American Journal of Transplantation 2016; 16: 3220–3234
Wiley Periodicals Inc.

doi: 10.1111/ajt.13837

Clinical Presentation and Determinants of Mortality of Invasive Pulmonary Aspergillosis in Kidney Transplant Recipients: A Multinational Cohort Study

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This study was partially presented at the 53rd Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC), Denver, CO (September 9–12, 2013).

The prognostic factors and optimal therapy for invasive pulmonary aspergillosis (IPA) after kidney transplantation (KT) remain poorly studied. We included in this multinational retrospective study 112 recipients diagnosed with probable (75.0% of cases) or proven (25.0%) IPA between 2000 and 2013. The median interval from transplantation to diagnosis was 230 days. Cough, fever, and expectoration were the most common symptoms at presentation. Bilateral pulmonary involvement was observed in 63.6% of cases. Positivity rates for the galactomannan assay in serum and bronchoalveolar lavage samples were 61.3% and 57.1%, respectively. *Aspergillus fumigatus* was the most commonly identified species. Six- and 12-week survival rates were 68.8% and 60.7%, respectively, and 22.1% of survivors experienced graft loss. Occurrence of IPA within the first 6 months (hazard ratio [HR]: 2.29; p-value = 0.027) and bilateral involvement at diagnosis (HR: 3.00; p-value = 0.017) were independent predictors for 6-week all-cause mortality, whereas the initial use of a voriconazole-based regimen showed a protective effect (HR: 0.34; p-value = 0.007). The administration of antifungal combination therapy had no apparent impact on outcome. In conclusion, IPA entails a dismal prognosis among KT recipients. Maintaining a low clinical suspicion threshold is key to achieve a prompt diagnosis and to initiate voriconazole therapy.

Abbreviations: AmB-D, amphotericin B deoxycholate; AmB-LC, amphotericin B lipid complex; auROC, area under the receiving operator characteristics curve; BAL, bronchoalveolar lavage; CI, confidence interval; CMV, cytomegalovirus; CT, computed tomography; 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; HCV, hepatitis C virus; HR, hazard ratio; HSCT, hematopoietic stem cells transplantation; ICU, intensive care unit; IFD, invasive fungal disease; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; KT, kidney transplantation; L-AmB, liposomal amphotericin B; SOT, solid organ transplantation

Received 22 January 2016, revised 24 March 2016 and accepted for publication 17 April 2016

Introduction

Invasive pulmonary aspergillosis (IPA) constitutes a devastating complication that affects severely immunosuppressed patients. Subjects with hematological malignancies represent the subpopulation with the highest incidence and the worst prognosis among those prone to developing IPA (1). In addition, this life-threatening infection is also described in other groups of patients, such as those with solid organ malignancies (1), chronic pulmonary diseases (2), admission to intensive care units (ICUs) (3), or solid organ transplantation (SOT) (4,5). Among this latter group, the highest incidence of IPA is described in lung transplant recipients, followed by heart and liver transplant recipients (4,6). The risk of IPA after kidney transplantation (KT) is relatively lower compared to those observed for other SOT populations, with incidence rates below 0.5% in most studies (6–8). Nevertheless, KT represents by far the most common transplant procedure. For example, more than 77 000 KT were performed worldwide in 2012 in comparison to 23 000 liver, 5900 heart, and 4300 lung transplants (9). Therefore, KT recipients suffer from the highest burden of IPA events in absolute terms (8,10–12).

Despite this reality, our knowledge about the clinical presentation of IPA in KT recipients and its impact on patient and graft survival is scarce and mostly based on small case series (13) or studies including different SOT populations (11,14–16) or invasive fungal diseases (IFD) due to both molds and yeasts (17–19). Only one single-center case–control study (which included 41 cases of IPA) has specifically analyzed the determinants of outcome among KT recipients, although no details on clinical or radiological features were provided (20).

The generalization of use of antimold prophylaxis has markedly reduced the incidence of IFD among patients with hematological malignancies or hematopoietic stem cells transplantation (HSCT). This approach has also been extended to specific high-risk subgroups of liver and heart transplant recipients (4,21), although so far no formal recommendation has been made regarding the need for antifungal prophylaxis in KT recipients or the optimal targeted therapy once the diagnosis of posttransplant IPA has been established. Such a lack of evidence raises further concern in view of the high mortality rates (ranging from 39% to 61%) observed in previous studies (15,16,19,20).

Thus, additional information is urgently needed to better define the clinical picture of IPA in KT recipients and to gain insight into its determinants of outcome and best therapeutic approaches, with the ultimate aim of improving the dismal prognosis associated with this opportunistic infection. The present study was designed to collect detailed data derived from the joint effort of a multinational group in order to override the intrinsic limitation imposed by the limited number of patients with this complication that may be seen at a single institution.

Patients and Methods

Study design

We performed a multinational retrospective cohort study in 29 hospitals located in Europe (Spain, Switzerland, Belgium, Portugal, France, and United Kingdom) and the Americas (United States, Brazil, Mexico, and Argentina). The Swiss Transplant Cohort Study contributed with the collective experience from six transplant centers in Switzerland (22,23). Participating centers were invited to include all the cases of proven or probable IPA diagnosed in KT recipients between January 1, 2000 and December 31, 2013. By using a standardized data collection form, anonymized information on demographic and baseline characteristics; posttransplant events; clinical, microbiological, and radiological features of IPA; therapeutic approaches; and patient and graft outcome were collected for each case by local investigators and entered in a protected electronic database.

The *primary outcome* was all-cause mortality at 6 weeks from diagnosis. This outcome was chosen in line with recent clinical trials (24) due to its objective nature and close relatedness with IPA-attributable mortality (25). *Secondary outcomes* included all-cause mortality at 12 weeks from diagnosis and graft loss among those patients who survived beyond that point. The retrospective design of the study and the lack of homogeneity in terms of follow-up schedules after the initiation of antifungal therapy (i.e. timing of follow-up thoracic computed tomography [CT] scan) precluded us from selecting clinical and radiological response as study outcome.

This 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 Ethics Committee of the coordinating center as well as by the local Ethics Committees of the different participating centers, as required. The study was performed in accordance with the Helsinki Declaration and the Declaration of Istanbul on Organ Trafficking and Transplant Tourism.

