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Risk Factors Associated With Early Invasive Pulmonary Aspergillosis in Kidney Transplant Recipients: Results From a Multinational Matched Case–Control Study

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Risk factors for invasive pulmonary aspergillosis (IPA) after kidney transplantation have been poorly explored. We performed a multinational case–control study that included 51 kidney transplant (KT) recipients diagnosed with early (first 180 posttransplant days) IPA at 19 institutions between 2000 and 2013. Control recipients were matched (1:1 ratio) by center and date of transplantation. Overall mortality among cases was 60.8%, and 25.0% of living recipients experienced graft loss. Pretransplant diagnosis of chronic pulmonary obstructive disease (COPD; odds ratio [OR]: 9.96; 95% confidence interval [CI]: 1.09–90.58; p = 0.041) and delayed graft function (OR: 3.40; 95% CI: 1.08–10.73; p = 0.037) were identified as independent risk factors for IPA among those variables already available in the immediate peritransplant period. The development of bloodstream infection (OR: 18.76; 95% CI: 1.04–339.37; p = 0.047) and acute graft rejection (OR: 40.73, 95% CI: 3.63–456.98; p = 0.003) within the 3 mo prior to the diagnosis of IPA acted as risk factors during the subsequent period. In conclusion, pretransplant COPD, impaired graft function and the occurrence of serious posttransplant infections may be useful to identify KT recipients at the highest risk of early IPA. Future studies should explore the potential benefit of antimold prophylaxis in this group.

Abbreviations: BAL, bronchoalveolar lavage; BSI, bloodstream infection; CI, confidence interval; CMV, cytomegalovirus; CoNS, coagulase-negative staphylococci; COPD, chronic obstructive pulmonary disease; EBNA, Epstein–Barr virus nuclear antigen; eGFR, estimated GFR; EORTC/MSG, European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group; GM, galactomannan; HBc, hepatitis B core antigen; HCV, hepatitis C virus; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; KT, kidney transplant; OR, odds ratio; ROC, receiver operating characteristic; SD, standard deviation; SOT, solid organ transplant; VIF, variance inflation factor

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Introduction

Patients who have undergone kidney transplant (KT) require life-long immunosuppressive treatment to prevent graft rejection. This circumstance increases their risk for developing severe opportunistic infections, including invasive pulmonary aspergillosis (IPA) (1,2). Of note, mortality rates ranging from 56% to 67% have been reported among KT recipients diagnosed with this complication (3,4).

The incidence rate of IPA after KT is lower than those observed for other solid organ transplant (SOT) populations. A multicenter survey in France revealed an incidence of 0.4% among KT recipients in comparison to 1.3% and 1.9% after heart and liver transplantation, respectively (3). Similar figures have been reported in other large studies, with incidence rates <0.5% (5,6). In view of such low incidence, the universal use of antimold prophylaxis in KT recipients is not feasible or advisable (2,7). Nevertheless, it should be noted that KT represents, by far, the most frequently performed transplant procedure worldwide. Consequently, KT recipients suffer from the highest burden of posttransplant IPA events in absolute terms, exceeded only by lung transplant recipients (5,8–10); for example, 47 cases of IPA in KT recipients were identified between 2001 and 2006 in the Transplant-Associated Infection Surveillance Network (TRANSNET) database, compared with only 42 and 23 cases among liver and heart transplant recipients, respectively (8).

Notwithstanding this fact and the dismal prognosis of this condition, our current knowledge about IPA after KT is limited mainly to single case reports, small case series (11), studies covering the overall SOT population (in which KT recipients are underrepresented) (9,10,12), or studies including invasive fungal diseases caused by both molds and yeasts (13–15). To date, only one single-center case–control study has been specifically aimed at

ascertaining the conditions leading to the development of IPA in KT recipients (16). The authors identified leukopenia and a longer duration of pretransplant renal replacement therapy as risk factors for early IPA (i.e. diagnosed within the first 3 mo), although only 15 cases were included in the multivariable model.

Most cases of IPA in SOT recipients are diagnosed during the first months following transplantation, when the overall amount of immunosuppression is higher (6,8); therefore, preventive efforts should be optimized throughout that period. The aim of our study was to assess the predisposing factors for the development of early IPA in a large representative population of KT recipients.

