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# Mainly Post-Transplant Factors Are Associated with Invasive Aspergillosis after Allogeneic Stem Cell Transplantation: A Study from the Surveillance des Aspergilloses Invasives en France and Société Francophone de Greffe de Moelle et de Thérapie Cellulaire

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A B S T R A C T

Invasive aspergillosis (IA) occurs in up to 23% of allogeneic hematopoietic stem cell transplantation (HSCT) patients. Although transplant procedures have changed over time, more late cases of IA are being observed. The objective of this study was to identify the pre- and post-transplant factors of IA in a large cohort of HSCT patients mainly transplanted with reduced-intensity conditioning. This multicenter, case-control study was carried out using data collected between 2005 and 2010 by the Surveillance des Aspergilloses Invasives en France program (Institut Pasteur, Paris) and the European Society for Blood and Marrow Transplantation ProMISe registry. Four control subjects without IA were individually matched to each case based on the center, patient age, and year of the transplant. We identified 185 cases of probable and proven IA and 651 control subjects. The median date of IA after the transplant was 133 days, with 35 cases (19%) of early IA (before day 40), 33 cases (18%) of late IA (days 40 to 100), and 117 cases (63%) cases of very late IA (after day 100). In the multivariate analysis early IA was significantly associated with a lack of engraftment, whereas late and very late IA were significantly associated with more than grade II acute graft-versus-host disease (GVHD); very late IA was also significantly associated with relapse and secondary neutropenia. Two-thirds of IA cases occurred more than 100 days after HSCT with different risk factors from those occurring earlier. Prophylactic strategies should consider the specific risk factors for late and very late IA, especially GVHD, relapse after transplant, and secondary neutropenia.



## INTRODUCTION

Invasive aspergillosis (IA) is a common complication after allogeneic hematopoietic stem cell transplantation (HSCT), occurring in 2.7% to 23% of HSCT recipients [1–4]. IA is the most common invasive fungal infection, representing about two-thirds of invasive fungal infection cases after allogeneic HSCT [2,5]. Despite improvements in antifungal prophylaxis and treatment, IA remains a life-threatening complication in allogeneic HSCT recipients and has a mortality rate of 20% to 75% [2,6–8].

Several risk factors of IA after allogeneic HSCT have been identified [9,10]. Pretransplant factors that increase the risk of

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IA include age, bone marrow transplant rather than peripheral blood stem cells, transplantation of cord blood units, an active hematologic malignancy, administration of antithymocyte globulin (ATG), and a pretransplant iron overload [7,9–12]. Geoclimatic conditions, such as temperature and precipitation, correlate with spore counts in the air and may also influence the incidence of IA [13]. Post-transplant factors include cytomegalovirus (CMV) infection and disease, delayed neutrophil and lymphocyte engraftment, secondary neutropenia, relapse of the underlying disease, administration of corticosteroids, and graft-versus-host disease (GVHD) [8–11,14–21].

Two decades ago IA was mainly considered to be an early complication of HSCT. However, more late cases of IA are being reported, and thus the role of the risk factors may vary according to the timing of the onset of IA [9,10]. In addition, transplant procedures have greatly evolved over the last 15 years. Although more than 80% of patients of previous large series of IA were transplanted using myeloablative regimens from HLA-identical donors [10,11], there has been a switch to reduced-intensity conditioning (RIC) and nonmyeloablative (NMA) regimes and more alternative stem cell sources [22–24].

The goal of this study was to determine if the risk factors for IA have changed with the evolution of conditioning. We identified the pre- and post-transplant risk factors that were independently associated with IA in a large cohort of allogeneic HSCT recipients transplanted in France. Of the cohort, 95% of the recipients were transplanted using RIC or NMA conditioning. We then assessed whether the impact of the factors varied according to the timing of the onset of IA after the transplant.

## METHODS

### *Design*

This multicenter, case-control study used data from 2 prospective programs: the Surveillance des Aspergilloses Invasives en France (SAIF) program from the Institut Pasteur in Paris, France, and the European ProMISe registry. SAIF anonymously collected consecutive cases of IA diagnosed in university hospitals from 3 regions of France (Paris-Ile de France, Grand Ouest, and Rhône-Alpes) between 2005 and 2010 through a web-based file [8]. The ProMISe registry prospectively collects data on all consecutive HSCTs within the European Society for Blood and Marrow Transplantation (EBMT). These 2 databases are complementary: The SAIF database contains mainly clinical, imaging, and microbiologic data focused on the definition criteria of IA, whereas the ProMISe database mainly contains transplant-related data. This study was registered with the Commission Nationale de l'Informatique et des Libertés (no. 1882064 v 0).