Study definitions

Posttransplant IPA was defined according to the 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 (26). We only included IPA cases that fulfilled modified EORTC/MSG definitions for probable or proven diagnosis categories. Cases were deemed as "proven IPA" 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 biopsy, 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 (6,27). The microbiological criteria included the recovery of *Aspergillus* spp. in culture from sputum, BAL, or bronchial brush samples, and/or a positive galactomannan (GM) assay (≥0.5 optical densities in plasma or serum specimens and ≥1.0 in BAL specimens). Imaging response in the follow-up CT scan was defined as complete (more than 90% radiographic improvement compared with baseline), partial (more than 50% radiographic improvement compared with baseline), stable (no change from baseline or less than 50% radiologic improvement), or failure (progression of disease). Mortality was assessed by review of medical chart records, institutional databases, and regional or national transplant registries and their corresponding mortality information systems, as appropriate for each participating center. Mortality was considered to be "IPA-attributable" when the patient died with microbiological, histological, or clinical evidence of active IPA and other potential causes of death could be excluded by the attending physician or the local site investigator. All cases were independently reviewed by an infectious disease specialist at the coordinating center. According to the interval between transplantation and diagnosis, cases of IPA were categorized as "early" (<180 days) or "late" forms (≥180 days). Given the long time frame of this study, an era effect was taken into account by dividing the cohort according to the date of diagnosis of IPA (2000–2006 and 2007–2013).

"Initial antifungal therapy" was that provided within the first 2 weeks of administration of systemic antifungal drugs with activity against *Aspergillus* for the episode of IPA. For the purpose of the present study, "active antifungal drugs" comprised the following classes: the different formulations of amphotericin B (amphotericin B deoxycholate [AmB-D], amphotericin B lipid complex [AmB-LC] or liposomal amphotericin B [L-AmB]), antimold triazoles (itraconazole [either as oral solution or capsules], voriconazole, or posaconazole), and echinocandins (caspofungin, anidulafungin, or micafungin). "Antifungal combination therapy" was defined as the concomitant use as initial therapy of two or more of these drugs for ≥72 h within the first 2 weeks of therapy. A patient who was given two or more different drugs initiated at least 2 weeks apart was considered to have received monotherapy (categorized according to the first drug used). "Sequential therapy" was defined as the use of one systemic antifungal followed by its discontinuation and replacement with another drug (an overlap period of 48 h or less was allowed), also within the first 2 weeks. For the purposes of multivariate analyses, patients receiving sequential therapy were classified according to the last drug used (provided it was administered for ≥50% of the overall length of therapy).

"Cytomegalovirus (CMV) disease" included viral syndrome and probable or definitive end-organ disease, as previously defined (28). The diagnosis of pneumonia included hospital-acquired, healthcare associated, and ventilator-associated forms. Only laboratory-confirmed cases of influenza were analyzed. "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 (29). Estimated glomerular filtration rate (eGFR) was assessed by the four-variable Modification of Diet in Renal Disease (4-MDRD) equation (30). "Graft loss" was defined as the need for permanent return to dialysis and/or retransplantation.

Statistical analysis

Quantitative data were shown as the mean ± standard deviation or the median with interquartile ranges (IQR). Qualitative variables were expressed as absolute and relative frequencies. Categorical variables were compared using the chi-square test, whereas Student's t-test or U Mann–Whitney test were applied for continuous variables. Survival curves were estimated by the Kaplan–Meier method, and differences between groups were compared with the log-rank test. Univariate and multivariate Cox regression models were used to identify factors predicting all-cause mortality at 6 and 12 weeks, as well as graft loss among survivors. For the latter outcome, the week 12 after diagnosis of IPA was used as reference time point for survival analysis. Those variables found to be significant (p-value <0.05) at the univariate level were included into the multivariate models in a backward stepwise fashion. To estimate survival curves, the date of diagnosis of IPA was set at the calendar day in which the first clinical sample yielding *Aspergillus* spp. or the first positive GM assay was obtained. Associations are expressed as hazards ratios (HRs) with 95% confidence intervals (CIs). Sensitivity analyses were performed using a bootstrap approach to adjust the estimated HR for overfitting. Bootstrap validation estimates how good the performance of the prediction model obtained from the development set would be on a hypothetical set of new patients (31). To this aim, 200 bootstrap samples of equal size were generated from the study population by sampling with replacement.

A potential center effect on the primary study outcome was accounted for through the Cochran's and Mantel–Haenszel statistics by grouping participating centers according to two criteria: the center contributing the largest number of cases versus all other centers pooled, and those contributing fewer than five cases versus those contributing five or more cases.

To partially overcome the limitation posed by the nonrandomized design of our study, and as a preliminary approach, we calculated the propensity for receiving antifungal combination therapy (vs. monotherapy or sequential therapy) given the patient's baseline characteristics and the clinical and radiological features of IPA at presentation. This score was estimated using a backward stepwise logistic regression model including variables with p-values <0.1 in the univariate analysis. In addition, and to increase the precision of the estimated exposure effect without increasing bias, we also adjusted the model for selected variables that were unrelated to the exposure (i.e. antifungal regimen), but significantly associated with the study outcome (6-week mortality). The fit of this model was assessed by means of the Hosmer–Lemeshow test and the area under the receiving operator characteristics curve (auROC). We then performed a 1:1 nearest neighbor matching on the propensity score with replacement and a caliper width of 0.25 to select two subgroups of patients (receiving combination therapy or other regimens) that were comparable in their pretreatment characteristics (32).

All the significance tests were two-tailed. Statistical analysis was performed using SPSS version 15.0 (Statistical Package for Social Sciences Inc., Chicago, IL), graphics were generated with Prism version 6.0 (GraphPad Software Inc., La Jolla, CA), and the propensity score matching was conducted using R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study cohort

We included 112 KT recipients from 33 different institutions (mean number of cases per center: 3.9 [range: 1–20]) (Table S1 in Supporting Information). According to the EORTC/MSG criteria, there were 28 cases (25.0%) of proven IPA, whereas the remaining 84 (75.0%) were classified as probable (of which 21 [25.0%] received this diagnostic category on the basis of the abovementioned modified radiological criteria). Three-quarters of the cases occurred between 2007 and 2013. The median interval between transplantation and diagnosis was 230 days (IQR: 95.5–1117.8), with 51 (45.5%) and 61 cases (54.5%) occurring before and after the sixth month (early and late forms, respectively) (Figure 1). Two cases (1.8%) were diagnosed at autopsy. Six- and 12-week follow-up were achieved in 76 and 66 patients (which accounted for 98.7% [76/77] and 97.0% [66/68] of patients surviving at each of these points, respectively).

Clinical presentation

The baseline and transplant-related characteristics according to the timing of diagnosis are shown in Table 1. As compared to those with late IPA, recipients suffering from early forms had older donors, were receiving higher doses of corticosteroids, and were more likely to be under tacrolimus-containing regimens at diagnosis, had been more frequently diagnosed with pneumonia, CMV disease, bloodstream infection, and acute graft rejection during the 3 preceding months, and had been more commonly admitted into the ICU within that period.