Materials and Methods

Study design

The present study was developed in 29 hospitals from 10 different countries (Spain, United States, Switzerland, Belgium, Brazil, Portugal, France, Mexico, Argentina, and United Kingdom). The Swiss Transplant Cohort Study contributed with the joint experience from six transplant centers in Switzerland, as detailed elsewhere (17,18). Participating centers were invited to include cases of early IPA (i.e. within the first 180 days after transplantation) diagnosed in KT recipients between January 1, 2000, and December 31, 2013 (IPA cases). Patients who underwent transplantation immediately before or after the index case at each center and with no evidence of IPA throughout the posttransplant period were selected as controls at a 1:1 ratio (control group). With matching by institution and date of transplantation, we attempted to control for potential imbalances in terms of posttransplant clinical management and institutional protocols across different periods. To be eligible, control participants must have survived at least until the time of diagnosis of IPA in the corresponding index case. To assess the impact of posttransplant risk factors (i.e. occurrence of acute graft rejection) on the occurrence of early IPA, control participants were assigned a "pseudo–date of diagnosis" to match their case with the aim of ensuring comparable periods of risk exposure in both groups. The date of diagnosis for IPA cases was defined as the calendar day on which the first clinical sample yielding *Aspergillus* spp. or the first detection of positive galactomannan (GM) assay was obtained. For cases in which the diagnosis of IPA was established only after autopsy, the date of death was used as the date of diagnosis.

The study was developed with the institutional support of the Spanish Network Research of Infectious Diseases and the Group for the Study of Infection in Transplant Recipients of the Spanish Society of Clinical Microbiology and Infectious Diseases. The study protocol was approved by the ethics committee of the coordinating center and by the individual participating centers, as required.

Study definitions

IPA was defined according to the revised criteria proposed in 2008 by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group (19). We included IPA cases that fulfilled modified EORTC/MSG definitions for *probable* or *proven* diagnosis categories. Cases were deemed proven when the diagnosis was established by the visualization of molds in a lung biopsy (or autopsy) with the simultaneous recovery of *Aspergillus* spp. in culture from lung tissue, sputum, bronchoalveolar lavage (BAL) or bronchial brush samples. Cases were categorized as probable IPA on the basis of the simultaneous presence of at least one host factor plus a radiological criterion plus a mycological criterion. The host factor was assumed to be the receipt of KT under chronic immunosuppressive therapy. The modified radiological criteria included the demonstration of not only dense, well-circumscribed lesions (with or without halo sign or cavitation) but also other lung infiltrates compatible with infection. This latter criterion responds to previous clinical experiences suggesting that IPA in SOT recipients may be accompanied by lung infiltrates (i.e. peribronchial consolidation or tree-in-bud pattern) that differ from the typical signs observed in hematological patients (20). The microbiological criteria included the recovery of *Aspergillus* spp. in culture from sputum, BAL or bronchial brush samples and/or a positive GM assay (cutoff value of 0.5 optical densities in plasma or serum specimens and 1.0 in BAL specimens). All IPA cases were independently reviewed by an infectious disease specialist at the coordinating center who rejected those cases that did not fulfill the above-mentioned criteria. Mortality was considered attributable to IPA when the patient died with microbiological, histological or clinical evidence of active IPA (proven or probable) and other potential causes of death were reasonably excluded by the attending physician (21). Cytomegalovirus (CMV) disease included viral syndrome (defined by the demonstration of CMV infection by pp65 antigenemia plus one or more of the following: fever, new-onset or increased malaise, leukopenia, atypical lymphocytosis, thrombocytopenia, or elevation of alanine aminotransferase or aspartate aminotransferase higher than two times the upper limit of normal) and probable or definitive end-organ disease, as defined previously (22). The diagnosis of pneumonia included community-acquired, hospital-acquired, health care–associated and ventilator-associated forms. Only laboratory-confirmed cases of influenza or other respiratory viruses were analyzed. Bloodstream infection (BSI) was defined as the presence of one microorganism or more in one blood culture along with clinical evidence of infection. For those microorganisms usually considered skin contaminants (i.e. coagulase-negative staphylococci [CoNS]), two consecutive positive cultures were required. Delayed graft function denoted the requirement for dialysis within the first 2 weeks after transplantation. Acute graft rejection was diagnosed by histological examination if possible or by response to empirical antirejection treatment (23). Estimated GFR (eGFR) was assessed using the four-variable MDRD equation (24).