### *Cases of IA*

Consecutive cases of IA were selected from the SAIF registry from January 1, 2005 to December 31, 2010. The IA cases were prospectively recorded from the laboratory of each participating center; the local microbiologist completed a standardized questionnaire about the demographics, underlying conditions, diagnostic tools used (imaging, direct examination and mycological culture, histology, and serum or bronchoalveolar galactomannan), dates of hospitalization, and first-line antifungal therapy. A local committee involving the mycologists and the hematologists in charge of the patient reviewed every recorded questionnaire, checked the diagnosis, and classified each episode according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group 2002 criteria [25] for patients included before 2008 and the European Organization for Research and Treatment of Cancer/Mycoses Study Group 2008 criteria [26] for patients included from 2008 to 2010. The date of IA was set as the date criteria were fulfilled. Patients were managed at HSCT centers according to the local practices for the diagnosis and treatment of IA. The timing of IA after transplant was classified as follows: early IA, diagnosed before day 40; late IA, diagnosed between days 40 and 100; and very late IA, diagnosed after day 100 [9].

All proven and probable cases of IA diagnosed in patients aged 15 years or over after an allogeneic HSCT were selected from the SAIF database. Only the first episodes of proven or probable IA after HSCT were considered. Patients with previous IA before transplantation were not excluded from the study. Of the 20 French HSCT centers that participated in the SAIF registry, 19 participated in the present study; 3881 allogeneic HSCT were performed during 2005 to 2010.

### *Control Patients*

Our objective was to individually match 4 control patients with each IA case in terms of HSCT center, age of patient (±5 years), and year of transplant. Control patients were recruited from the ProMISe registry among patients who did not develop IA after HSCT. Most patients (IA cases and control subjects) were housed in laminar air flow rooms at the initial phase of transplant, except those who received NMA conditioning with fludarabine and 2-Gy total body irradiation (TBI).

### *Data Collection and Definitions*

The pre- and post-transplant clinical and biologic data of the case and control patients were collected from the ProMISe registry. The EBMT definitions were used for the terms. For example, the day of engraftment was defined as the first day of 3 consecutive days $> .5 \times 10^9$/L. Acute GVHD was reported according to the staging by Glucksberg et al [27]. Secondary neutropenia was defined as the occurrence of a neutrophil count $< .5$ g/L after engraftment. According to the EBMT recommendations, all patients signed a consent form before transplant for their data to be entered into the ProMISe registry and used for retrospective studies.

### *Statistical Analysis*

For the present study the alpha risk was set at .05. Thus, 175 cases were needed to provide 80% power for detecting an odds ratio (OR) $> 1.65$ related to the factors present in 35% of the control population and an OR $> 5.21$ related to the factors present in 1% of the control population. The factors present in 1% of the population corresponded to the prevalence of secondary neutropenia, which was a less frequent, but well-known, risk factor.

Table 1 displays the pre- and post-transplant factors of the cases and control subjects with IA. The qualitative variables were given as numbers (%) and compared using the chi-square test or the Fisher exact test. The quantitative variables were given as the median (interquartile range, Q1 to Q3) and compared using the Kruskal-Wallis test.

Data were analyzed using the standard methods for estimating ORs and 95% confidence intervals (CIs) in case-control studies. For the univariate analyses the adjusted ORs (aOR) were estimated separately for each potential risk factor yielding a $P < .20$ using unconditional logistic regression models forcing the matching variables, such as HSCT center, age of patient, and year of transplant, into all models. The quantitative variables were converted into categorical variables using a cut-off according to the literature when available or a median split. The whole population was studied for preransplant factors. However, only the control subjects and their corresponding cases were studied for post-transplant factors; the control subjects were followed for the same duration as their cases. For the post-transplant risk factors we only analyzed the events that occurred before IA. Because aspergillosis prophylaxis with posaconazole has been recommended to be administered to patients with GVHD since 2008 [28], ORs were also adjusted for the periods 2005 to 2007 and 2008 to 2010.

Variables yielding $P < .10$ in the univariate analyses were considered for the multivariate analyses. First-order interactions and confounding factors were investigated using multiple 2-by-2 analyses. In the first multivariate logistic regression model we considered pretransplant factors and added post-transplant variables. The discrimination of the model was assessed using the C-index (area under the curve [AUC] of receiver operating characteristics [ROC]), whereas the Hosmer-Lemeshow calibration test was used to assess the goodness of fit.

Finally, we conducted similar analyses using a multinomial logistic regression model to compare early IA, late IA, and very late IA cases with the control population. Because of the small number of patients in some groups, we pooled some modalities of the following variables: disease status, stem cell source, and donor (D)/recipient (R) CMV serology.

All tests were 2-tailed, and $P \leq .05$ was considered statistically significant. $P$ values for comparisons between each group of IA (early, late, and very late) and the control group were corrected using the false discovery method for multiple comparisons [29].

Data were analyzed using Stata statistical software 12.0 (Stata Corp., College Station, TX). This observational study was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology statement [30].

## RESULTS

This study included 185 IA cases: 166 (90%) probable IA cases and 19 (10%) proven IA cases. The time from transplant to IA ranged from 3 days to 4308 days with a median of 133 days (range, 64 to 233). Thirty-five cases (19%) were classified as early IA, 33 (18%) were late IA, and 117 (63%) were very late IA. The median number of cases per HSCT center was 7 (range, 1 to 34).