Table 2 details the clinical, radiological, and microbiological features of IPA cases. Most of the patients presented with cough, fever, and expectoration, and had multilobar involvement in the form of nodules in the CT scan, whereas extrapulmonary involvement was uncommon. Of note, 20 patients (18.0%) had no fever, expectoration, or pleuritic chest pain at the time of diagnosis. We found no significant differences in clinical or radiological findings between early and late forms. Regarding the diagnostic procedures, patients with early IPA were more likely to undergo BAL in comparison to late cases. The positivity rates for the GM assay in serum and BAL

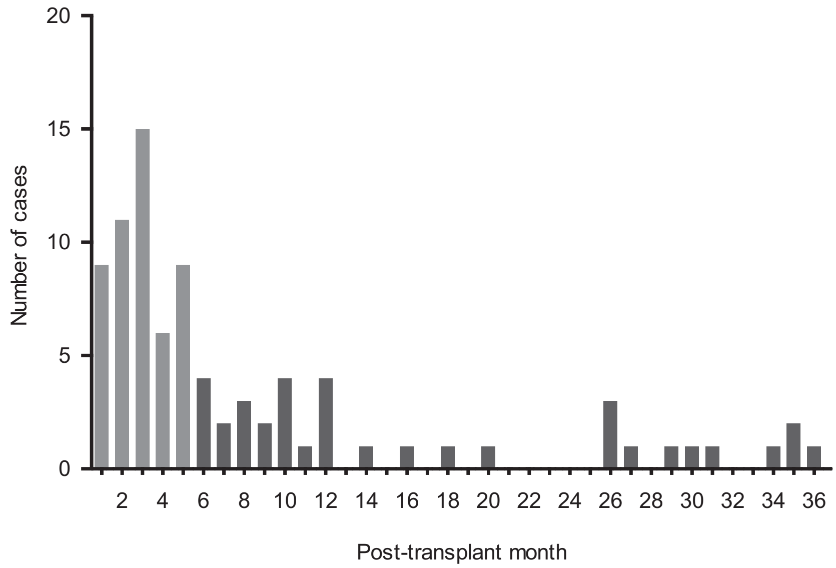

Figure 1: Temporal distribution of cases of invasive pulmonary aspergillosis occurring within the first 3 years after transplantation according to posttransplant month of diagnosis (light gray columns: early forms [<180 days after transplantation]; dark gray columns: late forms [≥180 days]).

samples were 61.3% (38/62) and 57.1% (12/21), respectively (60.0% [30/50] and 50.0% [9/18] when the analysis was restricted exclusively to probable cases). As expected, *A. fumigatus* was the most common species among the 99 episodes (88.4%) in which speciation was obtained.

Therapeutic approaches and outcome

One hundred and nine patients (97.3%) received some type of antifungal therapy with activity against *Aspergillus*. Therapy was initiated within the first 24 or 48 h from diagnosis in most of the patients (67.0% [73/109] and 77.1% [84/109], respectively). The median duration of therapy was 42.5 days (IQR: 13-108.8). Voriconazole was the agent most commonly used in monotherapy (43.1% [47/109]), followed by L-AmB (9.2% [10/109]). Patients treated with L-AmB in monotherapy had poorer graft function at diagnosis of IPA than those receiving other regimens (baseline eGFR: 32.1 ± 20.0 vs. 45.4 ± 33.7 mL/min/1.72 m^2; p-value = 0.037). On the other hand, patients who required ICU admission following diagnosis were also more likely to be initially given L-AmB in monotherapy (22.7% [5/22] vs. 5.7% [5/87]; p-value = 0.027). The median dose of L-AmB was 5.0 mg/kg/day (range: 3–10). As shown in Table 3, 22 patients (20.1%) received antifungal combination therapy for a median period of 14 days, with voriconazole plus echinocandin (45.5% [10/22]) being the most common regimen. Sequential therapy was used in 16 patients (14.7%). Overall, there were no significant differences in therapeutic approaches according to the timing of diagnosis, except for the higher use of L-AmB in early IPA cases compared to late cases (16.3% [8/49] vs. 3.3% [2/60], respectively; p-value = 0.019). Daily corticosteroid dose was reduced from baseline by ≥50% in 19.3% (21/109) of patients within the first month following diagnosis, particularly in cases of early IPA (40.0% [12/30] vs. 20.0% [9/45]; p-value = 0.059).

Following the diagnosis of IPA, 22 patients (19.6%) required ICU admission for a median of 10.5 days (IQR: 3–23.5). A follow-up CT scan was performed in 64 patients (57.1%) after a median interval of 90 days (IQR: 46–197), and showed complete response in 35.9% of cases (23/64), partial response in 46.9% (30/64), stable disease in 9.4% (6/64), and failure in 7.8% (5/64). Six- and 12-week survival rates were 68.8% (95% CI: 59.7–76.6) and 60.7% (95% CI: 51.5–69.3), respectively. Most of the deaths at week 12 (75.0% [33/44]) were considered attributable to IPA, including 7 out of 9 cases that underwent autopsy. The median interval from diagnosis to death was significantly shorter for IPA-attributable mortality than for other causes (8.5 vs. 38.5 days, respectively; p-value = 0.006). The 68 patients who survived beyond week 12 were additionally followed up for a median of 638.5 days (IQR: 119–1474.5), and graft loss was reported in 15 of them (22.1%).

Predictive factors for mortality

Table 4 shows the comparison between survivors and nonsurvivors at 6 weeks from diagnosis of IPA in terms of clinical characteristics and therapeutic approaches. By univariate analysis, it was found that survivors were more commonly diagnosed during the most recent study period (2007–2013) and treated with a voriconazole-based regimen. In contrast, nonsurvivors were more likely to have undergone invasive mechanical ventilation

Table 1: Demographics, pretransplant and donor-related characteristics, and posttransplant events in kidney transplant recipients with early (<180 days) and late (≥180 days) IPA

