Received Date : 28-Sep-2015

Revised Date : 13-Jan-2016

Accepted Date : 13-Jan-2016

Article type : O - Original Article

Risk factors associated with early invasive pulmonary aspergillosis in kidney transplant recipients: results from a multinational matched case-control study.

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This study was partially presented at the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Copenhagen (April 25-28, 2015).

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Running title: Risk factors for early IPA after KT

List of abbreviations

BAL:	bronchoalveolar lavage
BSI:	bloodstream infection
CI:	confidence interval
CMV:	cytomegalovirus
CoNS:	coagulase-negative staphylococci
COPD:	chronic obstructive pulmonary disease
eGFR:	estimated glomerular filtration rate
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
ICU:	intensive care unit
IFD:	invasive fungal disease
IPA:	invasive pulmonary aspergillosis
IQR:	interquartile range
KT:	kidney transplant
OR:	odds ratio
ROC:	receiving operating characteristics
SD:	standard deviation
SOT:	solid organ transplantation
VIF:	variance inflation factor

Abstract

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

Introduction

Patients undergoing kidney transplantation (KT) require life-long immunosuppressive treatment in order 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 compared to 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 below 0.5% [5,6]. In view of such a low incidence the universal use of anti-mold 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. Thus, KT recipients suffer from the highest burden of post-transplant IPA events in absolute terms only exceeded 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, as compared to only 42 and 23 cases among liver and heart transplant recipients [8].

Notwithstanding this fact and the dismal prognosis of this condition, our current knowledge about IPA after KT is mainly limited 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 due to 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 leucopenia and a longer duration of pre-transplant renal replacement therapy as risk factors for early IPA (i.e., that diagnosed within the first 3 months), 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]. Preventive efforts, therefore, 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 6 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 post-transplant period were selected as controls in a 1:1 ratio (control group). By matching by institution and date of transplantation we attempted to control for potential imbalances in terms of post-transplant clinical management and institutional protocols across different periods. To be eligible, controls must have survived at least until the time of diagnosis of IPA in the corresponding index case. In order to assess the impact of post-transplant risk factors (i.e., occurrence of acute graft rejection) on the occurrence of early IPA, controls were assigned a "pseudo-date of diagnosis" to match their case with the aim to ensure comparable periods of risk exposure in both groups. The date of diagnosis for IPA cases was defined as the calendar day in 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 (REIPI) and the Group for the Study of Infection in Transplant Recipients (GESITRA) of the Spanish Society of Clinical Microbiology and Infectious Diseases (SEIMC). The study protocol was approved by the Ethic Committee of the coordinating center, as well as 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 as “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 not only the demonstration of 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 abovementioned criteria. Mortality was considered to be IPA-attributable when the patient died with microbiological, histological, or clinical evidence of an 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, leucopenia, atypical lymphocytosis, thrombocytopenia, or elevation of ALT or AST higher than two times the upper limit of normal) and probable or definitive end-organ disease, as previously defined [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 or more microorganism(s) in one blood culture along with clinical evidence of infection. For those microorganisms usually considered as 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 two weeks after transplantation. Acute graft rejection was diagnosed by histological examination if possible or by response to empirical anti-rejection treatment [23]. Estimated glomerular filtration rate (eGFR) was assessed by using the 4-variable Modification of Diet in Renal Disease (4-MDRD) equation [24].

Statistical analysis

Continuous variables were summarized using the mean ± standard deviation (SD) or

the median with interquartile ranges (IQR), while categorical variables were summarized using absolute counts and percents. Categorical variables were compared using the McNemar test, whereas the Student's t-test for repeated measures or the Wilcoxon signed-ranks test were applied for continuous variables. Conditional logistic regression was used to identify independent risk factors for early IPA. Those variables found to be significant (*P*-value ≤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 cut-off values for distinguishing cases from controls on the basis of the Youden's index or J statistic (J = Sensitivity + Specificity − 1) [25]. Collinearity among explanatory variables was assessed using variance inflation factors (VIFs). VIF values over 3 will 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 as this ratio declines below such a threshold. In addition, we sought to identify a set of predictive criteria for the development of early IPA easily usable by the clinicians in order to identify a subgroup of high-risk KT recipients during either the peri-transplant period or throughout the following months. Therefore, we performed two separate models: the first one only included those variables already available at the time of transplantation or within the first two weeks (immediate peri-transplant period), whereas the second model was constructed on those events that occurred during the subsequent period (mostly post-transplant 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 in two parts (cases diagnosed in 2000-2009 and those diagnosed 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 receiving operating characteristics (ROC) curve.