**Table 1**
Univariate Analysis of Pre- and Post-Transplant Factors Associated with IA

| Pretransplant Factors | Cases (n = 185) | Control Subjects (n = 651) | P* | Analyses Adjusted For Matching Variables† aOR [95% CI] | P |
|---|---|---|---|---|---|
| Age, median (Q1-Q3), yr | 5.1 (36.7-57.6) | 49.3 (38.0-57.6) | .85 | | |
| Female sex | 71 (38.4) | 257 (39.5) | .79 | | |
| Body mass index, median (152/542) (range)‡ | 24.2 (21.9-26.6) | 23.6 (21.2-26.3) | .11 | .7 [.2-2.0] | .47 |
| Karnofsky score = 100 (161/560) | 68 (42.2) | 248 (44.3) | .64 | | |
| Underlying disease | | | .52 | | |
| Acute leukemia and myelodysplastic syndrome | 122 (66.0) | 453 (69.6) | | | |
| Lymphoproliferative disorder | 41 (22.2) | 120 (18.4) | | | |
| Myeloproliferative neoplasm | 16 (8.7) | 48 (7.4) | | | |
| Other§ | 6 (3.2) | 30 (4.6) | | | |
| Disease status (141/483) | | | .20 | | |
| Complete remission | 92 (65.3) | 333 (68.9) | | | |
| Partial remission | 0 | 9 (1.9) | | | |
| Stable disease | 2 (1.4) | 2 (.4) | | | |
| Failure/relapse | 46 (32.6) | 134 (27.7) | | | |
| Other | 1 (.7) | 5 (1.0) | | | |
| Stem cell source (185/649) | | | .91 | | |
| Bone marrow | 51 (27.6) | 169 (26.0) | | | |
| PBSCs | 111 (60.0) | 402 (61.9) | | | |
| Cord blood unit | 23 (12.4) | 76 (11.7) | | | |
| Bone marrow + PBSCs | 0 | 2 (.3) | | | |
| Unrelated donor (185/649) | 106 (57.3) | 350 (53.9) | .42 | | |
| Type of conditioning | | | .21 | | |
| Myeloablative conditioning | 10 (5.4) | 27 (4.2) | | | |
| Fludarabine + 2 Gy TBI | 48 (26.0) | 135 (20.7) | | | |
| RIC | 127 (68.7) | 489 (75.1) | | | |
| D/R CMV serology (183/638) | | | .11 | | |
| D−/R+ | 61 (33.3) | 156 (24.5) | | 1.54 [1.1-2.2] | .02 |
| D−/R− | 47 (25.7) | 198 (31.0) | | 1 | |
| D+/R− | 23 (15.6) | 89 (14.0) | | | |
| D+/R+ | 52 (28.4) | 195 (30.6) | | | |
| Transplantation during summer‖ | 44 (23.8) | 124 (19.1) | .16 | 1.3 [.9-2.0] | .16 |
| ATG-based conditioning | 64 (34.6) | 277 (45.6) | .05 | .7 [.5-1.0] | .05 |
| TBI (184/646) | 110 (94.4) | 316 (48.9) | .01 | 1.6 [1.1-2.2] | .01 |
| Clofarabine | 6 (3.2) | 8 (1.2) | .10 | 2.8 [1.0-8.3] | .06 |
| Invasive fungal infection before transplant (145/524) | 12 (8.3) | 52 (9.9) | .55 | | |
| Female donor (181/641) | 75 (41.4) | 272 (42.4) | .81 | | |
| Donor age (149/547), median (Q1-Q3),¶ yr | 41.7 (30.2-49.6) | 43.6 (32.2-51.4) | .18 | 1.3 [.9-1.9] | .21 |
| Post-Transplant Factors | Cases (n = 185) | Control Subjects (n = 526) | P | aOR [95% CI] | P |
| Acute GVHD (149/516) | | | <.001 | | |
| Grades 0-I | 349 (67.6) | 104 (56.2) | | 1 | |
| Grade II | 108 (20.9) | 37 (20.0) | | 1.2 [.7-1.8] | .54 |
| Grades III-IV | 59 (11.4) | 44 (23.8) | | 2.6 [1.7-4.1] | <.001 |
| CMV infection (185/526) | 36 (19.5) | 113 (21.5) | .56 | | |
| Time of engraftment** (185/526), median (Q1-Q3) | 19 (16-27) | 19 (15-25) | .76 | | |
| Absence of engraftment (182/515) | 10 (5.5) | 18 (3.5) | .24 | | |
| Relapse after transplant (175/500) | 40 (22.9) | 7 (1.4) | <.001 | 20.7 [9.0-47.5] | <.001 |
| Secondary neutropenia (138/418) | 55 (39.9) | 94 (22.5) | <.001 | 2.3 [1.5-3.5] | <.001 |

Values are expressed as n (%) unless otherwise defined. PBSCs indicates peripheral blood stem cells.
* *P* values of the chi-squared test, Fisher exact test, or nonparametric Kruskal-Wallis test as appropriate.
† Logistic regression analysis adjusted for matching variables, such as HSCT center, age of patient (±5 years), and year of transplant.
‡ aOR calculated for body mass index < 18.
§ Solid tumor, bone marrow failure, inherited disorder, histiocytic disorder, and hemoglobinopathy.
‖ July, August, and September.
¶ aOR calculated for age > 60 years.
** aOR calculated for number of days of neutrophil recovery after transplant > 18 days.

