant recipients

Characteristic		Number (%) or median (interquartile range)
Age (years)		54.6 (47.1-63.9)
Sex	Male	106 (63)
Ethnicity	Caucasian	106 (63)
	Asian	20 (12)
	Other	42 (25)
Cause of end stage renal failure	Diabetes	37 (22)
	Glomerulonephritis	31 (18)
	Polycystic kidney disease	18 (11)
	Other	
No. of previous transplants	0	150 (89)
	≥1	18 (11)
Transplant duration (years)		4.1 (1.6-7.8)
Medications	Tacrolimus	137 (81)
	Mycophenolate	140 (83)
	Azathioprine	22 (13)
	Prednisolone	144 (86)
	mTORi†	11 (6)

Tacrolimus level (µg/l)		4.6 (3.5-5.5)
Mycophenolate mofetil dose (mg/day)		1375 (1000-1500)
eGFR‡ mls/min/m^2		54.9 (41.0-73.2)
Cytomegalovirus donor/recipient serostatus	D¶-R-§	21(12)
	D-R+	60 (36)
	D+R+	68 (40)
	D+R-	19 (11)
CD45+ number cells/mL		7.2 (6.0-8.8)
CD4+number cells/mL		683 (427-986)
CD8+ number cells/mL		344 (244-520)
CD4+8+ ratio		1.9 (1.2-3.0).
CD19+ number cells/mL		103 (61- 212)
Natural killer cell number cells/mL		140.0 (77.5-211.5)
IgG g/L		8.8 (7.15-15.6)
IgM g/L		0.8 (0.5-1.1)
IgA g/L		1.7 (1.0- 2.7)

† mTORi = Mammalian Target of rapamycin inhibitor ‡ eGFR = estimated glomerular filtration rate ¶ D = Donor § R = Recipient

Table 2. Logistic regression for first severe infection

	Univariable Analysis	**Multivariable Analysis**

Variables	OR	95% CI	*p* value	Coefficient	OR	95% CI	*p* value
Age (per 10 years)	1.18	0.90 -1.52	0.236	-0.12	1.00	0.72-1.39	0.978
Sex (male)	0.77	0.38-1.56	0.473				
Transplant duration (per year)	0.95	0.89-1.02	0.167	-0.19	0.96	0.88-1.04	0.368
Number of previous transplants (per 1 increase)	0.71	0.35-1.47	0.365				
Mycophenolate use	2.62	0.93-7.40	0.068	1.44	3.86	1.06-13.94	**0.039**
Tacrolimus use	1.10	0.46-2.67	0.818				
Prednisolone use	1.31	0.47-3.64	0.599				
mTORi use	0.91	0.23-3.80	0.901				
Tacrolimus level	1.12	0.94-1.35	0.179				
History of rejection	1.13	0.49-2.62	0.760				
eGFR (per 10 ml/min/m^2)	0.74	0.61-0.87	**0.001**	-0.30	0.73	0.60-0.88	**0.001**
Total white cell number (per 1 increase)	1.01	0.89-1.15	0.861				
CD4+ cell number (per 100 increase)	0.85	0.77-0.94	**0.002**	-0.13	0.86	0.77-0.95	**0.007**
CD4+ cell % (per 1% increase)	0.99	0.97-1.02	0.925				
CD8+ cell number (per 100 increase)	0.89	0.77-1.01	0.880				
CD8+ cell % (per 1% increase)	1.00	0.97-1.03	0.818				
CD4+:CD8+ ratio	1.02	0.84-1.24	0.818				
CD19+ cell number (per 100 increase)	0.81	0.62-1.04	0.110				
CD19+ cell % (per 1% increase)	1.00	0.97-1.04	0.739				
NK number (per 100 increase)	0.71	0.51-0.99	**0.044**	-0.42	0.64	0.44-0.93	**0.022**
NK cell % (per 1% increase)	0.99	0.96-1.03	0.698				
IgG concentration (per 1 increase)	0.96	0.87-1.07	0.502				

IgM concentration (per 1 increase)	1.10	0.65-1.85	0.714	
IgA concentration (per 1 increase)	0.87	0.65-1.150.	0.322	

Bold indicates p value <0.05

Table 3. Predictions for the 2-year risk of severe infection using the level of immunosuppression score

Predictive variable	**Level of immunosuppression score**	**Examples** **Three hypothetical kidney transplant recipients**		
Characteristics of hypothetical patients		MMF use eGFR 90 ml/min/m^2 CD4+ 800 cells/mL NK 500 cells/mL	MMF use eGFR 50 ml/min/m^2 CD4+ 500 cells/mL NK 300 cells/mL	MMF use eGFR 30 ml/min/m^2 CD4+ 200 cells/mL NK 50 cells/mL
Mycophenolate use	6	6	6	6
eGFR per 10 ml/min/m^2	-1.2	-10.8	-6	-3.6
CD4+ per 100 cells/mL	-0.5	-4	-5	-1
NK per 100 cells/mL	-1.7	-8.5	-8.5	-0.85
Summative total points		-17.3	-13.5	0.55
Final level of immunosuppression score (addition of		0.7	4.5	18.55

constant of 18)

† Level of immunosuppression score derived by multiplying each coefficient from multivariable analysis by 4, summing the values and adding a constant of 18.

