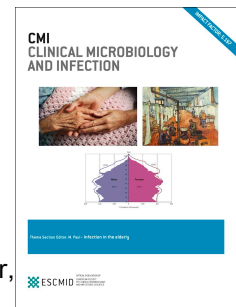# Accepted Manuscript

Multinational retrospective case-control study of risk factors for the development of late invasive pulmonary aspergillosis following kidney transplantation

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98

110 **Abstract** (250 words)

111 *Objectives*: To assess the risk factors for the development of late-onset invasive pulmonary

112 aspergillosis (IPA) after kidney transplantation (KT).

113 *Methods*: We performed a multinational case-control study that retrospectively recruited 112 KT

114 recipients diagnosed with IPA between 2000 and 2013. Controls were matched (1:1 ratio) by

115 center and date of transplantation. Immunosuppression-related events (IREs) included the

116 occurrence of non-ventilator-associated pneumonia, tuberculosis, cytomegalovirus disease

117 and/or *de novo* malignancy.

118 *Results:* We identified 61 cases of late (>180 days after transplantation) IPA from 24

119 participating centers (accounting for 54.5% [61/112] of all cases included in the overall study).

120 Most diagnoses (54.1% [33/61]) were established within the first 36 post-transplant months,

121 although 5 cases occurred more than 10 years after transplantation. Overall mortality among

122 cases was 47.5% (29/61). Compared to controls, cases were significantly older (*P*-value =

123 0.010) and more likely to have pre-transplant chronic obstructive pulmonary disease (*P*-value =

124 0.001) and a diagnosis of bloodstream infection (*P*-value = 0.016) and IRE (*P*-value <0.001)

125 within the 6 months prior to the onset of late IPA. After multivariate adjustment, previous

126 occurrence of IRE (odds ratio: 19.26; 95% confidence interval: 2.07 - 179.46; *P*-value = 0.009)

127 was identified as an independent risk factor for late IPA.

128 *Conclusion:* More than half of IPA cases after KT occur beyond the sixth month, with some of

129 them presenting very late. Late IPA entails a poor prognosis. We identified some risk factors

130 that could help the clinician to delimit the subgroup of KT recipients at the highest risk for late

131 IPA.

132 **Introduction**

133 Invasive pulmonary aspergillosis (IPA) constitutes one of the most feared complications

134 occurring in patients undergoing solid organ transplantation (SOT) in terms of both patient and

135 graft survival [1-3]. Apart from local susceptibility associated with specific surgical procedures

136 (e.g., ulcerative aspergillus tracheobronchitis at the bronchial anastomosis site after lung

137 transplantation) [4], it is conventionally assumed that the lifelong use of immunosuppression to

138 avoid graft rejection confers the most relevant risk for this event [5].

139 The intensity of the immunosuppressive therapy is usually higher during the first 6 months

140 following SOT, and therefore this period has been traditionally considered as carrying the

141 maximum risk for opportunistic infection including IPA [6]. Nevertheless, kidney transplant (KT)

142 recipients require potent triple-drug regimens —often containing steroids, calcineurin inhibitors

143 and antiproliferative agents— for indefinite time periods [7]. Although the relative risk of post-

144 transplant IPA after KT is lower compared to other types of grafts [1,3,8], KT recipients suffer

145 from the highest absolute disease burden due to the large number of procedures performed

146 worldwide [9,10]. In addition, recent decades have witnessed a continuous improvement in

147 long-term graft survival [11], thus increasing the population of aged KT recipients chronically

148 exposed to a high degree of immunosuppression.

149 By using a multicenter case-control design, we have recently analyzed the risk factors for the

150 occurrence of early IPA (i.e., diagnosed within the first 180 days) after KT [12]. Only one

151 previous study has analyzed the predisposing conditions for the late forms of infection, although

152 its results were limited by its single-center nature and by the inclusion of only 26 cases of late

153 IPA [13].

154 Transplant physicians may benefit from identifying, among the increasing population of long-

155 term KT recipients, that subgroup of patients at increased risk for late IPA in order to implement

156 individualized follow-up and prevention strategies. Unfortunately, such an approach remains an

157 unmet clinical need. To the best of our knowledge, this is the first study specifically aiming to

158 ascertain the predisposing factors for the development of late IPA from a large representative

159 population of KT recipients.