We were able to identify 651 control subjects (Figure 1). A total of 135 cases had 4 matched control subjects, 24 cases had 3 control subjects, 15 cases had 2 control subjects, 9 cases had 1 control subject, and 2 cases had no control subjects. For the post-transplant factor analysis 526 control subjects had a follow-up that matched the timing of IA in their corresponding case patient. The median follow-up was 1904 days (range, 1448 to 2505). The control patients were well matched with the case patients for age ($P = .85$), HSCT center ($P = 1.00$), and year of transplant ($P = 1.00$).

The characteristics of the patients are summarized in Table 1. Briefly, the median age at transplant was 49.6 years. More than two-thirds of the patients had acute leukemia or myelodysplastic syndrome. Two-thirds of the patients were in complete remission at transplant. Only 4.4% of the patients received a myeloablative regimen; all others received RIC or NMA conditioning. Peripheral blood stem cells were the main stem cell source. More than half of the patients were transplanted from an unrelated donor.

The median overall survival was .70 years (Q1 to Q3, .60 to .90) for the cases and 4.94 years (Q1 to Q3, 3.61 to 7.06) for the control subjects ($P < .001$). There was no difference in survival when the period before the introduction of posaconazole prophylaxis (2005 to 2007) was compared with the period after its introduction (2008 to 2010).

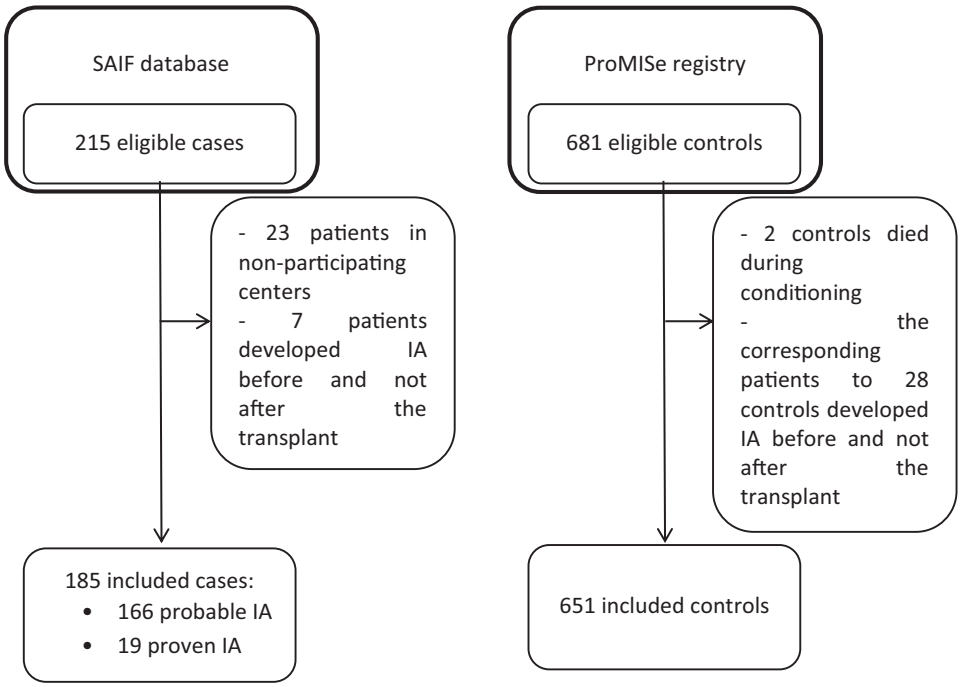


**Figure 1.** Flowchart.

### *Pre- and Post-Transplant Factors Associated with the Risk of IA*

*Univariate analysis*

The univariate analysis of the pretransplant factors adjusted for matching variables (Table 1) showed that IA was significantly associated with TBI, D and R CMV serology (D−/R + versus others), and the absence of the use of ATG-based conditioning. A trend for an association with IA was observed for the use of clofarabine.

Among the post-transplant factors (Table 1), IA occurrence was significantly associated with grades III to IV acute GVHD, a relapse after transplant, and secondary neutropenia. An adjustment for the period before 2008 did not affect the association between acute GVHD and IA (aOR, 2.65; 95% CI, [1.68 to 4.17]; $P < .001$). The time between acute GVHD and IA was 113 days (range, 56 to 259).

