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Risk factors and attributable mortality of late aspergillosis after T-cell depleted hematopoietic stem cell transplantation

C.N. Mihu, E. King, O. Yossepovitch, Y. Taur, A. Jakubowski, E. Pamer, G.A. Papanicolaou. Risk factors and attributable mortality of late aspergillosis after T-cell depleted hematopoietic stem cell transplantation.
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Abstract: *Aim.* Invasive aspergillosis occurs in 5–15% of allogeneic hematopoietic stem cell transplant (HSCT) recipients. Through the 1990s there has been an increase in the incidence of late aspergillosis (LA). We report on the incidence, risk factors, and attributable mortality of LA in a cohort of 398 adult and pediatric patients at Memorial Sloan-Kettering Cancer Center from January 1999 through December 2003.
Methods. LA was defined as occurring >40 days post HSCT. LA cases were identified by prospective surveillance and examination of a computerized database. Probable or definite aspergillosis was defined by standard EORTC/MSG criteria. Mortality was attributed to LA if it caused or significantly contributed to death.
Results. The overall incidence of LA in our cohort was 4.1%. Median time from stem cell infusion to diagnosis of LA was 164 days (range 68–677) after HSCT. The incidence of LA among unmodified, T-cell depleted, or reduced intensity HSCT was 2.2%, 4%, and 6.8%, respectively (P not significant). Risk factors for LA were grade II–IV acute graft-versus-host disease (GVHD) ($P = 0.002$), chronic GVHD ($P = 0.01$), secondary neutropenia ($P = 0.02$), and reduced intensity conditioning containing alemtuzumab ($P = 0.01$). LA was the immediate cause of death in 1 of 10 (10%) T-cell depleted, 2 of 2 (100%) unmodified, and 1 of 4 (25%) of reduced-intensity HSCT.
Conclusions. LA developed a median 164 days post HSCT. All-cause 30-day mortality of LA was 56.3%. The majority of LA cases died of concurrent infections and not from invasive aspergillosis.

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Key words: late aspergillosis; risk factors; mortality; T-cell depleted; allogeneic stem cell transplantation

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The incidence of invasive aspergillosis after allogeneic hematopoietic stem cell transplantation (HSCT) varies between 5% and 15% (1). The majority of cases occur after neutrophil engraftment. T-cell depletion of the graft has been associated with increased incidence of aspergillosis (2).

At our institution approximately two-thirds of HSCT patients receive T-cell depleted allografts. The methods used for T-cell depletion typically yield $<10^3$ T-cells/kg of patient's weight, a cut-off that has been demonstrated to be protective against graft-versus-host disease (GVHD). Complete T-cell depletion has been associated with delayed immune reconstitution and increased incidence of viral infections (3).

We report on the incidence, risk factors, and outcome of late aspergillosis (LA) in a cohort of 398 adult and pediatric patients who underwent HSCT at Memorial Sloan-Kettering Cancer Center.

Patients and methods

Patients

The study was approved by the Memorial Sloan-Kettering Cancer Center Institutional Review Board. Between January 1, 1999 and December 31, 2003, 432 consecutive patients

underwent HSCT. Excluded from the analysis were 34 patients with hereditary non-malignant disorders. The cohort consists of 398 consecutive adult and pediatric patients who underwent HSCT for malignant disorders. Patients who developed invasive fungal infections were identified by prospective surveillance and examination of a computerized database. Aspergillosis cases were confirmed by examination of histopathology and microbiology reports and medical record review. All patients were followed from stem cell infusion to development of aspergillosis, second HSCT, relapse, death, 365 days post HSCT or end of study (December 31, 2004), whichever occurred first.

Definitions

LA was defined as occurring >40 days post HSCT. Aspergillosis was defined by standard EORTC/MSG criteria (4). Only patients who met criteria for probable or definite aspergillosis are included in the analysis. GVHD was graded by standard criteria (5). Secondary neutropenia was defined as at least 2 values of absolute neutrophil count $<1000/mm^3$ within a week, after having achieved neutrophil engraftment.

Standard criteria were used for definition of infections other than aspergillosis. Diagnosis required demonstration of the organism in tissue from biopsy or autopsy specimen or culture of the organism from a sterile site with compatible clinical symptoms. Disseminated disease was defined as involvement of 2 organs. Cytomegalovirus (CMV) was diagnosed by viral inclusions and positive immunostaining for CMV in the affected organs. Toxoplasmosis was diagnosed by the characteristic appearance of the organisms in tissue.

Method of T-cell depletion

Peripheral blood stem cells were $CD34^+$ selected by the Isolex 300i (Miltenyi Biotech Inc., Auburn, California, USA) and further depleted of T cells by sheep erythrocyte rosetting. The median number of CD3/kg was 0.14×10^4 (range 0–7.4). Bone marrow-derived stem cells were depleted of T cells by soybean agglutination and sheep erythrocyte rosetting. The median number of CD3/kg was 3.5×10^4 (range 0.3–183).

Infection prophylaxis

Standard care for prevention of candidiasis included fluconazole (400 mg/day) until day 30 after HSCT.

Patients at high risk of invasive aspergillosis received mold-active prophylaxis. These patients included those with a history of fungal sinusitis, pneumonia, or hepatic/splenic abscesses, or prolonged course of corticosteroids (defined as >1 mg/kg of methylprednisolone or equivalent for more than 3 weeks), or chronic GVHD disease on systemic immunosuppression. From January 1999 through September 2001, prophylaxis consisted of liposomal amphotericin B at 3 mg/kg for 3–5 times weekly. Itraconazole suspension (200 mg daily) was used for patients intolerant to polyenes. After September 2001, first-line prophylaxis consisted of oral voriconazole 200 mg twice a day. Caspofungin or micafungin were used for patients intolerant to voriconazole.