Statistical analysis

Continuous variables were summarized using the mean plus or minus standard deviation or the median with interquartile range (IQR), whereas categorical variables were summarized using absolute counts and percentages. Categorical variables were compared using the McNemar test, whereas the Student t-test for repeated measures or the Wilcoxon signed rank test was applied for continuous variables. Conditional logistic regression was used to identify independent risk factors for early IPA. Those variables found to be significant ($p \leq 0.05$) in the univariate analysis were included into the multivariable models in a backward stepwise fashion. Continuous variables (i.e. total lymphocyte count) were entered after dichotomization by the optimal cutoff values for distinguishing cases from controls on the basis of the Youden index or J statistic (J = sensitivity + specificity − 1) (25). Collinearity among explanatory variables was assessed using variance inflation factors (VIFs). VIF values >3 suggest the presence of significant collinearity. It is conventionally assumed that regression models should be used with a minimum of 10 events per explanatory variable to avoid model overfitting, unreliable confidence interval coverage and convergence problems because this ratio declines below such a threshold. In addition, we sought to identify a set of predictive criteria for the development of early IPA that would be easily usable by clinicians to identify a subgroup of high-risk KT recipients during either

the peritransplant period or throughout the following months. Consequently, we performed two separate models: The first included only those variables already available at the time of transplantation or within the first 2 weeks (immediate peritransplant period), whereas the second model was constructed on those events that occurred during the subsequent period (mostly posttransplant complications) that had been identified at the univariate level as risk factors for the development of early IPA. The goodness of fit of both models was evaluated by the Hosmer-Lemeshow test. Results are given as odds ratios (ORs) with 95% confidence intervals (CIs). Given the long time frame of the study, an "era effect" was forced into the models by dividing the recruitment period into two parts (cases diagnosed in the period 2000–2009 and in 2010–2013).

In addition, we attempted to obtain an explanatory risk score based on the variables selected in the regression models by assigning a point value corresponding to the β-coefficient of that variable rounded to the nearest whole number. Summation of the points resulted in a weighted score that was assigned to each case and control. The accuracy of such score was assessed by means of the area under receiver operating characteristic (ROC) curve.

All significance tests were two-tailed. Statistical analysis was performed using SPSS version 15.0 (IBM Corp, Armonk, NY).

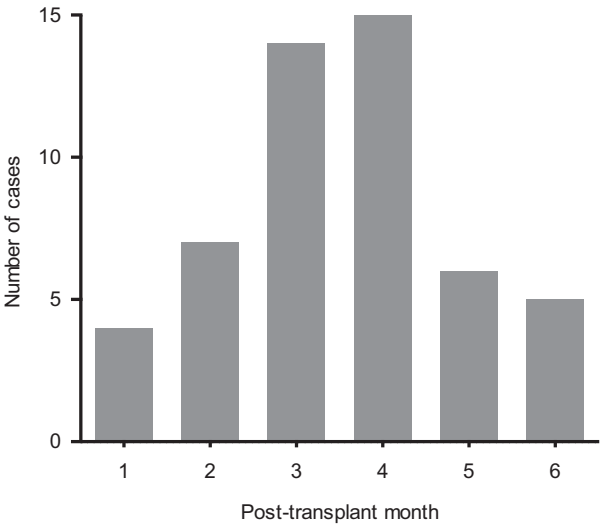

Figure 1: Temporal distribution of cases of early invasive pulmonary aspergillosis occurring according to posttransplant month of diagnosis.

Results

We included 51 early IPA cases (14 proven and 37 probable) and 51 controls from 19 institutions located in Europe and the Americas (16 and 3 centers, respectively). Approximately half of the IPA cases (25 of 51) were diagnosed between 2010 and 2013. The mean number of cases included from each center was 2.7 (range: 1–7). The median interval between transplantation and diagnosis was 91 days (IQR: 65–116 days), with 4 (7.8%), 7 (13.7%) and 14 (27.5%) cases occurring in the first, second and third posttransplant months, respectively (Figure 1). Overall mortality for IPA cases was 60.8% (31 of 51) and occurred at a median of 15 days (IQR: 6–59 days) from diagnosis, whereas the IPA-attributable mortality was 45.1% (23 of 51). Among living recipients, 25.0% (5 of 20) experienced definitive graft failure requiring return to permanent dialysis.