*Multivariate analysis*

The multivariate analysis (Table 2) showed that TBI, clofarabine use, and D−/R+ CMV serology were independently associated with IA. When pre- and post-transplant factors were taken into account, the analysis showed that TBI, grade ≥ II acute GVHD, and relapse after transplant were independently associated with IA. A trend for an association was observed for secondary neutropenia. The D−/R+ CMV serology and clofarabine use were not significant because of an inverse association with GVHD and relapse after transplant, respectively. The final model had a good calibration (goodness-of-fit test, $P > .20$) and a moderate discrimination (AUC under ROC curve, .77 95% CI[.73 to .82]).

### *Pre- and Post-Transplant Factors Associated with the Risk of Early, Late, or Very Late IA*

*Univariate analysis*

The univariate analysis (Table 3) showed that the occurrence of early IA was significantly associated with the use of cord blood units as the stem cell source, clofarabine use, absence of acute GVHD, lack of engraftment, and relapse after transplant. A trend for a high risk of early IA was observed for the absence of complete remission at transplant, an unrelated donor, transplantation during summer, and the absence of CMV infection. The occurrence of late IA was associated with grades III to IV acute GVHD regardless of the period (before or after 2008: aOR, 3.88; 95% CI, 1.58 to 9.57; $P = .003$), and relapse after transplant. A trend for a high risk of late IA was observed for the absence of the use of ATG-based conditioning. The time between acute GVHD and late IA was 39 days (range, 22 to 57). The occurrence of very late IA was associated with D−/R+ CMV serology, at least grade II acute GVHD regardless of the period (before or after 2008: aOR, 1.66; 95% CI, .98 to 2.79; $P = .057$ for grade II GVHD; and aOR, 4.12; 95% CI, 2.21 to 7.68; $P < .001$ for grades III to IV GVHD), relapse after transplant, and secondary neutropenia. A trend for a high risk of very late IA was observed for the absence of the use of ATG-based conditioning and TBI. The time between acute GVHD and very late IA was 158 days (range, 98 to 401).

**Table 2**
Multivariate Analysis of Pre- and Post-Transplant Factors Associated with IA

| Model 1: Pretransplant factors (185 cases and 651 control subjects)* | aOR [95% CI] | *P* |
|---|---|---|
| CMV D−/R+ serology | 1.5 [1.0-2.2] | .03 |
| Clofarabine | 4.1 [1.3-13.2] | .02 |
| TBI | 1.6 [1.1-2.4] | .01 |
| **Model 2: Pre- and post-transplant factors (185 cases and 526 control subjects)*** | **aOR [95% CI]** | ***P*** |
| TBI | 1.9 [1.1-3.2] | .02 |
| Acute GVHD | | |
| Grade II | 1.8 [1.0-3.2] | .05 |
| Grades III-IV | 4.3 [2.4-7.7] | <.001 |
| Relapse | 31.0 [10.4-92.4] | <.001 |
| Secondary neutropenia | 1.6 [1.0-2.8] | .07 |

*Multivariate logistic regression adjusted for matching variables, such as HSCT center, age of patient (±5 years), and year of transplant, and variables listed in the table.