‡ Normal ranges for the immune biomarkers were derived from cuts offs derived using 5th to 95th percentile measurements from a random cohort of staff controls who were well, with no known medical, inflammatory or infectious conditions.

§ The normal range of CD4+ cell number for healthy controls (5th-95th centile) at our institution was 389-1569 cells/mL

¶ The normal range of NK number for healthy controls (5th-95th centile) at our institution was 61-776 cells/mL

Table 4. Level of Immunosuppression score ranges and the observed number of patients, eGFR, CD4+, NK cell number and the number admitted with severe infection per range.

Level of Immunosuppression score range	Number (%) of patients	eGFR 10 ml/min/m^2	CD4+ number cells/mL	NK number cells/mL	Number (%) of patients with severe infection
0-5	25	76.7 (62.1-89.2)	951 (750-1308)	189 (115-287)	3 (12)
5.1-10	61	67.1 (51.0-81.4)	839 (603-1067)	167 (97-223)	13 (21)
10.1-15	61	46.8 (37.0-59.0)	531 (357-766)	130 (74-190)	27 (44)
15.1-20	19	27.7 (20.5-36.0)	379 (281-585)	62 (33-113)	16 (84)

† Level of immunosuppression score derived by multiplying each coefficient from multivariable analysis by 4, summing the values and adding a constant of 18.

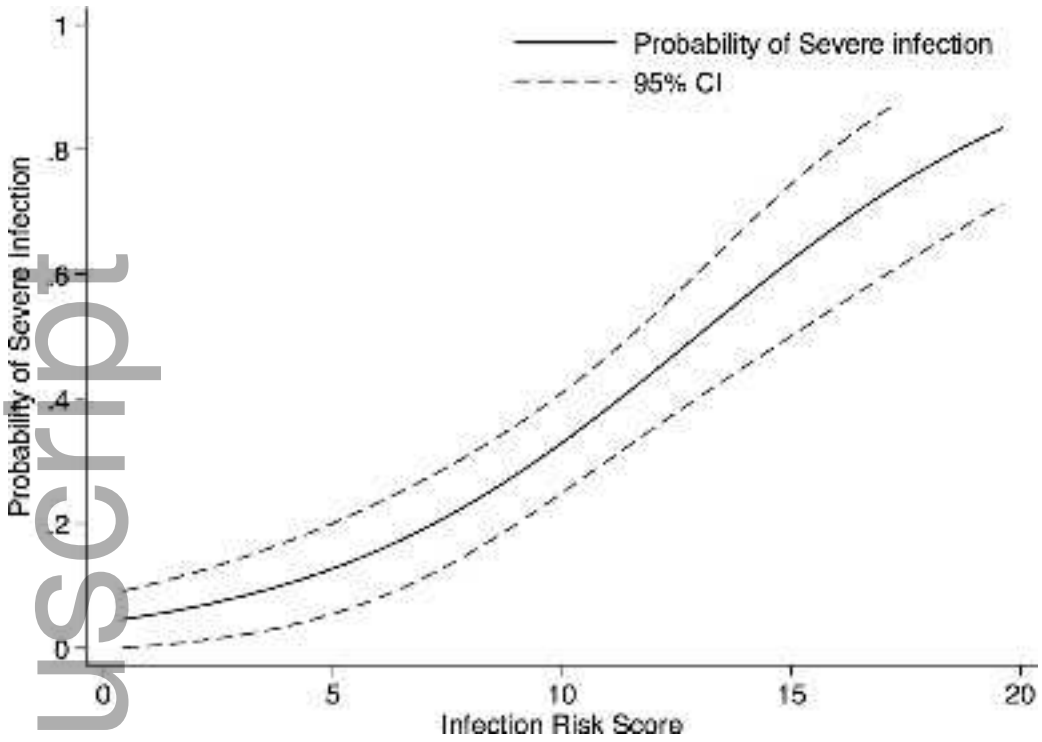
‡ Results are presented as median and interquartile range

Table 5. Diagnostic accuracy of calculated infection risk score.

Risk score	Probability of severe infection	Sensitivity	Specificity	Positive predictive value	Negative predictive value
8.4	0.25	84.7	51.4	49.0	86.0
10.25	0.35	71.2	68.2	55.2	81.1
13.0	0.50	50.8	83.2	62.5	75.4
17.6	0.75	11.9	99.1	87.5	67.1

FIGURE LEGEND

Figure 1. The probability of developing severe infection in the next two years according to level of immunosuppression score.



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