# UNIVERSITÀ DEGLI STUDI DI TORINO

# Incidence and Outcome of Invasive Fungal Diseases after Allogeneic Stem Cell Transplantation: A Prospective Study of the Gruppo Italiano Trapianto Midollo Osseo (GITMO)

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## Abstract

Epidemiologic investigation of invasive fungal diseases (IFDs) in allogeneic hematopoietic stem cell transplantation (allo-HSCT) may be useful to identify subpopulations who might benefit from targeted treatment strategies. The Gruppo Italiano Trapianto Midollo Osseo (GITMO) prospectively registered data on 1858 consecutive patients undergoing allo-HSCT between 2008 and 2010. Logistic regression analysis was performed to identify risk factors for proven/probable IFD (PP-IFD) during the early (days 0 to 40), late (days 41 to 100), and very late (days 101 to 365) phases after allo-HSCT and to evaluate the impact of PP-IFDs on 1-year overall survival. The cumulative incidence of PP-IFDs was 5.1% at 40 days, 6.7% at 100 days, and 8.8% at 12 months post-transplantation. Multivariate analysis identified the following variables as associated with PP-IFDs: transplant from an unrelated volunteer donor or cord blood, active acute leukemia at the time of transplantation, and an IFD before transplantation in the early phase; transplant from an unrelated volunteer donor or cord blood and grade II-IV acute graft-versus-host disease (GVHD) in the late phase; and grade II-IV acute GVHD and extensive chronic GVHD in the very late phase. The risk for PP-IFD was significantly higher when acute GVHD was followed by chronic GVHD and when acute GVHD occurred in patients undergoing transplantation with grafts from other than matched related donors. The presence of PP-IFD was an independent factor in long-term survival (hazard ratio, 2.90; 95% confidence interval, 2.32 to 3.62; $P < .0001$). Our findings indicate that tailored prevention strategies may be useful in subpopulations at differing levels of risk for PP-IFDs.

## Introduction

Invasive fungal diseases (IFDs), particularly invasive aspergillosis (IA), represent a leading cause of morbidity and mortality in recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14. Ongoing challenges are presented by the continuous evolution of the incidence, timing, and prognosis of IFDs owing to changes in transplant recipient populations and antifungal strategies. Therefore, ongoing epidemiologic investigation is advisable.

Given the poor outcomes associated with IFDs in transplant recipients, there is much interest in identifying risk factors and prognostic factors that may help guide the development of tailored prevention strategies and more aggressive diagnostic and treatment approaches 2, 3, 8, 9, 10, 12, 14 and 15. Although risk factors for IFDs have been evaluated extensively in allo-HSCT recipients, there are few studies describing specific variables related to outcome 1, 2, 8 and 9.

To assess the current incidence, risk factors, and prognostic factors of IFDs in allo-HSCT patients, the Gruppo Italiano Trapianto Midollo Osseo (GITMO) prospectively registered data of patients undergoing allo-HSCT between 2008 and 2010. These data provide the basis for promoting direct efforts in risk stratification, prevention, and management of IFDs in allo-HSCT recipients.

# Methods

The surveillance involved 30 transplantation centers in Italy for a period of 3 years from January 1, 2008, to December 31, 2010. Study start time could differ among the centers, but all consecutive transplants were enrolled. Second and third allo-HSCTs were analyzed as a separate category when appropriate.

Data collection for each case was interrupted at 12 months after transplantation, and thus the study end date was December 31, 2011. Data were entered into electronic case report forms, and all reported results are derived from the database frozen at June 30, 2012. The results of this study are reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement [16]. The study was approved by the Ethical Committee of each participating center, and informed consent was obtained from the patients.

### Data Collection

Variables analyzed included patient characteristics, diagnosis and phase of the underlying disease; previous autologous HSCT (auto-HSCT) or allo-HSCT; proven, probable, or possible IFD documented within the 180 days before transplantation; cytomegalovirus (CMV) serology of donor and recipient; stem cell donor; stem cell source; pretransplantation conditioning regimen; the use of total body irradiation; the use of in vivo T cell depletion with antithymocyte globulin (ATG) or alemtuzumab; and the transplantation activity of the center (with the median number of patients enrolled [n = 48] used to stratify 15 centers with high transplantation activity and 15 centers with low activity). Post-transplantation data included primary and secondary antifungal prophylaxis during the engraftment phase and, in the event of graft-versus-host disease (GVHD), grade II-IV acute GVHD (aGVHD) and extensive chronic GVHD (cGVHD) diagnosed and staged according to standard criteria [17], CMV reactivation, remission status of underlying diseases, proven and probable IFDs (PP-IFDs), survival, and causes of death. Information pertaining to PP-IFDs included timing after allo-HSCT, microbiological data, and site of infection.

### Case Definitions

Cases of PP-IFD were defined according to standard international criteria [18]. The clinical and laboratory data reported to define cases of PP-IFD were reviewed to determine the reliability of the diagnosis. Cases with IFD diagnosed before transplantation were analyzed as a separate category when appropriate. Onset of PP-IFD was defined as the day of the first positive radiologic exam, positive culture, or positive pathological test. PP-IFDs were classified as early (occurring ≤40 days after allo-HSCT), late (occurring 41 to 100 days after allo-HSCT), or very late (occurring 101 to 365 days after allo-HSCT), consistent with previous analyses 9 and 10. The cutoff of 40 days for early PP-IFDs was chosen to ensure that the model reflected the engraftment period. The day 41 to day 100 interval for late PP-IFDs reflects the immediate postengraftment period, the risk of developing aGVHD, and early immunologic recovery. The cutoff of 101 to 365 days for very late PP-IFDs reflects the late or post-aGVHD period, the cGVHD period, and late immunologic recovery.