160 **Materials and Methods**

161 *Study design*

162 This is a sub-analysis of a multinational retrospective case-control study performed in 29

163 hospitals from 10 European (Spain, Switzerland [6 centers included in the Swiss Transplant

164 Cohort Study [14]], Belgium, Portugal, France and United Kingdom) and American institutions

165 (United States, Brazil, Mexico and Argentina). Participating centers included cases of IPA

166 diagnosed in KT recipients between January 1, 2000 and December 31, 2013 [12,15]. In the

167 present *a priori* designed sub-analysis we focused on late episodes of IPA, defined as those

168 diagnosed beyond the first 180 days after transplantation ("IPA cases"). The "control group" was

169 selected (in an 1:1 ratio) among those patients that underwent transplantation at the same

170 center within a 3-month period before or after the calendar date of the corresponding case but

171 without the diagnosis of IPA throughout the post-transplant period. In addition, controls must

172 have survived at least until the time of diagnosis of IPA in the index case. To take into account

173 the effect of post-transplant events on the occurrence of late IPA, controls were assigned a

174 "pseudo-date of diagnosis" to match their cases with the aim of ensuring comparable risk

175 exposure periods in both groups. The criteria used to establish the date of IPA diagnosis is

176 available as **Supplementary Methods**. This research adhered to the STROBE guidelines for

177 observational studies. The study protocol was approved by the local Ethics Committee of the

178 coordinating center and of other participating sites as required.

179 *Study definitions*

180 IPA was defined according to the revised criteria proposed in 2008 by the European

181 Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative

182 Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group

183 (EORTC/MSG) Consensus Group (details provided as **Supplementary Methods**) [16]. It

184 should be noted that we added a modified radiological criterion (beyond the classic dense, well-

185 circumscribed lesions with or without halo sign or cavitation) based on the presence of certain

186 lung patterns that have been specifically associated with post-transplant IPA (peribronchial

187 consolidation or tree-in-bud pattern) [17]. Additional study definitions (including IPA-attributable

188 mortality, cytomegalovirus [CMV] disease, tuberculosis, pneumonia, respiratory tract viral

189 infection, bloodstream infection [BSI] or post-transplant lymphoproliferative disorder [PTLD]) are

7

190 available in **Supplementary Methods**.

191 To encompass the different post-transplant complications that may be attributable to over-
192 immunosuppression we constructed a composite variable (termed "immunosuppression-related
193 event" [IRE]) that included the occurrence of any of the following: non-ventilator-associated
194 pneumonia, tuberculosis, CMV disease and/or post-transplant *de novo* malignancy (both PTLD
195 and solid organ tumors). Community-acquired pneumonia has been previously recognized to be
196 more frequent among SOT recipients due to immunosuppression [18] and therefore
197 pneumococcal vaccination is strongly recommended for this population [19]. We did not
198 consider within the definition of IRE certain post-transplant infections (such as BSI or ventilator-
199 associated pneumonia) that may be arguably attributable to invasive procedures,
200 instrumentation (i.e., indwelling catheters) or anatomical abnormalities rather than to the
201 recipient's immune status.

202 *Statistical analysis*

203 Continuous variables were summarized by the mean ± standard deviation (SD) or the median
204 with interquartile ranges (IQR), while categorical variables were summarized using absolute
205 counts and percentages. Categorical variables were compared using the McNemar test,
206 whereas the Student's t-test for repeated measures or the Wilcoxon signed-ranks test were
207 applied for continuous variables. Conditional logistic regression was used to identify
208 independent risk factors for the development of late IPA. Those variables found to be significant
209 (*P*-value ≤0.1) at the univariate level were included into the multivariable models in a backward
210 stepwise fashion. Collinearity among explanatory variables was assessed by means of the
211 variance inflation factor (VIF), with VIF values over 3 suggesting significant collinearity. Results
212 are given as odds ratios (ORs) with 95% confidence intervals (CIs). As a secondary outcome,
213 we compared patient survival from the date (for cases) or the "pseudodate" (for controls) of IPA
214 diagnosis. Survival curves were plotted by the Kaplan-Meier method and differences between
215 groups were compared with the log-rank test. All the significance tests were two-tailed.
216 Statistical analysis was performed with SPSS version 20.0 (IBM Corp., Armonk, NY) and
217 graphics were generated with Prism v. 6.0 (GraphPad Software Inc., La Jolla, CA).