Table 1 details the demographics and pretransplant factors of cases and their control counterparts. Cases had a higher baseline prevalence of chronic obstructive pulmonary disease (COPD) and were more likely to be receiving chronic dialysis at the time of transplantation. Donor- and transplant-related and posttransplant variables are shown in Table 2. In comparison to controls, cases were more likely to suffer from delayed graft function; to have been diagnosed with pneumonia, CMV disease, BSI or acute graft rejection within the 3 mo prior to the diagnosis of early IPA; and to have required admission to the intensive care unit for at least 72 h during that period. The episodes of BSI were caused by CoNS (four cases), *Enterobacteriaceae* (three cases), *Pseudomonas aeruginosa* (three cases), *Staphylococcus aureus* and *Enterococcus* spp. (two cases each), and *Nocardia* (one case). In contrast, controls were more likely to have received a graft from a living donor. With regard to graft function, cases had consistently lower eGFR at the different time points preceding the diagnosis of IPA compared with controls. The total lymphocyte count at day 7 after transplantation was significantly lower in cases than in controls (Figure 2). The optimal cutoff value (i.e. value with the highest Youden index to distinguish cases and controls) for this variable was 1.75×10^3 cells/μL.

As detailed earlier in the Materials and Methods, we performed two separate conditional logistic regression models. There was no significant collinearity between the explanatory variables included in either of the models, with all VIF values <1.5 (data not shown).

The first explanatory model was limited to those variables that were already available at the time of transplantation or within the first 2 weeks (immediate peritransplant period). Pretransplant diagnosis of COPD (OR: 9.96; 95% CI: 1.09–90.58; p = 0.041) and delayed graft function (OR: 3.40; 95% CI: 1.08–10.73; p = 0.037) were independent risk factors for the occurrence of early IPA (Table 3). The Hosmer-Lemeshow test showed a good fit for the model (p = 0.789).

The second model included as explanatory variables those events occurring beyond the immediate posttransplant period (Table 4). The development of BSI (OR: 18.76; 95% CI: 1.04–339.37; p = 0.047) and acute graft rejection (OR: 40.73, 95% CI: 3.63–456.98; p = 0.003) within the 3 mo preceding the diagnosis of IPA (or the analogous pseudo–date of diagnosis in controls) were

Table 1: Comparison of demographics and pretransplant variables between kidney transplant recipients with and without IPA within the first 180 days after transplantation

Variable	IPA group (n = 51)	Control group (n = 51)	p-value[1]
Age, years, mean ± SD	57.3 ± 15.6	54.4 ± 14.5	0.211
Sex, male, n (%)	37 (72.5)	32 (62.7)	0.424
Pretransplant conditions, n (%)			
Diabetes mellitus	12 (23.5)	15 (29.4)	0.629
Chronic obstructive pulmonary disease	8 (15.7)	1 (2.0)	**0.039**
Pretransplant corticosteroid therapy, n (%)	6 (11.8)	7 (13.7)	1.000
ICU admission within 3 mo before transplantation, n (%)[2]	0 (0.0)	1 (2.2)	1.000
BMI at transplantation, kg/m^2, mean ± SD[3]	25.9 ± 5.6	25.2 ± 4.7	0.421
Previous kidney transplantation, n (%)	8 (15.7)	4 (7.8)	0.344
Underlying end-stage renal disease, n (%)			
Glomerulonephritis	12 (23.5)	11 (21.6)	1.000
Diabetic nephropathy	8 (15.7)	8 (15.7)	1.000
Nephroangiosclerosis	6 (11.8)	6 (11.8)	1.000
Polycystosis	9 (17.6)	6 (11.8)	0.581
Chronic interstitial nephropathy	3 (5.9)	5 (9.8)	0.727
Lupus nephropathy	0 (0.0)	5 (9.8)	0.063
Reflux nephropathy	1 (2.0)	0 (0.0)	1.000
Unknown	5 (9.8)	5 (9.8)	1.000
Other	8 (15.7)	6 (11.8)	0.791
Pretransplant serostatus, n (%)			
Hepatitis C virus[4]	5 (10.0)	1 (1.9)	0.125
Hepatitis B virus (anti-HBc)[5]	7 (15.2)	4 (9.3)	0.375
Hepatitis B virus (surface antigen)[4]	1 (2.0)	1 (1.9)	1.000
Epstein–Barr virus (anti-EBNA)[6]	40 (88.9)	39 (86.7)	1.000
CMV[7]	41 (82.0)	43 (91.5)	1.000
Renal replacement therapy, n (%)[7]			**0.008**
No (preemptive transplantation)	0 (0.0)	8 (17.0)	
Pretransplant maintenance dialysis	50 (100.0)	39 (82.9)	
Duration, mo, median (IQR)	30.0 (17.0–57.8)	24.0 (12.0–58.0)	0.152