**Analyses**

The 12-month cumulative incidence of PP-IFDs was calculated accounting for the competing risks of infection-free death, retransplantation, and relapse of the underlying disease. The cumulative incidence of PP-IFDs was calculated for any type and status of the underlying disease; any type of stem cell donor, stem cell source, and pretransplant conditioning; history of IFD before transplantation; type of primary antifungal prophylaxis; aGVHD and cGVHD; and CMV infection/reactivation. Cumulative incidence was assessed using the cmprsk package in R version 2.15 (R Project for Statistical Computing, Vienna, Austria). The associations among early, late and very late PP-IFDs and the foregoing risk factors were evaluated by landmark analysis. All factors were evaluated in multivariate models to control for potential confounders. Crude mortality rates at 100 days after PP-IFD and at 12 months post-transplantation were calculated. Death was attributed to a PP-IFD in patients who failed to respond to therapy (ie, who had stable disease or disease progression) and in patients who demonstrated a partial response to therapy who died as the result of an acute event involving any of the sites of infection and in the absence of any other causes considered to have primarily contributed to death.

Survival analyses were performed on the basis of the foregoing variables. The probability of 1-year survival from allo-HSCT was calculated using the Kaplan-Meier estimate, and the log-rank test was applied for univariate analyses. A 2-sided $P$ value <.05 was considered to indicate statistical significance. Multivariate analyses were performed with the Cox proportional hazards regression model. The variables PP-IFD, aGVHD, cGVHD, and CMV infection/reactivation in the model were considered time-dependent covariates, with their effect evaluated only in the period after their onset. All significant variables in univariate analysis were included in multivariate analysis; final models were also evaluated with backward and stepwise functions. In all, 95% confidence intervals (CIs) are reported for the main summary statistics, and all statistical comparisons were based on 2-tailed tests. Statistical analyses were performed with SAS version 9.2 (SAS Institute, Cary, NC). All comparisons are 2-sided, with a nominal significance level of 5%.

# Results

**Patient Characteristics**

The 1858 allo-HSCTs analyzed in the study included 1771 first allo-HSCTs, 85 second allo-HSCTs, and 2 third allo-HSCTs. Overall, 425 allo-HSCTs (22.9%) were preceded by an auto-HSCT. The median number of transplants per center was 48.5 (range, 9 to 228). Demographic data and patient characteristics at the time of allo-HSCT are summarized in Table 1.

**Table 1**
Characteristics of Patients at Allo-HSCT (n = 1858)

| Characteristic | Value |
|---|---|
| Age, yr, median (range) | 43 (1-72) |
| Age ≤18 yr, n (%) | 205 (11) |
| Male sex, n (%) | 1072 (57.7) |
| Underlying disease, n (%) | |
|   Acute myelogenous leukemia | 685 (36.9) |
|   Acute lymphoblastic leukemia | 325 (17.5) |
|   Biphenotypic acute leukemia | 7 (0.04) |
|   Myelodysplastic syndromes | 119 (6.4) |
|   Chronic myeloproliperative diseases | 142 (7.6) |
|   Non-Hodgkin lymphoma | 192 (10.3) |
|   Hodgkin lymphoma | 90 (4.8) |
|   Chronic lymphocytic leukemia | 48 (2.6) |
|   Aplastic anemia | 68 (3.7) |
|   Multiple myeloma and plasmacellular leukemia | 122 (6.6) |
|   Hemoglobinopaties | 19 (1.0) |
|   Solid tumors | 15 (0.8) |
|   Other diseases | 26 (1.4) |
| Phase of underlying disease at allo-HSCT, n (%) | |
|   Malignancies in complete remission | 977 (52.6) |
|   Malignancies not in complete remission remission/active* | 768 (41.3) |
|   Nonmalignant stable/ chronic diseases | 113 (6.1) |
| Previous HSCT, n (%) | |
|   Auto-HSCT alone | 418 (22.5) |
|   Allo-HSCT (in 7 cases preceded by auto-HSCT) | 87 (4.7) |
| Donor type, n (%) | |
|   HLA-matched related | 849 (45.7) |
|   HLA-mismatched related[†] | 110 (5.9) |
|   Unrelated volunteer | 720 (38.7) |
|   Unrelated cord blood | 179 (9.6) |
| Stem cell source, n (%) | |
|   Bone marrow | 581 (31.3) |
|   Peripheral blood | 1098 (59.1) |
|   Cord blood | 179 (9.6) |
| Pretransplantation conditioning, n (%) | |
|   Myeloablative | 1179 (63.4) |
|   Reduced-intensity | 368 (19.8) |
|   Nonmyeloablative | 679 (36.5) |
| CMV serostatus (n = 1803), n (%) | |
|   Recipient negative/donor negative | 188 (10.4) |
|   Recipient negative/donor positive | 137 (7.6) |
|   Recipient positive/donor positive | 969 (53.7) |
|   Recipient positive/donor negative | 509 (28.2) |
| IFD in the 180 d before allo-HSCT, n (%) | 93 (5.0) |
|   Proven/probable IA | 51 (54.8) |
|   Proven candidosis | 5 (5.4) |
|   Proven/probable mucormycosis | 3 (3.2) |
|   Other proven/probable IFD | 2 (2.1) |
|   Possible IFD | 32 (34.4) |
| Phase of previous IFD at allo-HSCT, n (%) | |
|   Infection in complete remission | 44 (47.3) |
|   Infection not in complete remission | 49 (52.7) |
| Patients enrolled in the centers with the greatest HSCT activity, n (%) | 1381 (74.3) |
| Patients enrolled in the centers with the least HSCT activity, n (%) | 477 (25.7) |

  * Among 1017 patients with acute leukemia, 231 (26.7%) were not in CR at the time of allo-HSCT.
  † The 110 transplants from HLA-mismatched related donors included 72 haploidentical transplants.

## Antifungal Prophylaxis

During the engraftment period, 92 patients who had experienced an IFD before transplantation (4.9%) received secondary antifungal prophylaxis, 1401 patients (75.4%) received primary

antifungal prophylaxis with fluconazole only, 263 (14.1%) received primary antifungal prophylaxis with a mold-active drug, and 102 (5.5%) received no antifungal prophylaxis. Among the 641 patients who experienced grade II-IV aGVHD and/or extensive cGVHD and did not receive antifungal therapy owing to a previous IFD, only 133 (20.7%) received mold-active primary antifungal prophylaxis. Considering that mold-active primary antifungal prophylaxis in patients with GVHD was given in a minority of patients and consisted of various drugs (liposomal amphotericin B, voriconazole, itraconazole, posaconazole, and caspofungin) administered alone or sequentially with heterogeneous start-and-stop timing after transplantation and frequently with intermittent administration, the variable "primary antifungal prophylaxis" administered after engraftment in patients with GVHD was not included in our risk analyses. In contrast, primary antifungal prophylaxis administered during the engraftment period was included in the variables considered for the PP-IFD risk analysis.