All the significance tests were two-tailed. Statistical analysis was performed using SPSS v. 15.0 (Statistical Package for Social Sciences, Inc., Chicago, IL).

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 (25/51) had been 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), with 4 (7.8%), 7 (13.7%) and 14 cases (27.5%) occurring in the first, second and third post-transplant months, respectively (**Figure 1**). Overall mortality for IPA cases was 60.8% (31/51) and occurred at a median of 15 days (IQR: 6-59) from diagnosis, whereas the IA-attributable mortality was 45.1% (23/51). Among survivors, 25.0% (5/20) patients experienced definitive graft failure requiring return to permanent dialysis.

Table 1 details the demographics and pre-transplant 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 post-transplant 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 three months prior to the diagnosis of early IPA, and to have required admission to the intensive care unit (ICU) for at least 72 hours during that period. The episodes of BSI were caused by CoNS (4 cases), *Enterobacteriaceae* (3 cases), *Pseudomonas aeruginosa* (3 cases), *Staphylococcus aureus* and *Enterococcus* spp. (2 cases each), and *Nocardia* (one case). On the other hand, controls were more likely to have received a graft from a living donor. With regards to the graft function, cases had consistently lower eGFR at the different time points preceding the diagnosis of IPA as compared to controls. The total lymphocyte count at day 7 after transplantation was significantly lower in cases than controls (**Figure 2**). The optimal cut-off value (i.e., that with the highest Youden's index to distinguish cases and controls) for this variable was 1.75 x 10^3 cells/μL.

As previously detailed in the Methods section, 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 two weeks (immediate peri-transplant period). Pre-transplant diagnosis of COPD (OR: 9.96; 95% CI: 1.09-90.58; *P*-value =

0.041) and delayed graft function (OR: 3.40; 95% CI: 1.08-10.73; *P*-value = 0.037) were independent risk factors for the occurrence of early IPA (**Table 3**). The Hosmer-Lemeshow test showed a good fit of the model (*P*-value = 0.789).

The second model included as explanatory variables those events occurring beyond the immediate post-transplant period (**Table 4**). The development of BSI (OR: 18.76; 95% CI: 1.04-339.37; *P*-value = 0.047) and acute graft rejection (OR: 40.73, 95% CI: 3.63-456.98; *P*-value = 0.003) within the three months preceding the diagnosis of IPA (or the analogous "pseudo-date of diagnosis" in controls) were identified as independent risk factors for early IPA. The Hosmer-Lemeshow test demonstrated again a good fit of the model (*P*-value = 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 an 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), pre-transplant diagnosis of COPD (2 points) and delayed graft function (1 point). As expected, the resulting weighted risk score significantly differed between cases and controls (median of 4 [IQR: 0-1] and 0 [IQR: 3-5] points, respectively; *P*-value <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% (4/41) of patients that eventually developed IPA (i.e., cases) were given 0 points in the score, as compared to 90.2% (37/41) of the controls. On the opposite, the presence of scores of 4-5 or ≥6 points would allow to correctly categorize as IPA cases 79.3% (23/29) and 100.0% (11/11) of patients, respectively.

Discussion

Early IPA represents a devastating complication among KT recipients. All-cause mortality rate in the present cohort was over 60%, with most of the deaths being directly attributable to aspergillosis. The above-mentioned single-center study (that analyzed both early and late forms of IPA) found an overall mortality of 39% [16]. These results highlight the imperative need to identify risk factors that could define a subgroup of KT recipients that 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: firstly, almost half of the episodes of IPA in this population occurs within the first 3-6 months 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), and this ultimately would lead to prescribe anti-mold prophylaxis for well-delimitated time periods; and secondly, KT recipients are more closely followed-up during this early post-transplant period, thus allowing a 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 could be also acting as a kind of "clinical surrogate" that summarized different conditions (i.e., longer hospital stay, urinary tract complications or higher transfusion requirements) that overall exert a deleterious effect 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], whereas graft failure has been also found to act as a risk factor for IPA in SOT recipients [6,12,26,27]. Of note, we identified that the pre-transplant diagnosis of COPD and the occurrence of post-transplant pneumonia also increase the risk of early IPA, suggesting the role of the 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 non-comparative studies [11,28]. Recent publications have underlined 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 an impairment in their phagocytic function, suggesting the existence of a compartmentalized immunologic defect [31].