CMV, cytomegalovirus; EBNA, Epstein–Barr virus nuclear antigen; HBc, hepatitis B core antigen; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; SD, standard deviation.
[1]Significant p-values (<0.05) are expressed in bold type.
[2]Data available for 48 cases and 46 controls.
[3]Data available for 34 cases and 34 controls.
[4]Data available for 50 cases and 51 controls.
[5]Data available for 46 cases and 43 controls.
[6]Data available for 45 cases and 45 controls.
[7]Data available for 50 cases and 47 controls.

identified as independent risk factors for early IPA. The Hosmer-Lemeshow test again demonstrated a good fit for the model ($p = 0.910$). These results remained unchanged when the era of diagnosis (2000–2009 or 2010–2013) was entered in both models (data not shown).

Finally, in an attempt to gain some preliminary insight into the potential feasibility of individualizing the risk of early IPA on the basis of these criteria, we constructed a score by assigning the following point values according to the β-coefficients of each factor: acute graft rejection (4 points), prior occurrence of BSI (3 points), pretransplant diagnosis of COPD (2 points) and delayed graft function (1 point). As expected, the resulting weighted risk scores differed significantly between cases and controls (median points: 4 [IQR: 0–1] and 0 [IQR: 3–5], respectively; $p < 0.001$). The area under the ROC curve for distinguishing cases from controls was 0.89 (95% CI: 0.83–0.96). As shown in Table 5, only 9.8% (4 of 41) of patients that eventually developed IPA (i.e. cases) were given 0 points in the score compared with 90.2% (37 of 41) of controls. In contrast, the presence of scores of 4–5 or ≥6 points would allow correct categorization of IPA cases in 79.3% (23 of 29) and 100.0% (11 of 11) of patients, respectively.

Discussion

Early IPA represents a devastating complication among KT recipients. The all-cause mortality rate in the present cohort was >60%, with most of the deaths directly attributable to aspergillosis. The above-mentioned single-center

Table 2: Comparison of donor- and transplant-related factors and posttransplant complications

Variable	IPA group (n = 51)	Control group (n = 51)	p-value[1]
Age of donor, years, mean ± SD	56.5 ± 14.0	51.1 ± 16.0	0.060
Living donor, n (%)	5 (9.8)	14 (27.5)	**0.022**
Double kidney transplantation, n (%)	0 (0.0)	2 (4.1)	0.500
Induction therapy, n (%)			
None	14 (27.5)	11 (21.6)	0.581
Anti-CD25 (basiliximab or daclizumab)	23 (45.1)	29 (56.9)	0.263
Anti–thymocyte globulin	13 (25.5)	9 (17.6)	0.454
Primary immunosuppression scheme including, n (%)			
Steroids	48 (94.1)	48 (94.1)	1.000
Tacrolimus	28 (54.9)	31 (60.8)	0.607
Cyclosporine	15 (29.4)	13 (26.0)	0.774
MMF/MPA	47 (92.2)	47 (92.2)	1.000
Azathioprine	0 (0.0)	1 (2.0)	1.000
mTOR inhibitor	1 (2.0)	1 (2.0)	1.000
Length of hospital admission for transplantation, days, median (IQR)[2]	19.5 (14.3–36.5)	10.0 (7.3–15.0)	**<0.001**
Delayed graft function, n (%)	22 (43.1)	8 (15.7)	**0.007**
Surgical reintervention, n (%)	8 (15.7)	3 (5.9)	0.065
Posttransplant events in the three preceding months, n (%)[3]			
Pneumonia or laboratory-confirmed viral respiratory tract infection	15 (29.4)	1 (2.0)	**0.001**
CMV disease	11 (21.6)	2 (3.9)	**0.012**
Bloodstream infection	14 (27.5)	1 (2.0)	**0.001**
ICU admission for ≥72 h	9 (17.6)	1 (2.0)	**0.021**
Invasive mechanical ventilation	6 (11.8)	1 (2.0)	0.125
Acute graft rejection	32 (62.7)	5 (9.8)	**<0.001**
Episode treated with steroid boluses	25 (49.0)	3 (5.9)	**<0.001**