Variable	Overall (n = 112)	Early IPA (n = 51)	Late IPA (n = 61)	p-value[1]
Age at transplantation, years (mean ± SD)	55.8 ± 14.9	57.3 ± 15.6	54.6 ± 14.3	0.340
Gender (male) (n [%])	70 (62.5)	37 (72.5)	33 (54.1)	**0.045**
Pretransplant conditions (n [%])				
Diabetes mellitus	30 (26.8)	12 (24.0)	18 (29.5)	0.516
COPD	19 (17.0)	8 (16.3)	11 (18.3)	0.784
Pretransplant corticosteroid therapy (n [%])	12 (10.7)	6 (12.8)	6 (10.3)	0.698
BMI at transplantation, kg/m^2 (mean ± SD)[2]	25.1 ± 4.7	25.9 ± 5.7	24.4 ± 3.6	0.163
Previous kidney transplantation (n [%])	15 (13.4)	8 (15.7)	7 (11.5)	0.515
Underlying end-stage renal disease (n [%])				
Glomerulonephritis	26 (23.2)	11 (21.6)	15 (24.6)	0.706
Diabetic nephropathy	20 (17.9)	8 (15.7)	12 (19.7)	0.583
Polycystosis	18 (16.1)	9 (17.6)	9 (14.8)	0.678
Nephroangiosclerosis	10 (8.9)	5 (9.8)	5 (8.2)	0.766
Chronic interstitial nephropathy	7 (6.3)	4 (7.8)	3 (4.9)	0.700
Congenital nephropathy	4 (3.6)	1 (2.0)	3 (4.9)	0.624
Unknown	12 (10.7)	5 (9.8)	7 (11.5)	0.776
Other	15 (13.4)	8 (15.7)	7 (11.5)	0.515
Pretransplant serostatus (n [%])[3]				
HCV	11 (10.0)	5 (10.0)	6 (10.0)	1.000
HBV surface antigen (HBsAg)	3 (2.7)	1 (2.0)	2 (3.3)	1.000
CMV	86 (78.2)	41 (83.7)	45 (73.8)	0.211
Pretransplant dialysis (n [%])	105 (93.8)	50 (98.0)	55 (90.2)	0.124
Dialysis vintage, months (median [IQR])	25 (15.5–47)	30 (17–57.8)	23 (15–41)	0.141
Age of donor, years (mean ± SD)	52.5 ± 15.6	56.5 ± 14.0	49.0 ± 16.2	**0.020**
Living donor (n [%])[3]	17 (15.5)	5 (10.0)	12 (20.3)	0.138
Prior antifungal prophylaxis (n [%])	2 (4.3)	2 (9.5)	0 (0.0)	0.194
Prior colonization with *Aspergillus* (n [%])	5 (4.5)	1 (2.0)	4 (6.6)	0.374
Induction therapy (n [%])[3]				
T cell–depleting agents (ATG or OKT-3)	29 (26.4)	13 (26.5)	16 (26.2)	0.972
Anti-CD25 monoclonal antibodies	47 (42.7)	24 (49.0)	23 (37.7)	0.235
None	36 (32.7)	13 (26.5)	23 (37.7)	0.214
Maintenance immunosuppression at diagnosis including (n [%])[4]				
Corticosteroids	101 (90.9)	47 (94.0)	54 (90.0)	0.507
Daily dose, mg (median [IQR])[5]	10 (5–20)	17.5 (10–21.3)	6 (5–10)	**<0.001**
Tacrolimus	63 (56.8)	35 (70.0)	28 (45.9)	**0.011**
Cyclosporine	32 (28.8)	10 (20.0)	22 (36.1)	0.063
MMF/MPA	80 (72.1)	36 (72.0)	44 (72.1)	0.988
Azathioprine	7 (6.3)	2 (4.0)	5 (8.2)	0.455
mTOR inhibitor	13 (11.7)	3 (6.0)	10 (16.4)	0.090
Posttransplant events in 3 months prior to diagnosis (n [%])				
Pneumonia	23 (20.5)	15 (29.4)	8 (13.1)	**0.033**
Laboratory-confirmed influenza	4 (3.6)	1 (2.0)	3 (4.9)	0.624
CMV disease	16 (14.3)	11 (21.6)	5 (8.2)	**0.044**
Bloodstream infection	18 (16.1)	14 (27.5)	4 (6.6)	**0.003**
ICU admission at any time for ≥48 h	15 (13.4)	13 (25.5)	2 (3.3)	**0.001**
Invasive mechanical ventilation	9 (8.0)	7 (13.7)	2 (3.3)	0.077
De novo malignancy	4 (3.6)	1 (2.0)	3 (4.9)	0.624
Acute graft rejection	46 (41.1)	32 (62.7)	14 (23.0)	**<0.001**

ATG, antithymocyte globulin; BMI, body mass index; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; HBV, hepatitis B virus; HCV, hepatitis B virus; 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]p-values refer to comparison between early and late cases of IPA. Significant values are indicated in bold characters.

[2]Data available for 78 patients.

[3]Data available for 110 patients.

[4]Data available for 111 patients.

[5]Prednisone or equivalent corticosteroid.

Table 2: Clinical, radiological, and microbiological features of IPA cases

Variable	Overall (n = 112)	Early IPA (n = 51)	Late IPA (n = 61)	p-value[1]
Age at diagnosis, years (mean ± SD)	58.3 ± 14.1	57.5 ± 15.6	58.9 ± 12.8	0.597
EORTC/MSG category (n [%])				0.584
Proven diagnosis	28 (25.0)	14 (27.5)	14 (23.0)	
Probable diagnosis	84 (75.0)	37 (72.5)	47 (77.0)	
Symptoms at presentation (n [%])[2]				
Cough	83 (75.5)	39 (79.6)	44 (72.1)	0.366
Fever	70 (63.1)	32 (64.0)	38 (62.3)	0.853
Expectoration	63 (57.3)	28 (57.1)	35 (57.4)	0.980
Pleuritic pain	42 (37.8)	20 (40.0)	22 (36.1)	0.671
Dyspnea	25 (22.5)	10 (20.0)	15 (24.6)	0.565
Hemoptysis	6 (5.4)	2 (4.0)	4 (6.6)	0.688
Septic shock	3 (2.7)	0 (0.0)	3 (4.9)	0.251
Extrapulmonary involvement (n [%])[3]	10 (8.9)	5 (9.8)	5 (8.2)	0.766
Findings on thoracic CT scan (n [%])				
Number of lesions (median [IQR])	2 (1–4)	2 (1–5)	2 (1–4)	0.970
Maximum diameter, cm (median [IQR])[4]	2.3 (1.5–5.0)	3.0 (2.0–4.5)	2.0 (1.4–5.8)	0.630
Nodules	74 (69.8)	32 (69.6)	42 (70.0)	0.961
Cavitation[5]	32 (29.9)	12 (25.5)	20 (33.3)	0.382
Halo sign[5]	26 (24.3)	10 (21.3)	16 (26.7)	0.519
Pleural effusion[5]	44 (41.1)	23 (48.9)	21 (35.0)	0.146
Multilobar involvement	83 (76.9)	38 (79.2)	45 (75.0)	0.610
Bilateral pulmonary involvement[5]	68 (62.9)	30 (63.8)	38 (63.3)	0.958
Diagnostic procedures (n [%])[5]				
Sputum culture	33 (30.6)	17 (34.7)	16 (27.1)	0.395
Positive identification	6/33 (18.2)	3/17 (17.6)	3/16 (18.8)	1.000
Bronchial brush	22 (20.4)	9 (18.4)	13 (22.0)	0.638
Positive identification	7/22 (31.8)	4/9 (44.4)	3/13 (23.1)	0.376
BAL	53 (49.1)	30 (61.2)	23 (39.0)	0.021
Positive identification	31/53 (58.5)	19/30 (63.3)	12/23 (52.2)	0.414
Lung biopsy	20 (18.5)	7 (14.3)	13 (22.0)	0.302
Positive identification	17/20 (85.0)	7/7 (100.0)	10/13 (76.9)	0.521
Detection of GM in serum sample	62 (55.4)	26 (51.0)	36 (59.0)	0.394
Positive (index ≥0.5 ODs)	38/62 (61.3)	15/26 (57.7)	23/36 (63.9)	0.621
Positive (index ≥0.5 ODs) in probable cases	30/50 (60.0)	11/19 (57.9)	19/31 (61.3)	0.812
Detection of GM in BAL sample	21 (18.8)	8 (15.7)	13 (21.3)	0.448
Positive (index ≥1.0 ODs)	12/21 (57.1)	5/8 (62.5)	7/13 (53.8)	1.000
Positive (index ≥1.0 ODs) in probable cases	9/18 (50.0)	3/6 (50.0)	6/12 (50.0)	1.000
Isolated species (n [%])[6]				0.862
Aspergillus fumigatus	78 (78.8)	36 (78.3)	42 (79.2)	
Aspergillus flavus	6 (6.1)	3 (6.5)	3 (5.7)	
Aspergillus niger	6 (6.1)	2 (4.3)	4 (7.5)	
Other[7]	9 (9.1)	5 (10.9)	4 (7.5)	