218 **Results**

219 We included 61 cases of late IPA (14/61 [23.0%] proven and 47/61 [77.0%] probable) and their

220 corresponding controls from 24 out of 29 participating centers (i.e., 5 centers did not contribute

221 to the present sub-analysis). This figure accounts for 54.5% (61/112) of all the cases enrolled in

222 the overall study. Twenty-nine out of 61 cases (47.5%) were diagnosed between 2010 and

223 2013. The median time interval between transplantation and diagnosis was 34.4 months (IQR:

224 11.8 - 78.5). Most diagnoses (54.1% [33/61]) were established within the first 36 months,

225 although this period spanned more than 27 years (with 5 very late-onset cases occurring after

226 the tenth year) (**Figure 1**). The median follow-up from the date (for cases) or the "pseudo-date"

227 of diagnosis (for controls) was 476 days (IQR: 70.0 - 1298.5). Overall and IPA-attributable

228 mortality among IPA cases was 47.5% (29/61) and 21.3% (13/61) and occurred at a median of

229 53.5 days (IQR: 14.5 - 171.5) and 15 days (IQR: 7.3 - 33.3), respectively, from diagnosis. There

230 were no significant differences in one-year survival rates between cases occurring in months 6

231 to 36 or >36 months after transplantation (55.0% versus 41.0%, respectively; log-rank test *P*-

232 value = 0.619). Among survivors, 9.4% (3/32) patients experienced definitive graft failure

233 requiring return to permanent dialysis. None of the patients in the control group died during the

234 follow-up. One-year survival was significantly lower among cases than controls (49.0% versus

235 100.0%; log-rank test *P*-value = 0.021).

236 The demographics and pre-transplant factors of patients who developed late IPA and their

237 controls are compared in **Table 1**. Cases were significantly older (54.6 ± 14.2 versus 48.6 ±

238 15.5 years; *P*-value = 0.010) and more likely to have pre-transplant chronic obstructive

239 pulmonary disease (COPD) (18.0% [11/61] versus 0.0% [0/61]; *P*-value = 0.001) than control

240 counterparts. The prevalence of underlying diabetic nephropathy as a reason for end-stage

241 renal disease requiring transplantation was also higher among cases, although not achieving

242 statistical significance (19.7% [12/61] versus 6.6% [4/61]; *P*-value = 0.077).

243 Donor- and transplant-related and post-transplant variables are compared in **Table 2**. Cases

244 were more likely to have been diagnosed with an IRE during the 6 months prior to the onset of

245 IPA (34.4% [21/61] versus 3.3% [2/61]; *P*-value <0.001), with significant (for non-ventilator-

246 associated pneumonia and CMV disease) or near significant differences (for post-transplant *de*

247 *novo* malignancy) observed for each of the different individual events included in this composite

248   variable. PTLD was the predominant type of malignancy diagnosed. A prior occurrence of BSI

249   was also more common among cases than controls (11.5% [7/61] versus 0.0% [0/61]; *P*-value =

250   0.016). No significant differences were observed between the groups regarding the prior

251   occurrence of acute graft rejection or the requirement of steroid boluses. None of these

252   episodes were treated with lymphocyte-depleting agents as anti-rejection therapy, and only one

253   of them (in the control group) received rituximab.