Cases developing early IPA were more likely to have been previously diagnosed with BSI and CMV disease, although only the former association remained in the

conditional logistic regression model. The link between bacterial infection and early IPA had been already described in a multicenter study that mainly included liver transplant recipients [6]. It could be speculated that the development of post-transplant BSI might represent a proxy for prolonged hospital stay, longer antibiotic exposure and higher rate of invasive procedures, which in turn would identify a subgroup of recipients prone to suffer from higher burden of systemic inflammation, malnutrition and cell-mediated immunity impairment. 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 due to the low numbers included in each group. Previous studies have suggested that chronic HCV infection may increase the incidence of severe infection in KT recipients [32]. On the other hand, CMV is known to cause a number of indirect effects due to its immunomodulatory mechanisms that lead to a non-specific 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 post-transplant events (such as acute rejection) were adjusted for in our multivariable model.

Total lymphocyte count at day 7 after transplantation was significantly lower in cases than controls in the univariate but not in the multivariable analysis. This parameter may be considered an affordable approach to the post-transplant 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 are a number of strengths to the present study, including the stringent application of uniform diagnostic criteria, comprehensive assessment of a large number of explanatory variables, multicenter design (that ensures appropriate external validity) and biological plausibility of the associations found. By performing two separate multivariable models we established different sets of predisposing factors that may be easily identified by the clinicians and that defines two different risk profiles. The first one includes those KT recipients that face an increased risk for IPA already from the very time of transplantation due to their pre-transplant comorbidities or impaired graft function. The second of these risk profiles takes into account the occurrence of different events during the first post-transplant months that modulate the individual susceptibility to IPA, such as graft rejection. On the basis of these variables we aimed

at constructing 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 (for example, only one control had pre-transplant COPD). The choice of matching each case with a single control was mainly made on practical grounds in order 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 post-transplant IPA, as 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 non-negligible proportion of cases (7.8% [4/51]) had a score of 0 points, a proportion that might be still considered as excessive in order to decide on the individualized use of anti-mold prophylaxis. Moreover, it should be stressed that, since the case-control design of our study did not allow the calculation of the incidence rates of post-transplant 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 post-transplant IPA and death should be taken with caution due to the retrospective nature of the research. Most of the 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 lead to some degree of heterogeneity in the immunosuppressive regimens and practices of post-transplant care. However, due to the rarity of early IPA in the specific population of KT recipients and the difficulty to obtain a large series from a single institution, we think that this methodological approach is a valid way to clarify critical aspects regarding this life-threatening complication.

Different regimens of anti-fungal 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 from estimating 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 anti-mold prophylaxis in KT recipients with the risk factors identified in the present study remains to be demonstrated.

Acknowledgments

The authors would like to acknowledge Mariano Barroso for his kind assistance with the creation of the database. This research was supported by Plan Nacional de I+D+i and Instituto de Salud Carlos III (Proyecto Integrado de Excelencia [PIE] 13/00045), Subdirección General de Redes y Centros de Investigación Cooperativa, Spanish Ministry of Economy and Competitiveness, Spanish Network for Research in Infectious Diseases (REIPI RD12/0015) - co-financed by European Development Regional Fund (EDRF) *"A way to achieve Europe"*. The study was also co-financed with an unrestricted grant from Pfizer Pharmaceutical (REI-ANT-2013-01). Mario Fernández-Ruiz holds a clinical research contract "Juan Rodés" (JR14/00036) from the Instituto de Salud Carlos III, Spanish Ministry of Economy and Competitiveness.

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 Dhome. 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 Dhome 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.