**Incidence of PP-IFDs**

Overall, in 164 of 1858 transplants (8.8%), a new PP-IFD or reactivation of a previous IFD was documented before the presence of any competing risk. IA was the most common infection (133 cases; 81.1%), followed by invasive candidiasis (18 cases, 11·0%), zygomycosis (6 cases, 3.7%), fusariosis (3 cases, 1.8%), and infections by other fungi (*Scedosporium* sp, *Scopulariopsis* sp, *Cryptococcus neoformans*, and *Geotrichum capitatum*, 1 case each).

The cumulative incidence of PP-IFDs was 5.1% at 40 days, 6.7% at 100 days, and 8.8% at 12 months from transplantation (Figure 1). One competing risk was present in 769 cases (41.4%). The rate of PP-IFDs at the transplantation centers ranged from 0% to 23.1% (Figure 2). The 12-month cumulative incidence of PP-IFD was 9.9% in the 15 centers with the highest transplantation activity and 5.7% in the 15 centers with the lowest activity ($P = .004$). The cumulative incidence of PP-IFDs according to demographics, underlying disease, and transplantation variables is presented in Table 2.



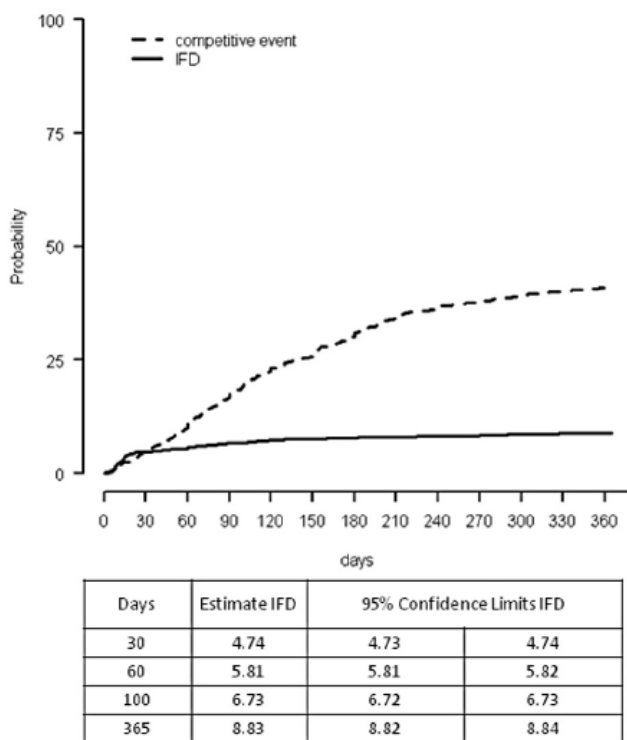| Days | Estimate IFD | 95% Confidence Limits IFD | |
|---|---|---|---|
| 30 | 4.74 | 4.73 | 4.74 |
| 60 | 5.81 | 5.81 | 5.82 |
| 100 | 6.73 | 6.72 | 6.73 |
| 365 | 8.83 | 8.82 | 8.84 |

**Figure 1.** Cumulative incidence curve for IFDs among allo-HSCT recipients in the GITMO epidemiologic survey.
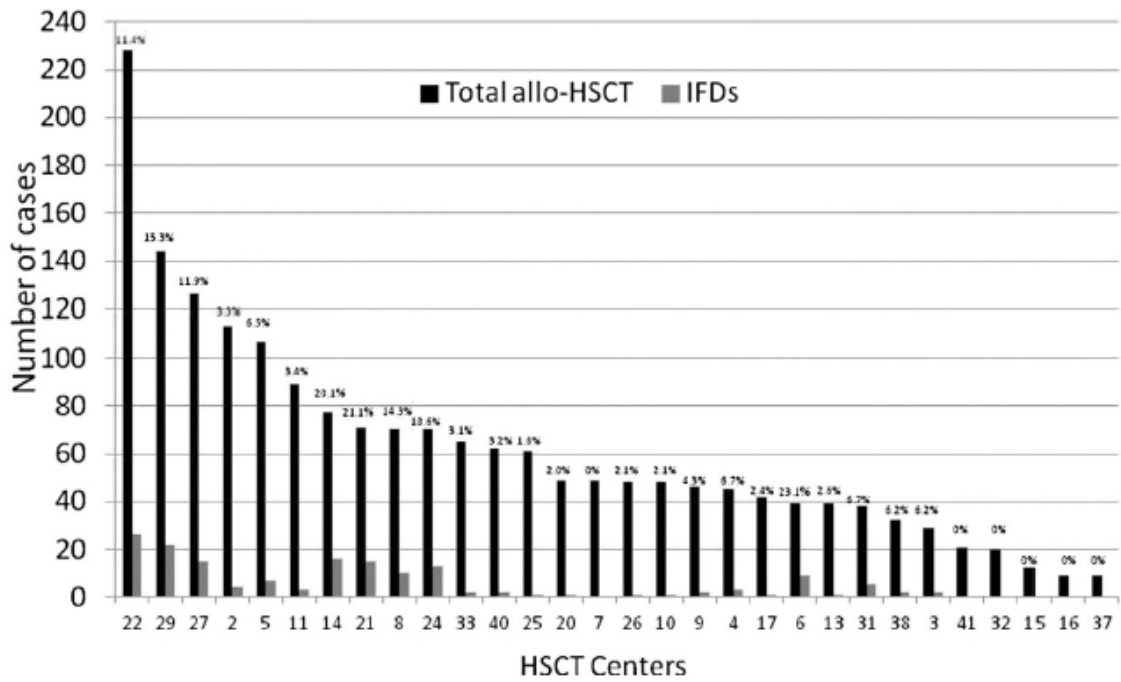
**Figure 2.** Distribution of total allo-HSCTs performed and the number and rate of IFDs in the 30 transplantation centers.