254   Finally, age at transplantation, pre-transplant COPD, underlying diabetic nephropathy, and the

255   diagnosis of an IRE or BSI within the preceding 6 months were entered into the conditional

256   logistic regression model (**Table 3**). Linear regression analysis showed no significant collinearity

257   among these explanatory variables, with all VIF values <1.5 (data not shown). After multivariate

258   adjustment, prior diagnosis of IRE (OR: 19.26; 95% CI: 2.07 - 179.46; *P*-value = 0.009) was

259   identified as the only independent risk factor associated with late IPA.

Discussion

260

261   To our knowledge, our multinational retrospective case-control study represents the largest

262 effort to date to explore the clinical outcome of and risk factors for IPA in the specific population

263 of KT recipients. Our experience highlights the poor prognosis conferred by the late forms of

264 this opportunistic infection, since more than half of the included patients had died at a median

265 time of less than two months from diagnosis. In addition, IPA-attributable mortality was

266 assumed in more than 20% of cases. Notwithstanding such an ominous picture, in our previous

267 study we reported even worse figures for early IPA (first 180 days), with global and attributable

268 mortality of 60.8% and 45.1%, respectively [12]. We hypothesize that this difference may be

269 explained by the relatively more intensive immunosuppression among patients in their first post-

270 transplant months [15].

271   Remarkably, although most of the episodes of late IPA occurred within the first three years,

272 almost 10% of them were diagnosed across a large time period covering more than a decade

273 after transplantation, including some very late onset episodes occurring more than ten years

274 post-transplantation. In a previous series of IPA among KT recipients [13], 43% of the 41 cases

275 were diagnosed beyond the sixth month, and 6 (14%) beyond the fifth year post-transplantation.

276 These concordant results reinforce the previously stated concept [20] that the period at risk for

277 severe opportunistic infection continues far beyond the classical time scheme proposed for SOT

278 recipients.

279   Despite the wide range of time between KT and the onset of late IPA, we were still able to

280 identify some factors associated to this event. Cases were more likely to have been diagnosed

281 with COPD, although such association only showed borderline univariate significance. The

282 presence of pre-transplant COPD may reflect underlying injury to the lung parenchyma [12,21]

283 or act as a surrogate marker for prolonged corticosteroid exposure. BSI during the six preceding

284 months was also more frequent among cases. Comparable associations have been previously

285 reported for the overall SOT population [8] or, specifically, KT recipients [12]. The occurrence of

286 BSI may identify patients commonly suffering from invasive procedures, impaired graft function

287 and antibiotic therapy exposure, which overall reflect increased patient frailty.

288   Following the example of previous studies [22], we created a composite variable (IRE) that

289 summarized post-transplant complications —such as severe non-device-associated infections,

290 CMV disease or *de novo* cancer— that are consistently assumed to indicate an excess of

291 immunosuppression. In the regression model this condition displayed a significant association

292 with the development of IPA during the following six months. Other authors have also reported

293 the observation of episodes of pneumonia preceding the onset of IPA [23,24]. On the other

294 hand, the deleterious impact exerted by CMV on the risk of IPA has been well established for

295 the SOT recipient [8,25,26]. In accordance with this rationale, the incidence of CMV disease in

296 our experience was ten times higher among cases than controls (16.4% versus 1.6%,

297 respectively). In a similar way, a recent diagnosis of *de novo* cancer (either PTLD or solid organ

298 tumor) had been made in almost one out of every ten cases as compared to none of the

299 controls. In a French nationwide epidemiological study, both hematologic and solid organ

300 malignancies have been described as an important risk factor for invasive aspergillosis [3]. In

301 addition to the direct deleterious effect of the oncologic therapies (B-cell-depleting agents such

302 as rituximab or cytotoxic chemotherapy) on the host's response and infection susceptibility, the

303 function of natural killer cells (which significantly contribute to the protective immunity against

304 fungi [27]) has been shown to be impaired in KT recipients with post-transplant cancer [28].