**Table 3**
Univariate Analysis of Pre- and Post-Transplant Factors according To the Timing of IA

| Pretransplant Factors | Control Subjects (n = 651) | Early IA (n = 35) | Late IA (n = 33) | Very late IA (n = 117) | P* | Analyses Adjusted for Matching VariablesaOR [95% CI]† Early IA OR [95% CI] | P† | Late IA OR [95% CI] | P† | Very late IA OR [95% CI] | P† |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age, median (Q1-Q3), yr | 49.3 (38.0-57.6) | 47.5 (35.9-60.8) | 54.8 (38.8-57.9) | 50.1 (36.7-57.4) | .87 | | | | | | |
| Female R | 257 (39.5) | 15 (42.9) | 10 (30.3) | 46 (39.3) | .73 | | | | | | |
| Body mass index (542/30/30/92), median (Q1-Q3) | 23.6 (21.2-26.3) | 24.1 (22.5-29.8) | 24.2 (19.6-27.4) | 24.3 (21.8-26.5) | .24 | | | | | | |
| Karnofsky score = 100 (560/30/33/98) | 312 (55.7) | 16 (53.3) | 15 (45.5) | 62 (63.3) | .30 | | | | | | |
| Underlying disease | | | | | .15 | | | | | | |
| Acute leukemia and myelodysplastic syndrome | 453 (69.6) | 22 (62.9) | 18 (54.6) | 82 (70.1) | | .7 [.2-3.1] | .62 | .3 [.9-1.0] | .18 | 5.8 [.8 -43.1] | .18 |
| Lymphoproliferative disorder | 120 (18.4) | 7 (20.0) | 7 (21.2) | 27 (23.1) | | .8 [.2-4.2] | .78 | .5 [.1-2.1] | .50 | 7.3 [.9-56.7] | .18 |
| Myeloproliferative disorder | 48 (7.4) | 4 (11.4) | 5 (15.2) | 7 (6.0) | | 1.2 [.2-7.5] | .96 | 1.0 [.2-4.6] | .96 | 4.2 [.5-36.6] | .57 |
| Other‡ | 30 (4.6) | 2 (5.7) | 3 (9.1) | 1 (.9) | | 1 | | 1 | | 1 | |
| Absence of complete remission (483/26/24/91) | 150 (31.1) | 14 (53.9) | 6 (25.0) | 29 (31.9) | .09 | 2.4 [1.1-5.4] | .09 | .7 [.3-1.8] | .65 | 1.1 [.7-1.7] | .82 |
| Cord blood unit stem cell source (649/35/33/117) | 76 (11.7) | 10 (28.6) | 3 (9.1) | 10 (8.6) | .03 | 2.9 [1.3-6.4] | .03 | .7 [.2-2.3] | .51 | .8 [.4-1.6] | .51 |
| Unrelated donor (649/35/33/117) | 350 (53.9) | 27 (77.1) | 16 (48.5) | 63 (53.9) | .05 | 2.7 [1.2-6.1] | .06 | .7 [.4-1.5] | .54 | 1.1 [.7-1.6] | .75 |
| Type of conditioning | | | | | .27 | | | | | | |
| Myeloablative conditioning | 27 (4.2) | 4 (11.4) | 1 (3.0) | 5 (4.3) | | | | | | | |
| Fludarabine + 2 Gy TBI | 135 (20.7) | 9 (25.7) | 10 (30.3) | 29 (24.8) | | | | | | | |
| RIC | 489 (75.1) | 22 (62.9) | 22 (66.7) | 83 (70.9) | | | | | | | |
| D−/R+ CMV serology | 156 (24.5) | 11 (31.4) | 8 (24.2) | 42 (36.5) | .05 | 1.5 [.6-3.6] | .51 | 1.0 [.5-2.4] | .94 | 1.7 [1.1-2.7] | .01 |
| Transplantation during summer§ | 124 (19.1) | 12 (34.3) | 6 (18.2) | 26 (22.2) | .16 | 2.3 [1.1-4.7] | .09 | 1.0 [.4-2.4] | .93 | 1.2 [.8-2.0] | .68 |
| ATG-based conditioning | 277 (42.3) | 20 (57.1) | 10 (30.3) | 34 (29.1) | .005 | 1.5 [.7-3.2] | .25 | .4 [.2 to .9] | .08 | .6 [.4-1.0] | .08 |
| TBI (646/35/33/116) | 316 (48.9) | 21 (60.0) | 18 (54.6) | 71 (61.2) | .07 | 1.7 [.9-3.5] | .20 | 1.4 [.7-2.9] | .33 | 1.6 [1.1-2.4] | .09 |
| Clofarabine | 8 (1.2) | 3 (8.6) | 2 (6.1) | 1 (.9) | .008 | 6.3 [1.5-25.6] | .03 | 3.9 [.8-19.6] | .15 | .9 [.1-7.0] | .88 |
| IFI before transplant (524/30/31/84) | 52 (9.9) | 2 (6.7) | 3 (9.7) | 7 (8.3) | .96 | | | | | | |
| Female donor (641/34/33/114) | 272 (42.4) | 15 (44.1) | 9 (27.3) | 51 (44.7) | .34 | | | | | | |
| Donor age (547/23/29/97), median (Q1-Q3), years | 41.7 (30.2-49.6) | 40.5 (26.8-48.1) | 45.4 (36.5-53.3) | 43.6 (33.8-50.8) | .26 | | | | | | |
| Post-Transplant Factors | Control Subjects (n = 526) | Early IA (n = 35) | Late IA (n = 33) | Very late IA (n = 117) | P | Early IA (n = 35) | P | Late IA (n = 33) | P | Very late IA (n = 117) | P |
| Acute GVHD (516/35/33/117) | | | | | <.001 | | | | | | |
| Grades 0 to I | 349 (67.6) | 33 (94.3) | 14 (42.4) | 57 (48.7) | | 1 | | 1 | | | |
| Grade II | 108 (20.9) | 1 (2.9) | 4 (12.1) | 32 (27.4) | | .1 [.0 to .8] | .05 | 1.0 [.3-3.1] | 1.00 | 1.7 [1.1-2.9] | .05 |
| Grades III-IV | 59 (11.4) | 1 (2.9) | 15 (45.5) | 28 (23.9) | | .2 [.0-1.3] | .09 | 6.2 [2.8-13.7] | <.001 | 3.2 [1.9-5.5] | <.001 |
| CMV infection | 113 (21.5) | 1 (2.9) | 5 (15.2) | 30 (25.6) | .03 | .1 [.0 to .8] | .09 | .7 [.3-1.8] | .45 | 1.3 [.8-2.1] | .41 |
| Time of engraftment‖ (117/35/33/117), median (Q1-Q3) | 19 (15-25) | 25 (17-40) | 17 (15-34) | 19 (15-23) | .13 | 1.6 [.8-3.3] | .32 | .6 [.3-1.2] | .32 | .9 [.6-1.4] | .67 |
| Absence of engraftment (515/34/32/116) | 18 (3.5) | 8 (23.5) | 2 (6.3) | 0 | <.001 | 8.7 [3.4-22.0] | <.001 | 1.8 [.4-8.4] | .65 | 2.6 10-7 [0-] | .99 |
| Relapse after transplant (500/32/33/110) | 7 (1.4) | 3 (9.4) | 2 (6.1) | 35 (31.8) | <.001 | 8.0 [1.9-32.6] | .004 | 5.0 [1.0-25.3] | .05 | 31.1 [13.2-73.1] | <.001 |
| Secondary neutropenia (418/23/25/90) | 94 (22.5) | 5 (21.7) | 8 (32.0) | 42 (46.7) | <.001 | .9 [.3-2.6] | .91 | 1.5 [.6-3.6] | .57 | 3.2 [2.0-5.2] | <.001 |