BAL, bronchoalveolar lavage; CT, computed tomography; 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; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; OD, optical density; SD, standard deviation.
[1]p-values refer to comparison between early and late cases of IPA.
[2]Data available for 111 patients.
[3]Included involvement of central nervous system (three cases), sinus (three cases), skin (three cases), liver (two cases), and endocardium (one case).
[4]Data available for 59 patients.
[5]Data available for 108 patients.
[6]Percentages calculated on the 99 cases (72 probable IPA and 27 proven) with *Aspergillus* speciation.
[7]Includes *A. terreus* (n = 4), *A. calidoustus* (n = 3), *A. nidulans* (n = 1), and *Aspergillus* section *Flavipedes* (n = 1).

prior to diagnosis, to have early forms of IPA and bilateral pulmonary involvement, to receive combination therapy, and to require ICU admission and acute renal replacement therapy. The GM indexes in serum and BAL samples were also significantly higher compared to survivors.

Table 3: Therapeutic approaches in patients who received any antifungal treatment (n = 109)

Variable	N (%)
Initial antifungal therapy[1]	
Monotherapy	71 (65.1)
Amphotericin B	
L-AmB	10 (9.2)
AmB-D	5 (4.6)
AmB-LC	1 (0.9)
AmB-D plus AmB-LC	1 (0.9)
Triazoles	
Voriconazole	47 (43.1)
Itraconazole	3 (2.8)
Echinocandins	
Caspofungin	2 (1.8)
Anidulafungin	2 (1.8)
Combination therapy[2]	22 (20.1)
Voriconazole plus echinocandin	10 (9.2)
Amphotericin B plus echinocandin	5 (4.6)
Amphotericin B plus triazole	4 (3.7)
Voriconazole plus echinocandin plus amphotericin B	3 (2.8)
Sequential therapy[3]	16 (14.7)
Amphotericin B followed by triazole	6 (5.5)
Echinocandin followed by triazole	6 (5.5)
Voriconazole followed by amphotericin B	2 (1.8)
Amphotericin B followed by echinocandin	1 (0.9)
Voriconazole followed by echinocandin	1 (0.9)
Adjuvant therapy	
G-CSF	7 (6.4)
Surgery	6 (5.5)
Reduction in overall immunosuppression[4]	80 (80.0)
Reduction by ≥50% in daily corticosteroid dose[5]	21 (28.0)
Requirement for ICU admission	22 (20.2)
Requirement for acute renal replacement therapy at diagnosis of IPA[6]	34 (31.2)

AmB-D, amphotericin B deoxycholate; AmB-LC, amphotericin B lipid complex; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; G-CSF, granulocyte colony-stimulating factor; L-AmB, liposomal amphotericin B.

[1]Refers to that administered during the first 2 weeks of therapy.

[2]Refers to the concomitant administration for ≥72 h of two or more active antifungal drugs within the first 2 weeks of therapy.

[3]Refers to the consecutive administration of two or more active antifungal drugs within the first 2 weeks of therapy (an overlap period of less than 72 h was allowed).

[4]Within the first month after diagnosis of IPA. Data available for 100 patients.

[5]Within the first month after diagnosis of IPA. Data available for 75 patients.

[6]Between 1 month before and 1 month after the diagnosis of IPA.

The Cox regression model revealed that early IPA (HR: 2.29; 95% CI: 1.10–4.79; p-value = 0.027) and bilateral involvement (HR: 3.00; 95% CI: 1.22–7.39; p-value = 0.017) were independently associated with a higher mortality, whereas the use of a voriconazole-based regimen as initial therapy exerted a protective effect (HR: 0.34; 95% CI: 0.15–0.74; p-value = 0.007). Although the administration of combination therapy appears to increase the risk of mortality at the univariate level, such an effect did not remain in the multivariate model. The HRs and corresponding 95% CIs of the bootstrap resampling procedure based on 200 samples were similar to those obtained in the original model (data not shown). Survival rates at 6 weeks were significantly lower in patients with early IPA (56.0% vs. 79.0%; log-rank p-value = 0.022) and in those receiving an antifungal regimen not based on voriconazole (55.0% vs. 84.0%; log-rank p-value = 0.002) (Figure 2). The Cochran's and Mantel–Haenszel statistics did not find any evidence of center effect on these associations. In addition, their direction and magnitude remained essentially unchanged when analyses were restricted to proven cases, although lacking statistical significance due the low number of patients analyzed (data not shown).

As expected, patients who were given antifungal combination therapy significantly differed in terms of baseline characteristics and markers of disease severity in comparison to those treated with monotherapy or sequential regimens, including higher rates of pretransplant dialysis, previous transplantation, chronic hepatitis C virus (HCV) infection and dyspnea and hemoptysis at presentation, higher number of lesions in the CT scan at diagnosis, and higher absolute neutrophil count at day 7, among others (Table S2). On the basis of these variables, we estimated the propensity score for receiving antifungal combination therapy compared to monotherapy or sequential therapy. In addition, the timing of diagnosis (early vs. late IPA) and the requirement for ICU admission at presentation were also entered into the model. The resulting score showed a good goodness-of-fit (Hosmer–Lemeshow test p-value = 0.794; auROC: 0.816; 95% CI: 0.698–0.935). There was an acceptable overlap between propensity scores for patients who were given one or other regimen, thus providing sufficient power for propensity adjustment in the survival analyses (Figure S1). Following assignment of propensity scores, each patient receiving combination therapy was matched to a single patient receiving monotherapy or sequential therapy who has the most similar estimated propensity score (i.e. the smallest distance). The lack of impact of combination therapy on the primary outcome was confirmed in this propensity-matched subanalysis (HR: 1.37; 95% CI: 0.43–4.31; p-value = 0.596).