CMV, cytomegalovirus; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; MMF/MPA, mofetil mycophenolate/mycophenolate acid; mTOR, mammalian target of rapamycin; SD, standard deviation.
[1]Significant p-values (<0.05) are expressed in bold type.
[2]Data available for 48 cases and 46 controls.
[3]Events occurring in the 3 mo previous to the date of diagnosis of IPA for cases or the analogous "pseudo–date of diagnosis" for their corresponding controls.

study (which analyzed both early and late forms of IPA) found an overall mortality rate of 39% (16). These results highlight the imperative need to identify risk factors that could define a subgroup of KT recipients who would benefit from targeted preventive strategies, and our study may provide preliminary evidence on this point. We decided to focus on patients developing early forms of IPA, for two reasons. First, almost half of the episodes of IPA in this population occur within the first 3–6 mo after transplantation (45% and 56% of the cases included in our multinational study [data not shown] and in the study by Heylen et al (16), respectively); ultimately, this would lead to prescription of antimold prophylaxis for well-delimitated time periods. Second, KT recipients are more closely followed during this early posttransplant period, allowing more accurate identification of predisposing conditions.

The presence of graft dysfunction, reflected by the requirement for dialysis within the first weeks following transplantation and by the development of acute rejection, was identified in our experience as a potential risk factor for early IPA. Apart from its direct impact on immune status, the occurrence of delayed graft function also could be acting as a kind of "clinical surrogate" that summarized different conditions (i.e. longer hospital stay, urinary tract complications or higher transfusion requirements) that exert a deleterious effect overall on the host's susceptibility to infection and that may remain hidden in a single-condition, deterministic model. Acute graft rejection has been previously reported to increase the incidence of invasive fungal disease after KT (13), and graft failure has been found to be a risk factor for IPA in SOT recipients (6,12,26,27). Of note, we noted that the pretransplant diagnosis of COPD and the occurrence of posttransplant pneumonia also increased the risk of early IPA, suggesting the role of previous injury to lung parenchyma as a sort of breeding ground for *Aspergillus*. The diagnosis of pneumonia preceding the onset of IPA has been reported in previous noncomparative studies (11,28). Recent publications have underscored the importance of COPD as a predisposing risk factor for aspergillosis (29,30), and it has been demonstrated that alveolar macrophages in patients with COPD exhibit impairment in their phagocytic function, suggesting the existence of a compartmentalized immunological defect (31).

Cases developing early IPA were more likely to have been diagnosed previously with BSI and CMV disease,

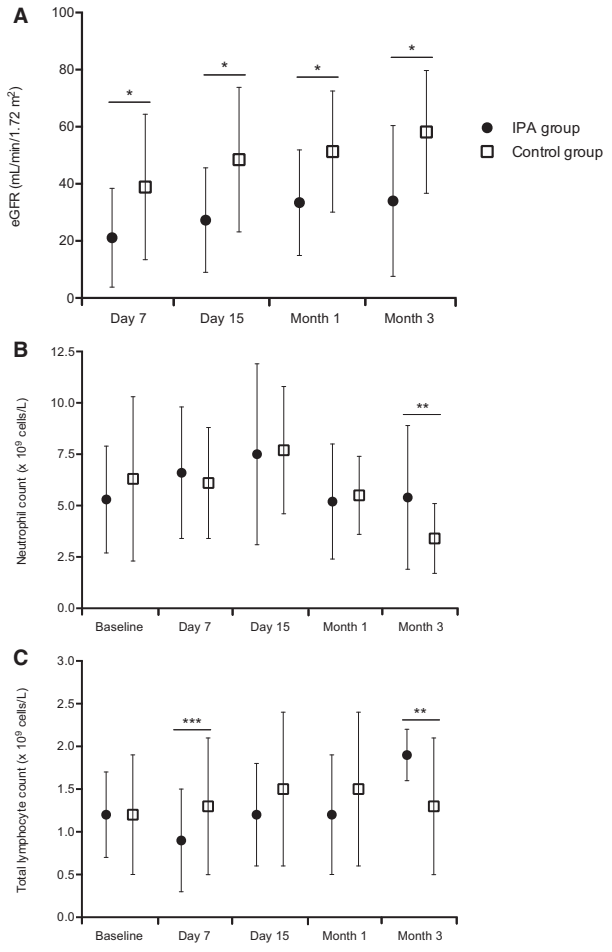

Figure 2: Comparison between kidney transplant recipients with and without early IPA in terms of (A) graft function, (B) leukocyte count, and (C) total lymphocyte count, at different time points. Only values determined before the date of diagnosis of IPA in cases (or the analogous "pseudo–date of diagnosis" in controls) were analyzed (points represent the mean values, and error bars denote the standard deviations). Student t-test for paired data: *$p < 0.001$; **$p < 0.05$; ***$p < 0.01$. eGFR, estimated GFR; IPA, invasive pulmonary aspergillosis.