**Table 2**
Cumulative Incidence of IFD at 1 Year According to Demographic, Underlying Disease, and Transplantation Variables

| Variable | Estimated Cumulative Incidence, % | 95% CI | P Value |
|---|---|---|---|
| **Sex** | | | |
| Male | 8.58 | 7.00-10.36 | .66 |
| Female | 9.16 | 7.27-11.31 | |
| **Underlying disease** | | | |
| Acute leukemia | 8.95 | 7.29-10.80 | .82 |
| Other | 8.68 | 6.90-10.71 | |
| **Phase of underlying disease at allo-HSCT** | | | |
| CR | 7.88 | 7.42-9.18 | .02 |
| No CR/chronic | 10.37 | 8.47-12.49 | |
| **Previous HSCT** | | | |
| No | 9.02 | 7.57-10.62 | .44 |
| Yes, autologous alone | 7.66 | 5.36-10.47 | |
| Yes, allogeneic | 11.49 | 5.86-19.22 | |
| **IFD in the 180 d before allo-HSCT** | | | |
| No | 8.27 | 7.05-9.62 | .0001 |
| Yes | 19.35 | 12.03-27.98 | |
| **CMV serostatus** | | | |
| Donor positive, recipient positive | 7.33 | 5.80-9.08 | .001 |
| Donor positive, recipient negative | 7.30 | 3.72-12.47 | |
| Donor negative, recipient positive | 12.97 | 10.22-16.05 | |
| Donor negative, recipient negative | 5.32 | 2.72-9.19 | |
| **Stem cell source** | | | |
| Bone marrow | 9.12 | 6.96-11.64 | <.0001 |
| Peripheral blood | 7.29 | 5.85-8.92 | |
| Cord blood | 17.32 | 12.17-23.23 | |
| **Donor type** | | | |
| HLA-matched related | 4.60 | 3.34-6.17 | <.0001 |
| HLA-mismatched related | 8.18 | 4.00-14.28 | |
| Unrelated volunteer | 11.77 | 9.55-14.25 | |
| Unrelated cord blood | 17.32 | 12.17-23.23 | |
| **Conditioning regimen** | | | |
| Myeloablative | 8.23 | 6.75-9.89 | .24 |
| Nonmyeloablative/ reduced-intensity | 9.87 | 7.77-12.25 | |
| **In vivo T cell depletion** | | | |
| No | 5.32 | 3.93-7.00 | <.0001 |
| Yes | 11.64 | 9.77-13.68 | |
| **Total body irradiation** | | | |
| No | 7.86 | 6.48-9.41 | .03 |
| Yes | 11.07 | 8.64-13.84 | |
| **Mold-active primary antifungal prophylaxis during engraftment** | | | |
| Yes | 4.58 | 4.14-5.48 | .02 |
| No | 8.95 | 7.90-10.15 | |
| **Center HSCT activity** | | | |
| Highest | 9.92 | 8.41-11.57 | .004 |
| Lowest | 5.66 | 3.83-7.99 | |

**Risk Factors for PP-IFD in the Early, Late, and Very Late Phases after Allo-HSCT**

The 164 cases of PP-IFD included 94 (57.3%) early, 39 (23.8%) late, and 31 (18.9%) very late cases. The early phase had the highest rate of IFD irrespective of fungal pathogen (Figure 3). In a landmark analysis, the cumulative incidence of PP-IFDs was 5,1% in the early phase, 1.9% in the late phase, and 2.9% in the very late phase. Risk factors for PP-IFD during each phase after allo-HSCT are shown in Table 3.

**Table 3**
Risk Factors for IFD in 1858 Allo-HSCTs during the Early (Days 1-40), Late (Days 41-100) and Very Late (Days 101-365) Phases