The comparison between survivors and nonsurvivors at 12 weeks from diagnosis of IPA is shown in Table S3. Early occurrence of IPA (HR: 2.11; 95% CI: 1.01–4.09; p-value = 0.028), bilateral pulmonary involvement (HR: 2.49; 95% CI: 0.99–6.32; p-value = 0.053), and the requirement for ICU admission (HR: 2.24; 95% CI: 1.12–4.48; p-value = 0.023) and acute renal replacement therapy (HR: 2.00; 95% CI: 0.99–4.05; p-value = 0.054) were predictors of all-cause mortality at 12 weeks. Of note,

Table 4: Uni- and multivariate analyses of risk factors predicting all-cause mortality at 6 weeks from the diagnosis of IPA

Variable	Survivors (n = 77)	Nonsurvivors (n = 35)	p-value[1]	Univariate			Multivariate		
				HR	95% CI	p-value	HR	95% CI	p-value
Age at diagnosis, years (mean ± SD)	58.6 ± 13.7	57.6 ± 15.1	0.720						
Male gender (n [%])	49 (63.6)	21 (60.0)	0.713						
Diagnosis in 2007–2013 compared to 2000–2006 (n [%])	62 (80.5)	22 (62.9)	**0.045**	0.50	0.25–0.99	0.048	–	–	–
Pretransplant diabetes mellitus (n [%])	21 (27.6)	9 (25.7)	0.833						
Pretransplant COPD (n [%])	12 (16.2)	7 (20.0)	0.627						
Positive HCV serostatus	6 (7.9)	5 (14.7)	0.310						
Previous kidney transplantation (n [%])	10 (13.0)	5 (14.3)	1.000						
Living donor (n [%])	14 (18.4)	3 (9.1)	0.217						
Prior CMV disease (n [%])[2]	9 (11.7)	7 (20.0)	0.244						
Prior ICU admission at any time for ≥48 h (n [%])[2]	9 (11.7)	6 (17.1)	0.550						
Prior invasive mechanical ventilation (n [%])[2]	3 (3.9)	6 (17.1)	**0.026**	2.79	1.15–6.75	0.023	–	–	–
Prior acute graft rejection (n [%])[2]	28 (36.4)	18 (51.4)	0.133						
Proven EORTC/MSG category (n [%])	23 (29.9)	5 (14.3)	0.077						
Early diagnosis (<180 days after transplant) compared to late (≥180 days) (n [%])	29 (37.7)	22 (62.9)	**0.013**	2.46	1.24–4.88	0.010	2.29	1.10–4.79	0.027
Fever at presentation (n [%])[3]	44 (57.9)	26 (74.3)	0.096						
Cough at presentation (n [%])[3]	58 (77.3)	25 (71.4)	0.503						
Dyspnea at presentation (n [%])[3]	17 (22.4)	8 (22.9)	0.954						
Extrapulmonary involvement (n [%])	9 (11.7)	1 (2.9)	0.168						
Number of lesions (median [IQR])	2 (1–4)	3 (1–4.8)	0.678						
Halo sign (n [%])[4]	21 (27.3)	5 (16.7)	0.251						
Cavitation (n [%])[4]	23 (29.9)	9 (30.0)	0.989						
Plural effusion (n [%])[4]	28 (36.4)	16 (53.3)	0.109						
Multilobar involvement (n [%])[4]	56 (72.7)	27 (87.1)	0.109						
Bilateral pulmonary involvement (n [%])	43 (56.6)	25 (80.6)	**0.019**	2.64	1.08–6.44	0.033	3.00	1.22–7.39	0.017
Serum GM index (ODs) (median [IQR])[5]	0.5 (0–1)	1.1 (0.5–3.4)	**0.024**						
BAL GM index (ODs) (median [IQR])[6]	1 (0–3.1)	6.5 (1.9–7.8)	**0.014**						

Table 4: Continued

Variable	Survivors (n = 77)	Nonsurvivors (n = 35)	p-value[1]	Univariate HR	Univariate 95% CI	Univariate p-value	Multivariate HR	Multivariate 95% CI	Multivariate p-value
Initial antifungal therapy (n [%])[7]									
Amphotericin B-based regimen	13 (16.9)	6 (18.8)	0.815						
Voriconazole-based regimen	47 (61.0)	9 (28.1)	**0.002**	0.32	0.15–0.68	0.003	0.34	0.15–0.74	0.007
Echinocandin-based regimen	2 (2.6)	4 (12.5)	0.060						
Combination regimen	10 (13.0)	12 (37.5)	**0.004**	2.60	1.27–5.34	0.009	–	–	–
Interval from diagnosis to initiation of treatment <48 h (n [%])	58 (75.3)	26 (81.2)	0.503						
Surgery (n [%])[7]	6 (7.8)	0 (0.0)	0.177						
Reduction by ≥50% in corticosteroid dose (n [%])[8]	19 (28.8)	2 (22.2)	1.000						
Requirement for ICU admission (n [%])[7]	11 (14.3)	11 (31.4)	**0.017**	2.06	1.01–4.20	0.048	–	–	–
Requirement for acute renal replacement therapy at diagnosis of IPA (n [%])[7,9]	17 (22.1)	17 (53.1)	**0.001**	2.47	1.27–4.81	0.008	–	–	–

BAL, bronchoalveolar lavage; CI, confidence interval; CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; 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; HCV, hepatitis C virus; HR, hazard ratio; ICU, intensive care unit; IPA, invasive pulmonary aspergillosis; IQR, interquartile range; OD, optical density; SD, standard deviation.

[1]Significant p-values at the univariate level are indicated in bold characters. Unless otherwise specified, these variables were entered into the Cox regression model.

[2]Within the 3 months prior to diagnosis of IPA.

[3]Data available for 111 patients.

[4]Data available for 108 patients.

[5]Data available for 45 patients. This variable was not included in the model due to the low number of patients in whom the assay was tested.

[6]Data available for 17 patients. This variable was not included in the model due to the low number of patients in whom the assay was tested.

[7]Percentages calculated on the 109 patients who received any antifungal treatment.

[8]Within the first month after diagnosis of IPA. Data available for 75 patients.

[9]Between 1 month before and 1 month after the diagnosis of IPA.

the administration of combination therapy exerted a negative impact on this outcome (HR: 2.11; 95% CI: 1.04–4.29; p-value = 0.039), although such effect disappeared in the propensity-matched cohort (HR: 2.32; 95% CI: 0.80–6.69; p-value = 0.120). The presence of a positive HCV serostatus was identified as a risk factor at univariate but not multivariate analysis. Again, the bootstrap estimates of the multivariate HRs and 95% CIs were similar to those obtained from the original sample (data not shown).