305 The design of our study (case-control study) prevents us from estimating the actual incidence of

306 late IPA among KT recipients that develop an episode of IRE. Case-control studies can

307 generate plausible associations rather than demonstrate direct causality. In our opinion, such a

308 circumstance and the heterogeneous distribution of IPA cases over a very long post-transplant

309 period would make it unreasonable to propose the use of antifungal prophylaxis for those

310 recipients fulfilling the characterized risk factors. Nevertheless, our findings do support the

311 recommendation of maintaining a low threshold for suspicion of post-transplant IPA in patients

312 with compatible respiratory symptoms and underlying COPD or recently diagnosed with a

313 serious infection, CMV disease or post-transplant cancer. In addition, this clinical awareness

314 should be maintained even for very long-term KT recipients, as IPA may occur many years after

315 transplantation. In this context, we have previously shown the protean clinical features of IPA

316 among KT recipients and the correlation between the timely initiation of antifungal therapy and

317 the outcome [15].

318 Strengths of the present collaborative effort include its multicenter nature, the use of uniform

319 diagnostic criteria, and the standardized collection of a large number of variables. However,

320   some limitations must be acknowledged, such as its retrospective design and the relatively low

321   sample size that may have limited statistical power. Therefore, confidence intervals for risk

322   estimates were wide. Most IPA cases were categorized as "probable" rather than "proven" [16].

323   The protracted inclusion period imposes heterogeneity among participating centers in

324   immunosuppression and standard of care. Nonetheless, the low incidence among KT recipients

325   of late-onset IPA made this approach the only practical method to collect a meaningful number

326   of cases. We lacked detailed data on certain relevant factors (such as the receipt of rituximab or

327   cytotoxic chemotherapy among patients with PTLD). Finally, we were unable to estimate the

328   incidence of late IPA due to the lack of denominator figures (i.e., number of transplant

329   procedures performed at each center or number of at-risk recipients during the study period)

330   since our research was conceived exclusively to ascertain the risk factors for developing such

331   condition. Thus, we chose a case-control design instead than other approaches (i.e., nested

332   case-control study within a multicenter cohort).

333   In conclusion, late IPA may develop among KT recipients even more than 10 years after

334   transplantation and entails a very poor prognosis. The preceding diagnosis of post-transplant

335   adverse events reflecting an excess of immunosuppression, such as serious or opportunistic

336   infection or *de novo* malignancy, may be useful to identify those patients at the highest risk for

337   this complication.

343 • **Authors´ contribution:** FLM designed the study; FLM and MFR analyzed data and wrote
344   the manuscript; FLM, MFR, JTS and JMA coordinated the study. FLM, JTS, PLC, CvD,
345   EM, MJPS, MM, JC, MAM, CC, LS, NS, AS, EC, LCV, PLMM, OL, ER, APL, MA, TL, MD,
346   CGR, FHP, JF, MN, OM, JRPP, MM, AV, BSS, AM, JP, JPH, TL, AM, JC, MB, DH,
347   EAHM, MCF and MPC collected data and critically reviewed the final version of the
348   manuscript.

349 • **Transparency declaration:** Francisco López-Medrano has been paid for talks on behalf
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351   talks on behalf of Pfizer and Gilead Sciences. Christian van Delden has been consultant
352   for Basilea, Debiopharm, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Astellas
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363   consultant to and on the speakers' bureau for Astellas Pharma, Pfizer, Gilead, Merck
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467   **Tables**

468   **Table 1.** Comparison of demographics and pre-transplant variables between KT recipients with

469   and without late IPA.