although only the former association remained in the conditional logistic regression model. The link between bacterial infection and early IPA was already described in a multicenter study that included mainly liver transplant recipients (6). It could be speculated that the development of posttransplant BSI might represent a proxy for prolonged hospital stay, longer antibiotic exposure and higher rates of invasive procedures, which in turn would identify a subgroup of recipients prone to a higher burden of systemic inflammation, malnutrition and impairment of cell-mediated immunity. Interestingly, the prevalence of hepatitis C virus (HCV) infection was five times higher among cases than controls, although this difference did not attain statistical significance because of the low numbers included in each group. Previous studies suggested that chronic HCV infection may increase the incidence of severe infection in KT recipients (32). In contrast, CMV is known to cause a number of indirect effects related to its immunomodulatory mechanisms that lead to nonspecific inhibition of the cell-mediated and humoral immune responses (33). The role of CMV infection as a risk factor for IPA has been well established in different SOT populations (6,15,34). In addition to the biological plausibility of this association, it should not be ruled out that the diagnosis of CMV disease may simply act as a surrogate marker for immunosuppression, as suggested by the lack of statistical significance when other posttransplant events (e.g. acute rejection) were adjusted for in our multivariable model.

Total lymphocyte count at day 7 after transplantation was significantly lower in cases than in controls in the univariate but not in the multivariable analysis. This parameter may be considered an affordable approach to the posttransplant cell-mediated immunity status, and previous studies have demonstrated the value of lymphopenia—particularly at the expense of $CD4^+$ and $CD8^+$ T cell subpopulations—for predicting the development of opportunistic infections in KT recipients (35,36).

The present study has a number of strengths, including stringent application of uniform diagnostic criteria, comprehensive assessment of a large number of explanatory variables, a multicenter design (that ensures appropriate external validity) and biological plausibility of the associations found. By using two separate multivariable models, we established different sets of predisposing factors that may be easily identified by clinicians and that define two different risk profiles. The first risk profile includes those

Table 3: Univariate and multivariable analyses (conditional logistic regression) of risk factors present at the immediate peritransplant period predicting the development of early IPA

	Univariate analysis			Multivariable analysis[1]			
Peritransplant factors	OR	95% CI	p-value	ß-coefficient	OR	95% CI	p-value
Pretransplant diagnosis of COPD	8.00	1.00–63.96	0.050	2.29	9.96	1.09–90.58	0.041
Pretransplant dialysis	7.00	0.96–56.89	0.069	–	–	–	–
Living donor	0.18	0.04–0.82	0.027	–	–	–	–
Delayed graft function	3.80	1.42–10.18	0.008	1.22	3.40	1.08–10.73	0.037

CI, confidence interval; COPD, chronic obstructive pulmonary disease; OR, odds ratio.
[1]Hosmer–Lemeshow p-value = 0.789.

Table 4: Univariate and multivariable analyses (conditional logistic regression) of risk factors occurring during the posttransplant period

Posttransplant events occurring before IPA diagnosis[2]	Univariate analysis			Multivariable analysis[1]			
	OR	95% CI	p-value	ß-coefficient	OR	95% CI	p-value
ICU admission for ≥72 h	9.00	1.14–71.04	0.037	–	–	–	–
Total lymphocyte count <1.75 × 10^3 cells/μL at day 7 after transplant[3]	10.00	1.28–78.12	0.028	–	–	–	–
Pneumonia or laboratory-confirmed viral respiratory tract infection	15.00	1.98–113.56	0.009	–	–	–	–
CMV disease	10.00	1.28–78.12	0.028	–	–	–	–
BSI	14.00	1.84–106.47	0.011	2.93	18.76	1.04–339.37	0.047
Acute graft rejection	28.00	3.81–205.79	0.001	3.70	40.73	3.63–456.98	0.003

BSI, bloodstream infection; CI, confidence interval; CMV, cytomegalovirus; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; OR, odds ratio.
[1]Hosmer–Lemeshow test, p = 0.910.
[2]Events occurring in the 3 mo previous to the date of diagnosis of IPA for cases or the analogous "pseudo–date of diagnosis" for their corresponding controls.
[3]Only values determined before the date of diagnosis of IPA in cases (or the analogous pseudo–date of diagnosis in controls) were taken into account.