Since the demonstration of bilateral lung involvement was identified to exert a negative impact on outcome, we further explored the factors associated with this condition at diagnosis. Patients with bilateral or unilateral infection did not significantly differ in terms of pretransplant comorbidities, prior occurrence of acute graft rejection or CMV disease, or leukocyte or lymphocyte counts at diagnosis. On the other hand, dyspnea at presentation, higher number of nodules in the CT scan, presence of pleural effusion, and poorer graft function (as measured by eGFR) were associated with bilateral involvement. In the multivariate logistic regression analysis the presence of dyspnea, number of nodules, and graft function remained as independent predictor factors (Table S4).

Predictive factors for graft loss

Finally, we assessed the risk factors for the occurrence of graft loss among those patients who remained alive at 12 weeks from diagnosis of IPA (Table S5). The use of acute renal replacement therapy (HR: 16.89; 95% CI: 4.59–60.60; p-value <0.001) and the requirement for ICU admission, either within the 3 months prior to (HR: 4.24;

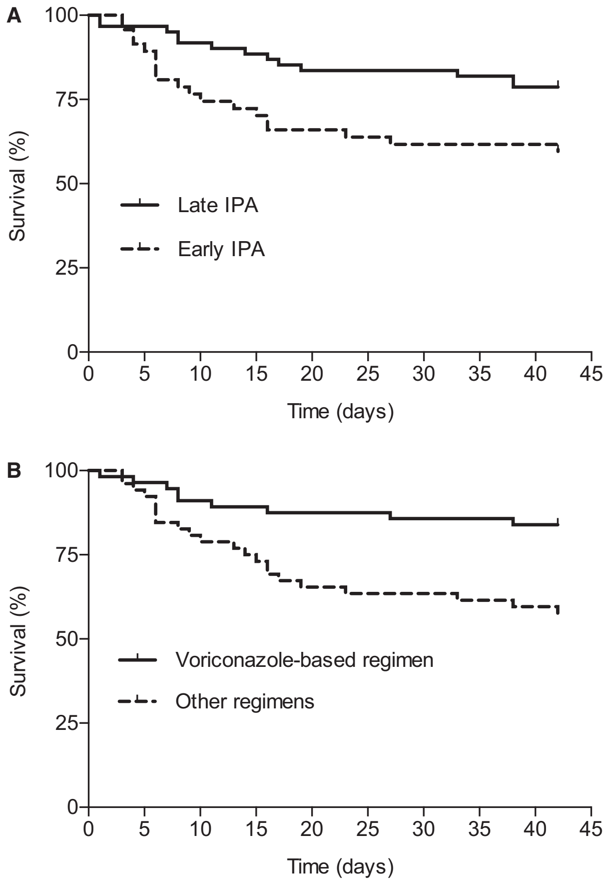

Figure 2: Kaplan–Meier survival curves at week 6 after diagnosis of IPA according to (A) timing of diagnosis (log-rank p-value = 0.022) and (B) type of initial antifungal regimen (log-rank p-value = 0.002). IPA, invasive pulmonary aspergillosis.

95% CI: 1.28–14.05; p-value = 0.018) or at the time of diagnosis (HR: 3.68; 95% CI: 1.04–12.99; p-value = 0.043), emerged as independent predictors, although it should be noted that the number of events analyzed was low (n = 15). The administration of any amphotericin B-containing regimen was identified as a risk factor at the univariate level but not after adjustment for potential confounders.

Discussion

To our knowledge, the present study represents the largest cohort assessing the clinical characteristics and outcome of IPA among KT recipients and the first one to include detailed data on microbiological and radiological features. Some relevant clinical and prognostic information may be derived from our series. First, IPA was found to be a devastating disease in the setting of KT, as 20% of the patients had to be admitted to the ICU following diagnosis, 31% had died at 6 weeks (43% if IPA occurred within the first 180 days after transplantation), and as many as 20% of survivors suffered from graft lost and required permanent return to dialysis or retransplantation.

It should be highlighted that almost half of the cases of IPA were diagnosed within the first 6 months after transplantation. Similar results have been communicated from previous smaller cohorts (20). Therefore, a low threshold for suspicion of this complication must be maintained throughout such period, particularly in view of the fact that the time interval to the onset of infection also exerted a relevant prognostic effect. Early cases of IPA had increased risk of death at 6 and 12 weeks, even after adjusting for a large range of potential confounders. The lack of obvious differences between early and late cases in variables reflecting disease severity (such as the number of nodules in CT scan or the requirement for ICU admission) or appropriateness of therapeutic approach suggests that the worse prognosis observed for the former would be primarily driven by the host's factors. Indeed, subgroup comparisons suggest that patients diagnosed with IPA during the first 180 days had a greater immunosuppression (i.e. higher daily corticosteroid dose, previous occurrence of CMV disease or acute graft rejection) and poorer status (prior ICU admission) compared to those with later infection.

From a clinical perspective, it is noteworthy that about one-fifth of the patients presented with no typical symptoms of respiratory tract infection (fever, expectoration, or pleuritic chest pain), and that a nonspecific manifestation, such as cough, was the most commonly observed complaint. With regard to the findings of the CT scan, the presence of cavitation or halo sign—classically deemed as specific signs of IPA, particularly in the hematological patient (33)—was reported in only one-third and one-fourth of the cases, respectively. In contrast, well-circumscribed nodules constituted the most common radiological sign (about 70% of cases), in line with previous studies (34). We found that bilateral pulmonary involvement acted as an independent predictor for all-cause mortality. A similar association has been reported in cancer patients (35). It seems plausible that the demonstration of bilateral infection may represent a surrogate marker for delayed diagnosis and advanced disease, a hypothesis further supported by the fact that we found no apparent differences in the net state of immunosuppression between patients with unilateral or bilateral involvement.

Our experience confirms that the more invasive the diagnostic procedure, the greater the possibility of achieving a microbiological confirmation to support the suspicion of IPA. Indeed, *Aspergillus* spp. were isolated in 18.2%, 31.8%, 58.5%, and 85.0% of sputum, bronchial brush, BAL, and lung biopsy samples, respectively. On the other hand, the GM assay was positive in about 60% of samples in which it was tested, suggesting an acceptable sensitivity, although the design of our study did not allow us to assess the diagnostic performance of this biomarker. A previous meta-analysis reported a pooled sensitivity in SOT recipients noticeably lower (41%) (36). Heylen et al observed in their single-center experience that the serum GM assay was positive in one-third of KT recipients (20), whereas a recent study obtained high sensitivity but low specificity values among liver transplant recipients (37). Interestingly, the GM indexes in both serum and BAL samples were significantly higher in nonsurvivors at 6 weeks compared to survivors, although we could not demonstrate the impact of this variable in the multivariate Cox model, likely due to the limited number of patients in whom the assay was performed. In agreement with this finding, Heylen et al also reported that the height of the GM index (>2 optical densities) acted as a risk factor for mortality in their experience (20). In view of this, we propose that the GM assay should be ordered in KT recipients with suspected posttransplant IPA not only on the grounds of its diagnostic value, but also due to its prognostic significance.