Values are expressed as n (%) unless otherwise defined. IFI indicates invasive fungal infection.

* *P* values of the chi-square test, Fisher exact test, or nonparametric Kruskal-Wallis test as appropriate comparing the 4 groups overall.

† Multinomial logistic regression adjusted for matching variables, such as center, age of patient (±5 years), and year of transplant, comparing each group with the control population (reference category).

‡ Solid tumor, bone marrow failure, inherited disorder, histiocytic disorder, and hemoglobinopathy.

§ July, August, and September.

‖ aOR calculated for number of days of neutrophil recovery after transplant > 18 days.

*Multivariate analysis*

The multivariate analysis showed that among the pretransplant factors, only the absence of a complete remission at transplant and an unrelated donor tended to be associated with early IA (Table 4). Clofarabine use was not associated with early IA in the multivariate analysis because of its association with the absence of a complete remission. When the pre- and post-transplant factors were taken into account, the analysis showed that only the absence of engraftment remained significantly associated with early IA. The absence of a complete remission tended to be associated with early IA. The final model had a good calibration (goodness-of-fit test, $P$ = .32) and a moderate discrimination (AUC under ROC curve, .72 [.60 to .84]).

The multivariate analysis (Table 4) showed that among the pretransplant factors, only the absence of ATG-based conditioning tended to be associated with late IA. When the pre- and post-transplant factors were taken into account, only grades III to IV acute GVHD remained associated with late IA. There was a trend for relapse after transplant to be associated with late IA. The final model had good calibration (goodness-of-fit test, $P$ = .33) and a moderate discrimination (AUC under ROC curve, .69 [.58 to .80]).

Among the pretransplant factors the multivariate analysis (Table 4) showed that D−/R+ CMV serology was associated with very late IA. When the pre- and post-transplant factors were taken into consideration, the analysis showed that grade ≥ II acute GVHD, relapse after transplant, and secondary neutropenia were independently associated with very late IA. CMV infections were not associated with very late IA in the multivariate analysis because of their association with GVHD. The final model had good calibration (goodness-of-fit test, $P$ = .54) and discrimination (AUC under ROC curve, .80 [.74 to .85]).

## DISCUSSION

Our study showed that the changes in allogeneic HSCT procedures over time did not change the impact that GVHD has on the onset of IA except for early IA cases, which occur before day 40. It also showed that, currently, 63% of IA cases occur after day 100. These findings were drawn from robust data from 2 prospective registries and the use of a 1:4 ratio of case patients to control patients from the HSCT centers of 19 university hospitals over 6 years. This is the largest study on the occurrence of IA after allogeneic HSCT in Europe.

Our study also showed that the risk of IA after allogeneic HSCT cannot be reliably predicted from pretransplant characteristics and thus should be regularly assessed after transplant. As previously reported from a cohort with a majority of myeloablative-conditioned patients [9], the risk factors of IA in our study varied according to the timing of the onset of IA. The factors intervened after transplant; for example, the occurrence of early IA was associated with the absence of engraftment, late IA was associated with grades III to IV acute GVHD, and very late IA was associated with at least grade II acute GVHD, post-transplant relapse, and secondary neutropenia. Contrary to previous studies [1,9,10], we found that pretransplant factors, such as sex, donor type, stem cell source, and underlying disease, were not associated with the occurrence of IA. Because age was 1 of the matching criteria for choosing the control subjects, it was not studied as a risk factor. In our study the pretransplant factors that showed a trend for an association with early IA were supplanted by the lack of engraftment. The pretransplant factors had less impact on early IA, late IA, and very late IA when the post-transplant factors were added to the model, despite a trend for an association between absence of complete remission and early IA [9,10].