Table 5: Distribution of the risk score values between IPA cases and their corresponding controls

Risk score[1]	Overall (n = 102)	IPA group (n = 51)	Control group (n = 51)
0	41	4 (9.8)	37 (90.2)
1–3	21	13 (61.9)	8 (38.1)
4–5	29	23 (79.3)	6 (20.7)
≥6	11	11 (100.0)	0 (0.0)

IPA, invasive pulmonary aspergillosis.
[1]Includes the following variables: pretransplant chronic obstructive pulmonary disease (2 points), delayed graft function (1 point), posttransplant bloodstream infection (3 points), and acute graft rejection (4 points).

KT recipients who already face an increased risk of IPA from the time of transplantation because of their pretransplant comorbidities or impaired graft function. The second risk profile takes into account the occurrence of different events during the first posttransplant months that modulate individual susceptibility to IPA, such as graft rejection. On the basis of these variables, we aimed to construct a single weighted risk score, although it is far from our intention to encourage its application to the clinical decision-making process.

Our study also has some limitations. Despite the collaborative effort to include a large number of early IPA cases, the effective sample size was low, and results are offered with wide CIs (e.g. only one control participant had pretransplant COPD). The choice of matching each case with a single control was made mainly on practical grounds to optimize the data-collection effort, although this design might have compromised the statistical power. We can only infer potential associations rather than demonstrate direct causality in the pathogenesis of posttransplant IPA because the impact of unmeasured confounders cannot be excluded. The proposed score must be regarded as merely explanatory rather than predictive and should be tested in an appropriately sized validation cohort. In addition, the combination of variables derived from two different models may have inflated ORs. A nonnegligible proportion of cases (7.8% [4 of 51]) had a score of 0 points, a proportion that might be still considered excessive to determine individualized use of antimold prophylaxis. Moreover, it should be stressed that because the case–control design of our study did not allow the calculation of incidence rates of posttransplant IPA across participating centers, we were not able to formally estimate the positive and negative predictive values of the score. The attribution of direct causality between the occurrence of posttransplant IPA and death should be made with caution because of the retrospective nature of the research. Most analyzed cases were probable forms of IPA, according to the EORTC/MSG criteria. Finally, the long case-inclusion period and the considerable number of participating centers led to some degree of heterogeneity in the immunosuppressive regimens and the practices of posttransplant care. Nevertheless, because of the rarity of early IPA in the specific population of KT recipients and the difficulty of obtaining a large series from a single institution, we think this methodological approach is a valid way to clarify critical aspects of this life-threatening complication.

Different regimens of antifungal prophylaxis have been used in heart (34) and liver transplantation (37). Notwithstanding its exploratory and hypothesis-generating nature, our study may entail both experimental and clinical implications, although, as mentioned, its case–control design prevents estimation of the number of patients that should be exposed to a prophylaxis to prevent a single case of IPA. Ultimately, the potential usefulness of preventive strategies based on tapered immunosuppression, close clinical and diagnostic follow-up, and targeted administration of antimold prophylaxis in KT recipients

with the risk factors identified in the present study remains to be demonstrated.

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Disclosure

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Francisco López-Medrano has been paid for talks on behalf of Pfizer, Gilead Sciences and Astellas Pharma. Mario Fernández-Ruiz has been paid for talks on behalf of Pfizer and Gilead Sciences. Peggy L. Carver has been paid for talks on behalf of Merck Sharp and Dohme. Oscar Len has been paid for talks on behalf of Astellas Pharma and Merck, Sharp and Dohme and has received grants from Merck Sharp and Dohme and Astellas. Oriol Manuel has received unrestricted grants for research from Roche and Lophius Bioscience. Mariano Arriola has been consultant for Novartis, Pfizer and Astellas Pharma. Jesús Fortún has received grant support from Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Instituto de Salud Carlos III. Ricardo Lazurica has been paid for talks on behalf of Novartis and Astellas Pharma. Marino Blanes has been paid for talks on behalf of Astellas, Pfizer, Gilead and Merck Sharp and Dohme. José María Aguado has been a consultant to and on the speakers' bureau for Astellas Pharma, Pfizer, Gilead, Merck Sharp and Dohme, and Roche. The other authors have no conflicts of interest to disclose.

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