In relation to the therapeutic approaches, voriconazole monotherapy was administered as first-line treatment in more than 40% of the patients included in our multinational series, and its use was independently associated with a better outcome at 6 weeks. All-cause mortality was lower among cases diagnosed during the most recent study era (2007–2013), probably reflecting the increasing use of voriconazole (approved in 2002 [38]) over recent years as well as overall improvements in posttransplant management. A similar trend has been observed for patients with heart (39) and liver (40) transplantation and hematological malignancies (35). Likewise, the positive impact of voriconazole-based regimens on mortality was also found in other high-risk populations, including HSCT recipients (15), patients with hematological malignancies (35), overall SOT recipients (15), and liver transplant recipients (40). Voriconazole acts as a potent inhibitor of cytochrome P450 isoform CYP2C19, which poses an increased potential for clinically relevant interactions with certain immunosuppressive drugs (41). This threat must be carefully taken in consideration whenever prescribing this antifungal agent in SOT recipients.

On the other hand, we found no significant association at the multivariate level between the use of voriconazole and 12-week mortality. Although it has been suggested that mortality at 6 weeks from diagnosis acts as a good surrogate for IPA-attributable mortality (25), later outcomes may be influenced to a greater extent by concurrent factors such as the loss of graft function or the severity of respiratory failure, even outweighing the protective role of antifungal therapy. In support of this, the requirement for ICU admission and renal replacement therapy following diagnosis emerged as independent risk factors for 12-week mortality. In that sense, voriconazole-based therapy remained significant in the multivariate analysis when both variables were removed from the Cox model (data not shown). Moreover, we cannot rule out that our study lacked statistical power to demonstrate differences in 12-week mortality according to the antifungal regimen. Interestingly, positive HCV serostatus exerted a negative impact on this outcome at the univariate level. Various studies have reported poorer patient and graft survival among HCV-positive KT recipients (42–44), and some of these series identified infectious complications as one of the leading causes of mortality (45), suggesting a reciprocal deleterious effect between HCV infection and the level of immunosuppression.

The initial use of antifungal combination therapy—mostly voriconazole plus echinocandin—was found to be related to poorer outcomes at the univariate level. However, the demonstration of imbalances in baseline and clinical characteristics suggests that this finding was likely subject to confounding by indication bias due to the observational design of our study, as further suggested by the lack of association observed in the propensity-matched cohort. Nevertheless, this subanalysis should be regarded as merely exploratory due to the low number of patients who were given combination regimens. The efficacy of the combination of voriconazole and an echinocandin as primary therapy for IPA remains controversial, with some retrospective studies suggesting a beneficial impact in SOT recipients compared to L-AmB monotherapy (46). A recent trial failed to demonstrate a clear benefit in patients with hematological malignancies or HSCT (24).

In contrast to other SOT populations, a distinctive issue concerning the clinical management of KT recipients who develop severe opportunistic infections lies in the possibility of performing a more intensive reduction of immunosuppression, even by assuming the risk of graft rejection and graft loss. Indeed, the overall amount of immunosuppression at the time of diagnosis of IPA was tapered in 80% of the cases analyzed. It is likely that such a high rate precluded us from demonstrating the potential role of this approach in terms of patient outcome.

A point of key interest in the setting of KT is the impact of posttransplant IPA on graft function and subsequent mortality. We observed that one-third of patients required acute renal replacement therapy at the time of diagnosis, and this factor was independently associated with all-cause mortality at 12 weeks. Renal failure is a well-described risk factor for poor outcome in HSCT recipients, patients with hematological or solid organ malignancies, and SOT recipients with IFD (5,15,35). Furthermore, more than 20% of survivors beyond week 12 developed graft loss, a complication that was more frequent among those requiring prior ICU admission and acute renal replacement therapy, as previously described for other

SOT recipients (40). Although not achieving statistical significance in the multivariate model, we observed that the use of amphotericin B increased the risk of graft loss, thus emphasizing the role of voriconazole as first-line therapy not only on the grounds of better recipient survival, but also long-term graft function.

Certain limitations to our study should be noted, mostly derived from its retrospective design. Some clinical data could not be retrieved for all cases (i.e. follow-up imaging). As previously mentioned, the administration of antifungal combination therapy was not random but based on physician's criteria, and therefore particularly susceptible to confounding bias that cannot be completely removed through propensity score analysis. Moreover, these results should be interpreted with particular caution due to the reduced sample of the propensity-matched cohort. The prolonged inclusion period and the large number of participating centers, albeit strengthening the generalizability of our findings, entail some heterogeneity in terms of posttransplant and critical care practices. However, we have attempted to take into account this "era effect" when assessing determinants of outcome. Finally, and despite our study being the largest series exclusively focused on IPA after KT to date, the relatively low number of analyzed events may limit the stability of the multivariate analysis, since it has been demonstrated that the results of Cox models suffer from increasing bias and variability, unreliable confidence interval coverage, and problems with model convergence as the ratio of events to predictor variables declines below 10 (47).

In summary, clinicians in charge of KT recipients must keep in mind the possibility of IPA in patients presenting with nonspecific respiratory symptoms (such as cough), fever, and nodular lung lesions, especially during the first months after transplantation. An aggressive diagnostic approach—which should include GM testing in serum and BAL samples—and the prompt initiation of voriconazole-based therapy may be life-saving in this setting. Future studies are needed to better delineate the role of tapering immunosuppression in an attempt to improve the dismal prognosis of this opportunistic infection without threatening the survival of the renal graft.

Acknowledgments

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 MSD. Oscar Len has been paid for talks on behalf of Astellas Pharma and MSD and has received grants from MSD 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, MSD, Pfizer, and Instituto de Salud Carlos III. Ricardo Lauzurica 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 MSD. José María Aguado has been a consultant to and on the speakers' bureau for Astellas Pharma, Pfizer, Gilead, MSD, and Roche. The other authors have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Box-and-whisker diagram depicting the median (dot), interquartile range (box), and range (whiskers) for propensity scores for receiving antifungal combination therapy.

Table S1: List of participating centers.

Table S2: Comparison of baseline clinical characteristics and markers of disease severity between patients initially treated with antifungal combination therapy or other regimens.

Table S3: Uni- and multivariate analyses of risk factors predicting all-cause mortality at 12 weeks.

Table S4: Uni- and multivariate logistic regression analyses of factors associated with bilateral lung involvement at diagnosis.

Table S5: Uni- and multivariate analyses of risk factors predicting graft loss among patients that remained alive at 12 weeks from diagnosis of IPA (n = 68). IPA, invasive pulmonary aspergillosis.