| Variable | Early IFD (n = 94) | | | | Late IFD (n = 31) | | | | Very Late IFD (n = 39) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value | HR (95% CI) | P Value |
| Age | 1.0 (0.99-1.01) | .81 | | | 0.97 (0.95-0.99) | .02 | | | 0.99 (0.97-1.01) | .57 | | |
| Sex, female versus male | 1.06 (0.70-1.59) | .8 | | | 1.88 (0.92-3.83) | .08 | | | 0.70 (0.36-1.35) | .29 | | |
| Underlying disease, acute leukemia versus other | 1.18 (0.78-1.77) | .43 | | | 0.59 (0.29-1.2) | .15 | | | 1.18 (0.63-2.24) | .6 | | |
| Phase of any underlying disease at allo-HSCT, CR versus no CR | 0.51 (0.34-0.78) | .0017 | | | 0.76 (0.38-1.53) | .44 | | | 1.01 (0.53-1.91) | .98 | | |
| Phase of the underlying disease at allo-HSCT | | | | | | | | | | | | |
|   Acute leukemia in CR versus no CR | 0.27 (0.16-0.46) | <.0001 | 0.29 (0.17-0.49) | <.0001 | 1.23 (0.27-5.56) | .78 | | | 1.55 (0.37-6.53) | .55 | | |
|   Other in CR versus acute leukemia no CR | 0.30 (0.13-0.68) | .004 | 0.39 (0.17-0.91) | .03 | 1.96 (0.36-10.68) | .44 | | | 0.64 (0.09-4.52) | .65 | | |
|   Other no CR versus acute leukemia no CR | 0.38 (0.23-0.63) | .0002 | 0.45 (0.27-0.77) | .003 | 2.04 (0.46-8.97) | .35 | | | 1.44 (0.33-6.29) | .63 | | |
| Previous allo-HSCT, no versus yes | 0.51 (0.25-1.06) | .73 | | | 1.34 (0.18-9.83) | .77 | | | 1.62 (0.23-11.6) | .63 | | |
| IFD in the 180 d before allo-HSCT, no versus yes | 0.26 (0.15-0.45) | <.0001 | 0.27 (0.15-0.48) | <.0001 | 0.39 (0.12-1.27) | .12 | | | Not converged | | | |
| CMV serostatus | | | | | | | | | | | | |
|   Donor+/recipient− versus donor+/recipient+ | 1.31 (0.58-2.93) | .51 | | | 1.30 (038-4.44) | .68 | | | Not converged | | | |
|   Donor−/recipient+ versus donor+/recipient+ | 1.98 (1.26-3.09) | .003 | | | 1.25 (0.57-2.75) | .58 | | | | | | |
|   Donor−/recipient− versus donor+/recipient+ | 0.67 (0.27-1.69) | .4 | | | 0.31 (0.04-2.36) | .26 | | | | | | |
| Stem cell source | | | | | | | | | | | | |
|   Peripheral blood versus bone marrow | 1.13 (0.69-1.86) | .6 | | | 0.62 (0.29-1.34) | .23 | | | 0.52 (0.27-1.01) | .053 | | |
|   Cord blood versus bone marrow | 3.23 (1.81-5.77) | <.0001 | | | 1.67 (0.59-4.71) | .34 | | | 0.94 (0.32-2.78) | .91 | | |
| Donor type | | | | | | | | | | | | |
|   Mismatched related versus matched related | 2.37 (0.94-5.92) | .07 | 1.90 (0.75-4.81) | .18 | 1.79 (0.21-15.1) | .59 | 1.41 (0.16-12.14) | .75 | 1.31 (0.30-5.73) | .72 | | |
|   Unrelated volunteer versus matched related | 2.76 (1.64-4.66) | .0002 | 2.86 (1.68-4.84) | .0001 | 5.05 (1.89-13.5) | .001 | 3.73 (1.38-10.02) | .009 | 1.80 (0.90-3.59) | .09 | | |
|   Unrelated cord blood versus matched related | 5.45 (2.98-9.95) | <.0001 | 4.60 (2.43-8.69) | <.0001 | 6.05 (1.75-20.9) | .004 | 4.64 (1.32-16.31) | .017 | 1.83 (0.60-5.54) | .29 | | |
| Myeloablative conditioning, yes versus no | 0.89 (0.59-1.34) | .56 | | | 0.77 (0.38-1.56) | .47 | | | 0.69 (0.37-1.3) | .26 | | |
| T cell depletion in conditioning, yes versus no | 2.53 (1.58-4.04) | .0001 | | | 1.8 (0.85-3.83) | .12 | | | 2.26 (1.13-4.53) | .02 | | |
| Total body irradiation in conditioning, yes versus no | 1.51 (1.0-2.29) | .05 | | | 1.54 (0.75-3.17) | .24 | | | 1.18 (0.61-2.29) | .63 | | |
| Mold-active primary antifungal prophylaxis during engraftment, yes versus no | 0.46 (0.20-1.06) | .06 | | | 0.69 (0.21-2.28) | .54 | | | 0.53 (0.16-1.71) | .29 | | |
| Grade II-IV aGVHD | 0.83 (0.25-2.74) | .76 | | | 6.81 (3.14-14.8) | <.0001 | 5.93 (2.71-12.98) | <.0001 | 5.06 (2.60-9.85) | <.0001 | 3.80 (1.90-7.59) | .0002 |
| Extensive cGVHD | NA | NA | | | NA | NA | | | 5.99 (3.10-11.57) | <.0001 | 3.98 (2.02-7.82) | <.0001 |
| CMV reactivation/infection | 1.07 (0.33-3.54) | .91 | | | 1.86 (0.92-3.79) | .09 | | | 1.85 (0.98-3.50) | .06 | | |
| Center HSCT activity, highest versus lowest | 2.39 (1.31-4.39) | .005 | 1.85 (0.98-3.48) | .06 | 1.88 (0.72-4.91) | .19 | | | 1.08 (0.53-2.20) | .84 | | |

NA indicates not applicable.

**Table 4**

Cumulative Incidence of IFD According to the Variables Identified as Independent Risk Factors during the Early, Late, and Very Late Phases after Allo-HSCT

| Variable | Early IFD | | Late IFD | | Very Late IFD | |
|---|---|---|---|---|---|---|
| | Estimate, % | 95% CI | Estimate, % | 95% CI | Estimate, % | 95% CI |
| Underlying disease and disease phase at allo-HSCT | | | | | | |
|   Acute leukemia in CR | 3.43 | 3.43-3.44 | NS | NS | NS | NS |
|   Acute leukemia not in CR | 12.12 | 12.03-12.21 | | | | |
|   Other disease in CR | 3.78 | 3.74-3.82 | | | | |
|   Other disease not in CR | 4.88 | 4.86-4.89 | | | | |
| Previous IFD in the 180 d before allo-HSCT | | | | | | |
|   No | 4.48 | 4.47-4.48 | NS | NS | NS | NS |
|   Yes | 16.13 | 15.84-16.42 | | | | |
| Donor type | | | | | | |
|   Matched related | 2.47 | 2.46-2.48 | 0.633 | 0.631-0.634 | NS | NS |
|   Mismatched related | 5.45 | 5.36-5.54 | 1.14 | 1.11-1.16 | | |
|   Unrelated volunteer | 6.37 | 6.35-6.39 | 3.17 | 3.16-3.18 | | |
|   Unrelated cord blood | 11.86 | 11.75-11.98 | 3.82 | 3.76-3.87 | | |
| aGVHD grade | | | | | | |
|   0-I | NS | NS | 0.793 | 0.791-0.794 | 1.348 | 1.346-1.351 |
|   II-IV | | | 4.36 | 4.34-4.37 | 6.52 | 6.49-6.55 |
| cGVHD | | | | | | |
|   None or limited | NA | NA | NA | NA | 1.704 | 1.701-1.707 |
|   Extensive | | | | | 8.06 | 8.0-8.12 |

NS indicates not significant; NA, not applicable.