Two-thirds of the cases of IA occurred after day 100 independently of the type of conditioning, which confirmed the increasing trend in delayed onset of IA over the last decades [9,31]. This result suggests that it may be possible to reduce the risk of IA during the first months after transplant but that the risk is underestimated later on. The later occurrence of IA raises major concerns for the transplant community. First, because the clinical and imaging presentation may be different, the diagnosis of IA may be more difficult several months after transplant than during neutropenia [32]. The serum galactomannan may be less often positive in these patients than in neutropenic patients [33], and thus the requirement for bronchoalveolar lavage may increase. However, neutropenic patients and patients with GVHD share the same consensus diagnostic criteria [26]. Moreover, the presence of several types of mold in the bronchoalveolar lavage fluid may be more difficult to interpret in highly immunodepressed patients suffering from severe chronic GVHD than in standard patients [34]. Thus, the current definitions of probable IA, which have been mainly tailored for neutropenic patients, should be refined for patients with GVHD. Second, the late occurrence of IA should prolong the duration of antifungal prophylaxis for months or even years, which raises potential concerns about

**Table 4**
Multivariate Analysis of Pre- and Post-Transplant Factors for Early, Late, and Very Late IA

| | Early IA aOR [95% CI] | $P$* | Late IA aOR [95% CI] | $P$* | Very late IA aOR [95% CI] | $P$* |
|---|---|---|---|---|---|---|
| *Pretransplant factors* | | | | | | |
| Absence of complete remission | 2.4 [.9-6.3] | .07 | | | | |
| Unrelated donor | 2.4 [.9-6.4] | .10 | | | | |
| D−/R+ CMV serology | | | | | 1.6 [1.0-2.5] | .04 |
| ATG-based conditioning | | | .4 [.2-1.0] | .06 | | |
| *Pre- and post-transplant factors* | | | | | | |
| Absence of complete remission | 2.6 [.9-7.2] | .06 | | | | |
| Acute GVHD | | | | | | |
| Grade II | | | 1.2 [.3-4.4] | .76 | 2.6 [1.3-5.3] | .006 |
| Grades III-IV | | | 4.7 [1.8-12.0] | .001 | 6.9 [3.1-15.3] | <.001 |
| Absence of engraftment | 16.4 [2.9-92.6] | .002 | | | | |
| Relapse after transplant | | | 11.4 [.9-142.5] | .06 | 24.1 [7.3-79.6] | <.001 |
| Secondary neutropenia | | | | | 2.2 [1.1-4.1] | .02 |

*$P$ value from multinomial logistic regression adjusted for matching variables, such as HSCT center, age of patient (±5 years), and year of transplant, and for the variables listed in the table comparing each group with the control population (reference category).

resistance, prolonged toxicity, and drug interferences. Studies should now focus on diagnostic criteria of IA in this very specific population and also on prophylaxis approach for very late IA.

Finally, despite the progress in the management of fungal infections over the last 2 decades, IA remains associated with a very poor outcome in cases when compared with control subjects. It can be hypothesized that IA acts both as a deadly infection and as a marker of deep immunosuppression associated with GVHD and other infectious complications. It should be noticed that in our study the poor outcome was not modified by the introduction of posaconazole in 2008.

We acknowledge the following limitations in this study. First, because of its design, some data were not available, including ferritin levels; neutrophil, lymphocyte, or monocyte counts; and post-transplant factors, such as chronic GVHD and steroid use. However, all IA data were prospectively collected in the HSCT centers, and the transplant data were exhaustively registered in the ProMISe registry, which ensured the consecutive patterns of the collected data available for study. Second, we were unable to obtain individual data about antimold prophylaxis. However, posaconazole prophylaxis had become routine in most French HSCT centers for patients with GVHD in the second semester of 2007 after the publication of the study by Ullmann et al. [28]. The ORs adjusted for the period before or after 2008 for IA and the overall survival were unchanged according to this study, and GVHD remained the main risk factor regardless of the time period. Third, we did not explore the genetic risk factors that have been shown to increase the risk of fungal infection after HSCT [35–37]. Other main post-transplant risk factors, such as relapse of the underlying disease or secondary neutropenia, should be taken into account for prophylaxis indication and be considered in future prospective studies. Finally, we did not have any correction for multiple comparisons because it would have eliminated most of the risk factors identified.

In conclusion, in this comparative study we found that in a population of patients mainly conditioned with NMA or RIC, GVHD remained the main risk factor for IA. Among pretransplant factors absence of complete remission tended to be associated with early IA. Clearly, IA cannot be reliably predicted before HSCT. Post-transplant factors have a major impact on IA, especially the lack of engraftment for early IA cases and acute GVHD, relapse, and secondary neutropenia for late and very late cases of IA. Although the presence of GVHD in transplant centers should indicate the need to start antimold prophylaxis, relapse and secondary neutropenia that is often consecutive to the use of ganciclovir should also be considered as major indicators of prophylaxis and the targets of future studies.

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