Figure legends

Figure 1. Temporal distribution of cases of early invasive pulmonary aspergillosis occurring according to post-transplant month of diagnosis.

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 (eGFR: estimated glomerular filtration rate; IPA: invasive pulmonary aspergillosis; points represent the mean values and error bars denote the standard deviation). Student's t-test for paired data *P*-values: *<0.001; ** *P*-value <0.05; *** *P*-value <0.01.

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Tables

Table 1. Comparison of demographics and pre-transplant 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[a]
Age, years [mean ± SD]	57.3 ± 15.6	54.4 ± 14.5	0.211
Gender (male) [n (%)]	37 (72.5)	32 (62.7)	0.424
Pre-transplant conditions [n (%)]			
Diabetes mellitus	12 (23.5)	15 (29.4)	0.629
Chronic obstructive pulmonary disease	8 (15.7)	1 (2.0)	**0.039**
Pre-transplant corticosteroid therapy [n (%)]	6 (11.8)	7 (13.7)	1.000
ICU admission within 3 months before transplantation [n (%)][b]	0 (0.0)	1 (2.2)	1.000
BMI at transplantation, Kg/m^2 [mean ± SD][c]	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
Policystosis	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
Pre-transplant serostatus [n (%)]			
Hepatitis C virus[d]	5 (10.0)	1 (1.9)	0.125

Hepatitis B virus (anti-HBc)[e]	7 (15.2)	4 (9.3)	0.375
Hepatitis B virus (surface antigen)[d]	1 (2.0)	1 (1.9)	1.000
Epstein-Barr virus (anti-EBNA)[f]	40 (88.9)	39 (86.7)	1.000
CMV[g]	41 (82.0)	43 (91.5)	1.000
Renal replacement therapy [n (%)][g]			**0.008**
No (preemptive transplantation)	0 (0.0)	8 (17.0)	
Pre-transplant maintenance dialysis	50 (100.0)	39 (82.9)	
Duration, months [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.

[a] Significant *P*-values (<0.05) are expressed in bold.

[b] Data available for 48 cases and 46 controls.

[c] Data available for 34 cases and 34 controls.

[d] Data available for 50 cases and 51 controls.

[e] Data available for 46 cases and 43 controls.

[f] Data available for 45 cases and 45 controls.

[g] Data available for 50 cases and 47 controls.

Table 2. Comparison of donor- and transplant-related factors and post-transplant complications.

Variable	IPA group (n = 51)	Control group (n = 51)	*P*-value[a]
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)][b]	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
Post-transplant events in the three preceding months [n (%)][c]			
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 hours	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.

[a] Significant *P*-values (<0.05) are expressed in bold.

[b] Data available for 48 cases and 46 controls.

[c] Events occurring in the three months previous to the date of diagnosis of IPA for cases or the analogous "pseudo-date of diagnosis" for their corresponding controls.

Table 3. Uni- and multivariable analyses (conditional logistic regression) of risk factors present at the immediate peri-transplant period predicting the development of early IPA.

Peri-transplant factors	**Univariate analysis**			**Multivariable analysis[a]**			
	OR	95% CI	*P*-value	ß-coefficient	OR	95% CI	*P*-value
Pre-transplant diagnosis of COPD	8.00	1.00 - 63.96	0.050	2.29	9.96	1.09 - 90.58	0.041
Pre-transplant 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.

[a] Hosmer-Lemeshow *P*-value = 0.789.

Table 4. Uni- and multivariable analyses (conditional logistic regression) of risk factors occurring during the post-transplant period.

Post-transplant events occurring before IPA diagnosis[b]	**Univariate analysis**			**Multivariable analysis**[a]			
	OR	95% CI	*P*-value	ß-coefficient	OR	95% CI	*P*-value
ICU admission for ≥72 hours	9.00	1.14 - 71.04	0.037	-	-	-	-
Total lymphocyte count <1.75 x 10^3 cells/µL at day 7 post-transplant[c]	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.

[a] Hosmer-Lemeshow *P*-value = 0.910.

[b] Events occurring in the three months previous to the date of diagnosis of IPA for cases or the analogous "pseudo-date of diagnosis" for their corresponding controls.