**Risk of PP-IFD in Patients with aGVHD or cGVHD**

After excluding 20 patients who developed a PP-IFD before the occurrence of GVHD, 641 patients who experienced grade II-IV aGVHD and/or extensive cGVHD were included in this analysis. A PP-IFD was documented in 28 of 388 patients (7.2%) with aGVHD not followed by cGVHD, in 25 of 129 patients (19.4%) with aGVHD followed by cGVHD, and in 4 of 124 patients (3.2%) with cGVHD not preceded by aGVHD (ie, "de novo" cGVHD). The median interval from the onset of GVHD to detection of IFD was 38 days for patients with aGVHD not followed by cGVHD, 80 days for patients with aGVHD followed by cGVHD, and 25.5 days for patients with cGVHD not preceded by aGVHD (Figure 4).
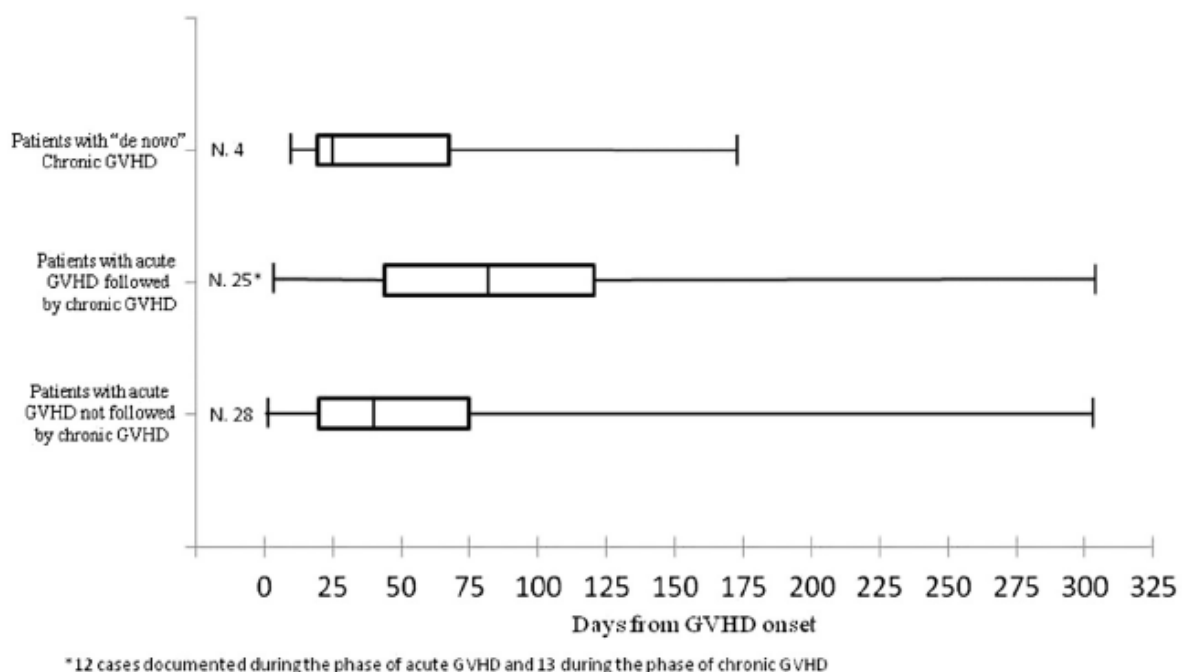


*12 cases documented during the phase of acute GVHD and 13 during the phase of chronic GVHD

**Figure 4.** Distribution of time to IFD stratified by type of GVHD.

The cumulative incidence of PP-IFD, starting from GVHD onset, is shown in Figure 5 according to GVHD group and donor type. In patients with aGVHD not followed by cGVHD, the cumulative incidence of PP-IFD was 2.3% in recipients of allo-HSCT from a matched related donor (MRD) and 10% in recipients of allo-HSCT from an alternative donor (mismatched related donor [MMRD], UD, or CB) (P = .0039). In allo-HSCT recipients with aGVHD followed by cGVHD, the cumulative incidence of PP-IFD was 10% in recipients of an MRD graft and 25.3% in recipients of a graft from an alternative donor (P = .06). In patients with de novo cGVHD, the cumulative incidence of PP-IFD was 3% for the former group 3.5% for latter (P = .90). The cumulative incidence of PP-IFDs at 6 months after onset of GVHD was 7.1% in 334 patients with a grade II aGVHD and 12.3% in 178 patients with grade III-IV aGVHD (P = .049).
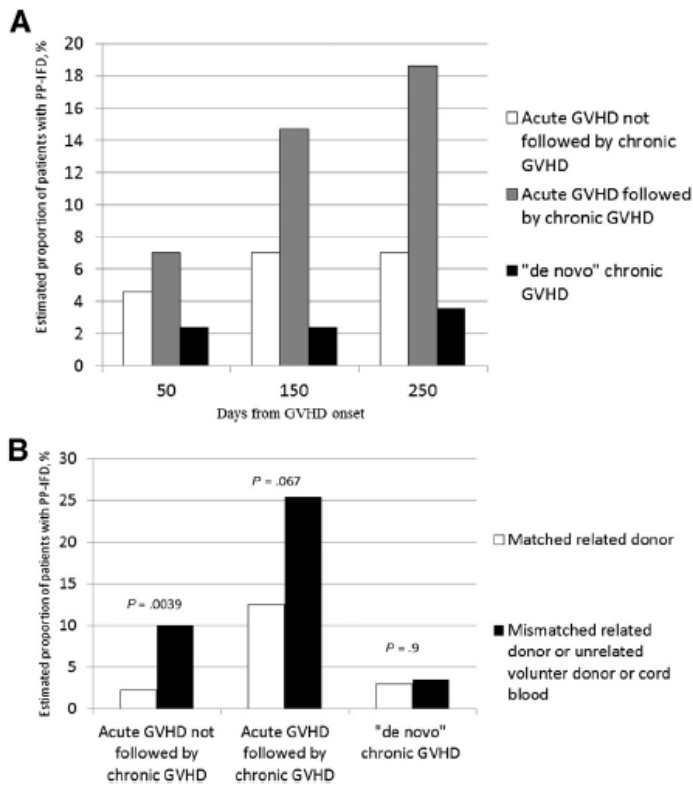
**Figure 5.** Cumulative incidence of IFD from GVHD onset by type of GVHD (A) and by type of donor (B).