Variable	Late IPA group (n = 61)	Control group (n = 61)	P-value[a]
Age, years [mean ± SD]	54.6 ± 14.2	48.6 ± 15.5	**0.010**
Gender (male) [n (%)]	33 (54.1)	38 (62.3)	0.458
Pre-transplant conditions [n (%)]			
Diabetes mellitus	18 (29.5)	9 (14.7)	0.093
Chronic obstructive pulmonary disease	11 (18.0)	0 (0.0)	**0.001**
Pre-transplant corticosteroid therapy [n (%)][b]	6 (10.3)	5 (8.8)	0.754
BMI at transplantation, Kg/m^2 [mean ± SD][c]	24.3 ± 3.6	26.7 ± 7.3	0.074
Previous kidney transplantation [n (%)]	7 (11.5)	8 (13.1)	1.000
Underlying end-stage renal disease [n (%)]			
Glomerulonephritis	14 (23.0)	14 (23.0)	1.000
Diabetic nephropathy	12 (19.7)	4 (6.6)	0.077
Nephroangiosclerosis	8 (13.1)	8 (13.1)	1.000
Polycystic kidney disease	8 (13.1)	11 (18.0)	0.824
Chronic interstitial nephropathy	3 (4.9)	3 (4.9)	1.000
Congenital nephropathy	2 (3.3)	3 (4.9)	1.000
Lupus nephropathy	1 (1.6)	1 (1.6)	1.000
Reflux nephropathy	0 (0.0)	1 (1.6)	1.000
Unknown	6 (9.8)	9 (14.8)	0.388
Other	7 (11.5)	7 (11.5)	0.549
Pre-transplant positive serostatus [n (%)]			
Hepatitis C virus	6 (9.8)	1 (1.6)	0.125
Hepatitis B virus (surface antigen)	2 (3.3)	4 (6.6)	0.625
Epstein-Barr virus (anti-EBNA)[d]	49 (87.5)	47 (83.9)	0.754
CMV[e]	45 (73.8)	45 (75.0)	1.000
Pre-transplant maintenance dialysis [n (%)]	55 (90.2)	54 (88.5)	1.000
Duration, months [median (IQR)]	23 (15 - 41)	19.5 (12 - 45.8)	1.000

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 58 cases and 57 controls.

[c] Data available for 43 cases and 43 controls.

[d] Data available for 56 cases and 56 controls.

[e] Data available for 61 cases and 60 controls.

470 **Table 2.** Comparison of donor- and transplant-related factors, post-transplant events and

471 outcomes.

Variable	Late IPA group (n = 61)	Control group (n = 61)	*P*-value[a]
Age of donor, years [mean ± SD]	49.8 ± 16.3	46.8 ± 13.5	0.283
Living donor [n (%)]	12 (19.7)	12 (19.7)	1.000
Double kidney transplantation [n (%)]	3 (4.9)	0 (0.0)	0.250
Induction therapy [n (%)][b]			
None	22 (36.7)	20 (33.9)	1.000
Anti-CD25 (basiliximab or daclizumab)	22 (36.7)	20 (33.9)	0.815
Anti-thymocyte globulin	16 (26.7)	19 (32.2)	0.648
Primary immunosuppression regimen including [n (%)][b]			
Steroids	54 (88.5)	57 (93.4)	0.375
Tacrolimus	29 (48.3)	30 (50.8)	1.000
Cyclosporine	19 (31.7)	20 (33.9)	1.000
MMF / MPA	47 (78.3)	50 (84.7)	0.375
Azathioprine	5 (8.5)	7 (11.9)	0.375
mTOR inhibitor	6 (10.0)	2 (3.4)	0.219
Length of hospital admission for transplantation, days [median (IQR)]	12 (8 - 18.8)	11 (6.3 - 18.8)	0.314
Delayed graft function [n (%)]	13 (21.3)	8 (13.1)	0.388
Surgical reintervention [n (%)][c]	6 (10.2)	2 (3.7)	0.687
eGFR at month 3 after transplantation, mL/min/1.72 m^2 [mean ± SD][d]	23.8 ± 3.2	25.6 ± 3.4	0.873
eGFR at month 6 after transplantation, mL/min/1.72 m^2 [mean ± SD][e]	22.9 ± 3.1	20.5 ± 2.8	0.159
Leukopenia (<3.0 x 10^9 cells/L) [n (%)][f,g]	10 (16.9)	6 (10.2)	0.388
Neutropenia (<1.5 x 10^9 cells/L) [n (%)][f,h]	6 (12.2)	3 (6.2)	0.687
Serum IgG levels, mg/dL [mean ± SD][i]	879 ± 627	763 ± 571	0.750
Post-transplant events within the previous 6 months [n (%)][j]			
IRE[k,l]	21 (34.4)	2 (3.3)	**0.000**
CMV disease	10 (16.4)	1 (1.6)	**0.004**
Non ventilator-associated pneumonia	9 (14.8)	1 (1.6)	**0.021**
De novo malignancy[m]	5 (8.2)	0 (0.0)	0.063
Laboratory-confirmed respiratory tract viral infection[n]	5 (8.2)	0 (0.0)	0.063
Bloodstream infection[o]	7 (11.5)	0 (0.0)	**0.016**
ICU admission for ≥72 hours	2 (3.3)	0 (0.0)	0.500
Acute graft rejection	4 (6.6)	5 (8.2)	1.000
Episode treated with steroid boluses	4 (4.9)	5 (8.2)	0.687
Overall mortality [n (%)]	29 (47.5)	0 (0.0)	0.001