[c] 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[a]	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)

[a] Includes the following variables: pre-transplant COPD (2 points), delayed graft function (1 point), post-transplant BSI (3 points), and acute graft rejection (4 points).

Figure 1.

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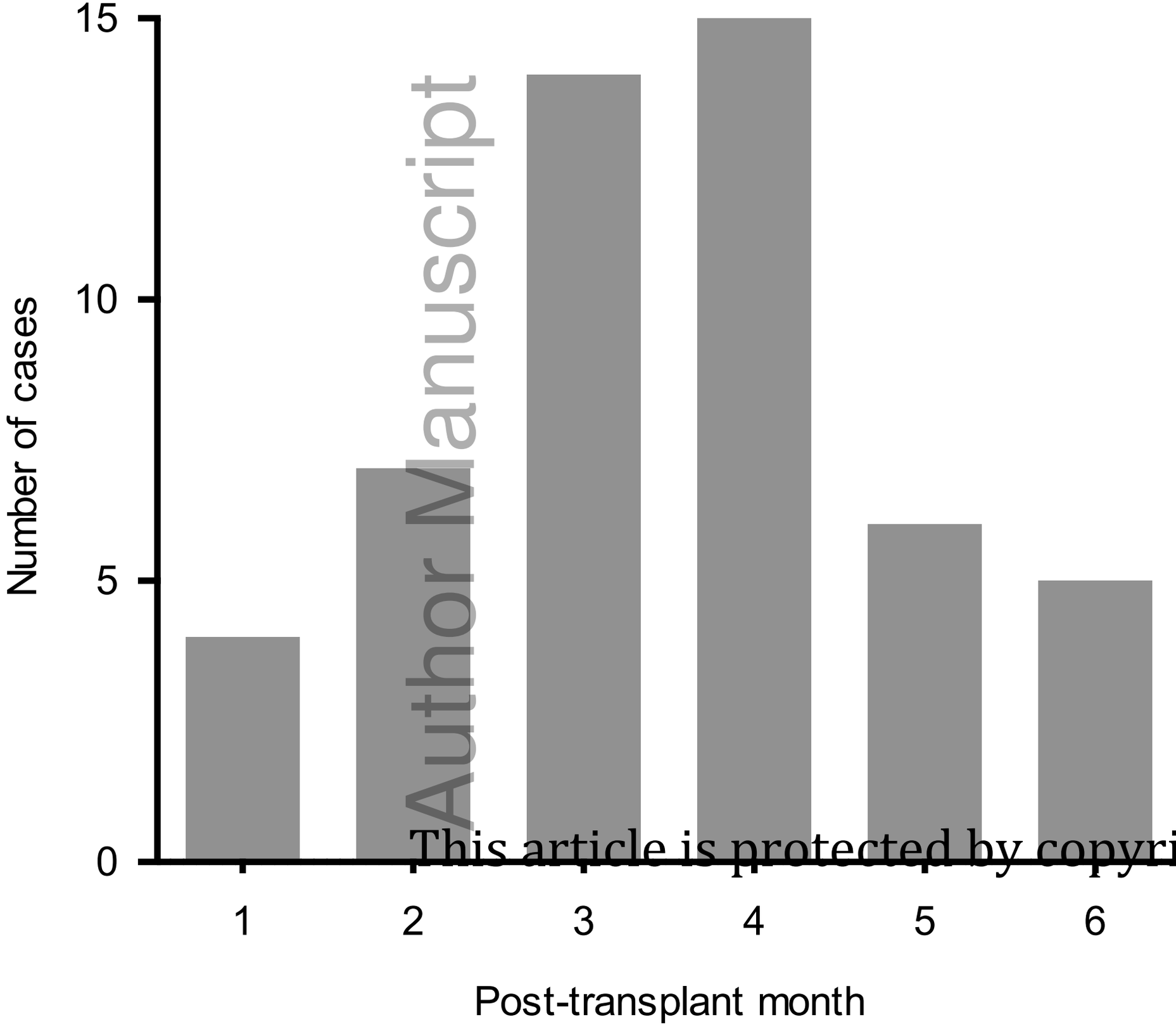

Figure 2.