## Survival

The mortality rate at 100 days from the diagnosis of PP-IFD was 46.3% (76 of 164 patients). In patients with IA, invasive candidiasis, and non-*Aspergillus*–non-*Candida* infections, it accounted for 48.5%, 39%, and 75%, respectively. Among the 76 patients who died within 100 days from the diagnosis of a PP-IFD, the infection was considered the primary cause of death in 34 (44.7%), with an attributable mortality rate of 20.7%. The probability of survival to 6 months after diagnosis of PP-IFD was roughly 55% in patients in the early, late and very late phases after receipt of allo-HSCT ( Figure 6).
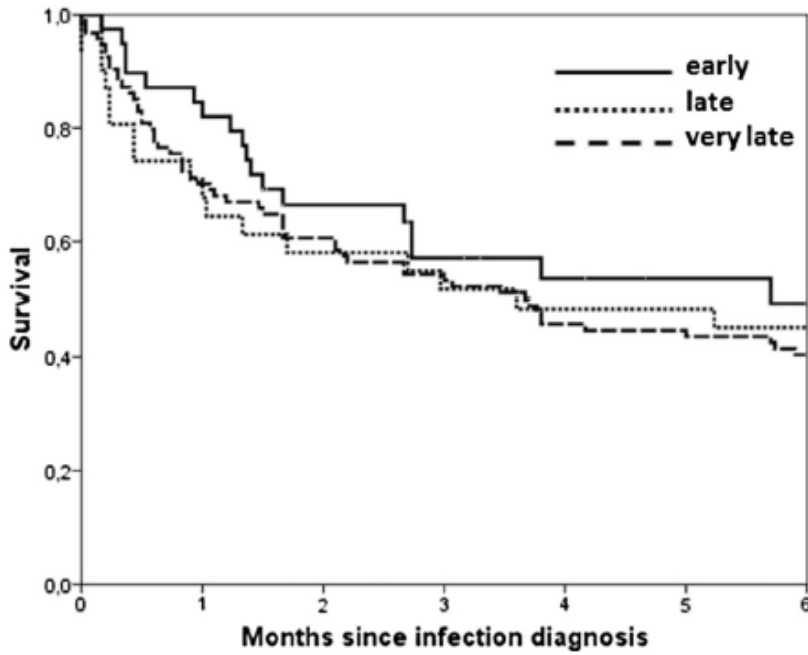
**Figure 6.** Probability of 6-month survival after diagnosis of early PP-IFD, late PP-IFD, and very late PP-IFD.

The overall survival of the entire population at 1 year after allo-HSCT was 64.4% (95% CI, 62.2% to 66.6%). Multivariate analysis identified older age, active acute leukemia at transplantation, IFD in the 180 days before transplantation, receipt of a CB transplant, receipt of a UD transplant, aGVHD, and PP-IFD (hazard ratio [HR], 2.90; 95% CI, 2.32 to 3.62; *P* < .0001) as independent survival factors ( Table 5).

**Table 5**
Probability of Overall Survival at 1 Year from Allo-HSCT

| Variable | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|
| | HR (95% CI) | P Value | HR (95% CI) | P Value |
| Male versus female | 0.99 (0.85-1.16) | .9 | | |
| Age (increased by 10) | 1.12 (1.06-1.17) | <.0001 | 1.14 (1.10-1.19) | <.0001 |
| Underlying disease, acute leukemia versus other | 1.09 (0.93-1.27) | .28 | | |
| Phase of any underlying disease at allo-HSCT, CR versus no CR | 0.56 (0.48-0.66) | <.0001 | | |
| Phase of the underlying disease at allo-HSCT | | | | |
| Acute leukemia in CR versus acute leukemia no CR | 0.29 (0.23-0.35) | <.0001 | 0.36 (0.29-0.45) | <.0001 |
| Other in CR versus acute leukemia no CR | 0.31 (0.23-0.42) | <.0001 | 0.41 (0.30-0.56) | <.0001 |
| Other no CR versus acute leukemia no CR | 0.39 (0.32-0.48) | <.0001 | 0.49 (0.40-0.61) | <.0001 |
| Previous allo-HSCT, no versus yes | 0.59 (0.43-0.80) | .0006 | | |
| Previous IFD in the 180 d before allo-HSCT, no versus yes | 0.49 (0.37-0.65) | <.0001 | 0.57 (0.43-0.76) | 0.0001 |
| CMV serostatus | | | | |
| Donor+/recipient− versus donor+/recipient+ | 0.99 (0.73-1.35) | .94 | | |
| Donor−/recipient+ versus donor+/recipient+ | 1.20 (1.01-1.43) | .04 | | |
| Donor−/recipient− versus donor+/recipient+ | 0.85 (0.64-1.12) | .27 | | |
| Stem cell source | | | | |
| Peripheral blood versus bone marrow | 1.11 (0.93-1.32) | .24 | | |
| Cord blood versus bone marrow | 1.77 (1.37-2.29) | <.0001 | | |
| Donor type | | | | |
| Mismatched related versus matched related | 2.04 (1.52-2.74) | <.0001 | 1.81 (1.34-2.44) | <.0001 |
| Unrelated volunteer versus matched related | 1.40 (1.18-1.66) | .0001 | 1.34 (1.13-1.60) | <.0001 |
| Unrelated cord blood versus matched related | 2.02 (1.58-2.59) | <.0001 | 1.81 (1.40-2.35) | <.0001 |
| Myeloablative conditioning, yes versus no | 0.87 (0.75-1.02) | .09 | | |
| T cell depletion in conditioning, yes versus no | 1.25 (1.07-1.46) | .004 | | |
| Total body irradiation in conditioning, yes versus no | 0.98 (0.83-1.16) | .79 | | |
| Center HSCT activity, highest versus lowest | 1.08 (0.91-1.29) | .39 | | |
| Grade II-IV aGVHD | 1.58 (1.34-1.86) | <.0001 | 1.46 (1.23-1.72) | <.0001 |
| Extensive cGVHD | 1.69 (1.31-2.19) | <.0001 | | |
| CMV reactivation/infection | 1.46 (1.24-1.72) | <.0001 | | |
| PP-IFD after allo-HSCT, yes versus no | 3.89 (3.14-4.79) | <.0001 | 2.90 (2.32-3.62) | <.0001 |

# Discussion

Few large, prospective surveillance studies on IFDs in allo-HSCT recipients have been published to date 12 and 13. A study from the sentinel surveillance system of the Transplant-Associated Infections Surveillance Network (TRANSNET) evaluated the incidence and burden of IFDs in 6666 allogeneic HSCTs performed between March 2001 and March 2006 in 23 transplantation centers (representing approximately 20% of HSCT recipients in the United States during the study period) with the aim of estimating the cumulative incidence of PP-IFDs and collecting clinical, diagnostic, and outcome information for each reported infection [12]. A study by the Prospective Antifungal Therapy (PATH) Alliance registry collected data and monitored trends in epidemiologic characteristics, diagnosis, treatment, and outcomes of 234 allo-HSCT recipients with PP-IFD observed in 23 centers in the United States and Canada between July 2004 and September 2007 [13].