IPA-attributable mortality [n (%)]	13 (21.3)	-	NA

CMV: cytomegalovirus; eGFR: estimated glomerular filtration rate; ICU: intensive care unit; IgG: immunoglobulin G; IPA: invasive pulmonary aspergillosis; IQR: interquartile range; IRE: immunosuppression-related event; MMF / MPA: mofetil mycophenolate / mycophenolate acid; mTOR: mammalian target of rapamycin; NA: not applicable; SD: standard deviation.

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

[b] Data available for 60 cases and 59 controls.

[c] Data available for 59 cases and 54 controls.

[d] Data available for 56 cases and 56 controls.

[e] Data available for 54 cases and 54 controls.

[f] At any point during the first 6 months after transplantation.

[g] Data available for 59 cases and 59 controls.

[h] Data available for 49 cases and 48 controls.

[i] Serum IgG levels measured within the 6-month period prior to or following the date of diagnosis of IPA (for cases) or the analogous "pseudo-date" of diagnosis (for controls). Data available for 10 cases and 4 controls.

[j] Events occurring within the 6-month period prior to the date or the "pseudo-date" of diagnosis of IPA.

[k] The total number of IREs may be less than the sum of each conditions since more than one event was consecutively present in some patients.

[l] There were 3 cases of post-transplant tuberculosis, although none of them occurred within the 6-month period prior to the date or the "pseudo-date" of diagnosis of IPA.

[m] Includes PTLD (3 cases), colorectal adenocarcinoma and metastatic adenocarcinoma of unknown primary origin (one case each).

[n] Includes influenza virus infection (4 cases).

[o] Includes BSI due Enterobacteriaceae (3 cases), *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *S. aureus* and *Candida albicans* (one case each).

472 **Table 3.** Uni- and multivariable analyses (conditional logistic regression) of risk factors

473 predicting the occurrence of late IPA.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Age at transplantation, years[a]	1.04	1.01 - 1.08	0.017	-	-	-
Diabetic nephropathy	3.00	0.97 - 9.30	0.057	-	-	-
Pre-transplant COPD	65.29	0.51 - 8324.28	0.091	-	-	-
Prior IRE[b,c]	20.00	2.68 - 149.02	0.003	19.26	2.07 - 179.46	0.009
Prior BSI[b]	7.00	0.86 - 56.89	0.069	-	-	-

BSI: bloodstream infection; CI: confidence interval; COPD: chronic obstructive pulmonary disease; IPA: invasive pulmonary aspergillosis; IRE: immunosuppression-related event; OR: odds ratio.

[a] OR per unitary increment.

[b] Events occurring within the 6 months previous to the date of diagnosis of IPA for cases or the analogous "pseudo-date of diagnosis" for corresponding controls.

[c] Includes non-ventilator-associated pneumonia, CMV disease and post-transplant *de novo* malignancy.

474   **Figure legend**

475   • **Figure 1.** Temporal distribution of cases of late invasive pulmonary aspergillosis occurring

476   according to post-transplant month of diagnosis.

477   **Supporting Information**

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

479   • **Supplementary Materials and Methods:** Definitions used for date of IPA diagnosis, IPA-

480   attributable diagnosis, CMV disease, tuberculosis, pneumonia, respiratory tract viral

481   infection, BSI, PTLD, delayed graft function, acute graft rejection and eGFR.

Figure 1.