The present GITMO survey, which includes approximately 40% of allo-HSCTs performed in Italy during the years 2008 to 2010, was a prospective study designed to critically assess not only the incidence, but also the risk factors for and prognostic role of PP-IFDs at 1 year from allo-HSCT. A

valuable characteristic of this study is the availability of complete denominator data collected prospectively for consecutive allo-HSCT recipients at each center.

The overall 12-month cumulative incidence of PP-IFDs was 8.8%. Of these PP-IFDs, 81% were caused by IA and 11% were caused by *Candida* species, with other infections relatively uncommon. The incidence of PP-IFDs by allo-HSCT graft source was 4.6% for MRD, 8.2% for MMRD, 11.8% for UD, and 17.3% for CB. These results are comparable in some respects to those reported in the TRANSNET survey, with 12-month cumulative incidences of 5.8% for MRD, 8.1% for MMRD, and 7.7% for UD (with specific data for the few CB transplants not reported) [12]. The incidence of PP-IFDs among centers ranged from 0% to 23%, with the highest incidences in the larger centers. A potential center effect may represent a limit of our survey and of any multicenter trial. To evaluate the potential role of the different clinical care habits, we stratified the centers according to transplantation activity, which may reflect the different types of transplants and care capabilities. Multivariate analysis did not identify transplantation activity as an independent risk factor, although it was borderline significant in the early phase after allo-HSCT. The site-specific variation in the incidence of PP-IFDs may reflect differences in patient populations, transplantation programs, and diagnostic accuracy, particularly for mold infections.

The timing of PP-IFDs in our study was rather unexpected: indeed, early infections represented 57.3% of all infections documented during the first year after allo-HSCT. This result contrasts with previous studies in which the vast majority of cases were documented after the engraftment period 8 and 12. Several factors may explain the high incidence of PP-IFDs during the early transplantation phase in our series. Transplant with a UD or CB graft, active acute leukemia at the time of transplantation, and an IFD before transplantation were found to be independent factors for the development of an early PP-IFD. One or more of these conditions were present in more than one-half of the patients in our study, in line with the increasing use of transplants from alternative donors and a trend toward less-restrictive eligibility criteria worldwide. The high risk for early IFDs in patients who receive a transplant from an alternative donor, particularly CB, or in those with a history of infection before transplantation is well known 5, 6, 7, 10 and 19; however, the high rate of early PP-IFDs in patients with active acute leukemia at the time of transplantation is an original finding. The severe immunologic impairment associated with active disease, multiple intensive chemotherapy treatments, and late engraftment frequently seen in these patients may justify the high early post-transplantation risk of PP-IFD 20, 21 and 22.

GVHD represents a known risk factor for IFD, and mold-active primary antifungal prophylaxis is recommended in patients with GVHD while receiving initiation of immunosuppressive therapy 2, 23 and 24. Our study confirms that aGVHD and cGVHD are independent risk factors for the development of late and very late PP-IFDs. The subanalysis of patient populations with different types and severity of GVHD and different donors revealed a significantly different risk profile, however. The cumulative incidence of PP-IFDs was high in patients with aGVHD developing after transplantation from an alternative donor, and was even higher when aGVHD was followed by cGVHD regardless of donor type, up to 25% in recipients of an alternative donor graft. Of interest, the cumulative incidence of PP-IFDs was relatively low (<4%) in patients with de novo cGVHD regardless of donor type. As expected, the risk for PP-IFD was significantly higher in patients with grade III-IV aGVHD compared with those with grade II aGVHD [2].

In this study, we were not able to evaluate the impact of mold-active primary antifungal prophylaxis on the epidemiology of post-transplantation PP-IFDs either during the engraftment period or in the event of GVHD, likely related to the wide variability in prophylaxis according to clinical indication, type of antifungal drug, and timing and duration of administration. The real life data from our study demonstrate the difficulty of applying the guidelines for antifungal prophylaxis in the various

clinical settings of allo-HSCT, and suggest the need for risk- and time-adapted indications 14 and 24.

Reported mortality rates in allo-HSCT recipients with IFD have been as high as 80% 1, 13 and 25; however, more recent data seem to show improving outcomes, particularly for IA, possibly related to the extensive use of voriconazole 8, 15 and 26. In the PATH Alliance study, the overall mortality rate at 12 weeks after documented IA was 35.5% [13], compared with the 48.5% rate in our study. Of interest, mortality attributed to PP-IFD was 20.7%, confirming the improvement in therapeutic approaches to these infections with favorable impacts on short-term outcome.

Whereas in previous studies, outcomes of IFDs were usually evaluated at 3 months after transplantation, we chose to evaluate outcomes at 1 year. In our study, in addition to the well-known factors predicting poor outcome, including older age, active acute leukemia at transplantation, IFD before transplantation, alternative donor graft, and aGVHD, PP-IFDs represent an independent prognostic factor for survival. This finding suggests that although PP-IFDs may be cured or controlled in most cases, they are of poor prognostic significance, likely because of the underlying condition that favored the development of the infection, and also because of the possible interference of IFD with the management of other post-transplantation complications, such as GVHD, viral infection, and hematologic relapse.

In conclusion, our study has identified important risk factors for PP-IFD during the preengraftment and postengraftment phases in a real life allo-HSCT scenario. The demonstration of an independent impact of these complications on transplantation outcomes suggests the need for changes in supportive care strategies to decrease the burden of IFD in allo-HSCT recipients. Of relevance, our experience shows an absence of consensus in the practice of antifungal prophylaxis in the allo-HSCT community, likely related to the lack of specific epidemiologic information. Our present findings may be useful in identifying the subpopulations of allograft recipients who might benefit from targeted prevention strategies.

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