Prophylaxis was continued until all immunosuppressive medications were discontinued and the patient had a CD4 count $>200/mm^3$ and a phytohemagglutinin response at least 50% of normal.

Pneumocystis jiroveci prophylaxis was provided with aerosolized pentamidine. Acyclovir was used for herpes simplex virus and varicella zoster virus prophylaxis. Patients who received HSCT from mismatched related or unrelated donors received ganciclovir for CMV prophylaxis from engraftment until day $+100$ post HSCT, if donor or recipient were CMV seropositive. All other patients were monitored for CMV reactivation by the pp65 antigenemia assay and treated preemptively.

Statistical analysis

Simple logistic regression was used for the bivariate analysis. The following variables were examined: age, gender, donor type (matched related sibling, mismatched related, unrelated identical, and unrelated non-identical), stem cell source (peripheral blood versus bone marrow), conditioning regimen (total body irradiation [TBI] versus non-TBI containing regimens), and GVHD requiring systemic immunosuppressants. The χ^2 test was used to look at associations between univariate variables.

Stepwise multiple logistic regression analysis was used for multivariate analysis. Kaplan–Meier survival analysis was used to examine comparative survival time. A P value ≤ 0.05 was considered significant in all cases. Stata software (version 7) was used for statistical analysis.

Results

Table 1 shows the baseline characteristics of the cohort. The majority of patients received a myeloablative conditioning regimen. Sixty-three percent received a T-cell depleted HSCT.

Baseline characteristics of the cohort of 398 hematopoietic stem cell transplant recipients

Characteristic	Number (%)
Median age (range), years	34.2 (0.5–67.9)
Male gender	231 (58.0)
Diagnosis	
Acute lymphocytic leukemia	67 (16.8)
Acute myelogenous leukemia	122 (30.7)
Chronic myelogenous leukemia	46 (11.6)
Lymphoma	70 (17.5)
Myelodysplastic syndrome	52 (13.1)
Aplastic anemia	10 (2.6)
Other[1]	31 (7.7)
HLA match	
Matched-related donor	21 (52.8)
Mismatched-related donor	32 (8.0)
Matched-unrelated donor, identical	103 (25.9)
Matched-unrelated donor, non-identical	53 (13.3)
Stem cell source	
Peripheral blood	180 (45.3)
Bone marrow	218 (54.8)
T-cell depletion	
Unmodified	90 (22.6)
T-cell depleted	249 (62.5)
Conditioning regimen	
Myeloablative	
Containing total body irradiation (TBI)	235 (69.3)
Non-TBI, all chemotherapy	104 (30.7)
Non-myeloablative	59 (14.8)

[1]Multiple myeloma, melanoma, renal cell carcinoma.

Table 1

Characteristics of 16 cases with late aspergillosis

Characteristic	Number	%
Certainty of diagnosis		
Definite	10	62.5
Probable	6	37.5
Method of diagnosis		
Cytology/histopathology	10	62.5
Culture	13	81.2
Cytology and culture	7	43.8
Microbiology	13	81.2
Aspergillus fumigatus	9	56.2
Aspergillus flavus	3	18.7
Aspergillus terreus[1]	2	12.5
Sites of disease		
Lung	15	93.7
Skin	2	12.5
Radiographic appearance[2]		
Nodules	9	69.2
Mass	3	23
Opacity	11	84.6
Other[3]	8	61.5

[1]*Aspergillus terreus* was found along with *Aspergillus fumigatus* in 1 patient.
[2]Thirteen patients had computerized tomography of the chest, 2 patients had chest x-ray.
[3]Ground glass opacities, pleural effusions, reticulonodular pattern.

Table 2

Incidence and timing of invasive aspergillosis

During the study period, 22 (5.52%) patients developed aspergillosis. The incidence of early aspergillosis and LA was 1.5% and 4.1%, respectively. There was no variation in the annual or seasonal incidence of aspergillosis. LA occurred at a median 164 days (range 68–677) post HSCT.

Table 2 shows the microbiology and radiographic characteristics of the 16 cases of LA. Two-thirds of the cases met criteria for definite aspergillosis. Ten patients had fungal elements observed at cytology or histopathology (7/10 also had positive culture for *Aspergillus* spp.). In the remaining 6 patients, *Aspergillus* was recovered in culture from skin biopsy, pleural fluid, sputum, or endotracheal aspirate. The lung was the site of LA in 15 of 16 (94%) cases.

Aspergillus fumigatus accounted for the majority of isolates (69.2%). *Aspergillus terreus* accounted for 15%. One patient had co-infection with *A. fumigatus* and *A. terreus*.

Twelve of 16 (75%) patients had nodules or mass on computerized tomography of the chest or chest x-ray. We did not observe the 'halo sign' in any of our patients.

Ten of 16 (62.5%) cases of LA occurred in T-cell depleted HSCT. The median number of days from T-cell depleted HSCT to diagnosis of LA was 218 days (range 92–677). Figure 1 shows the cumulative incidence of LA in T-cell depleted HSCT.

Risk factors for LA

We compared the characteristics of 16 patients with LA to 376 patients who did not develop early aspergillosis or LA. LA was associated with acute GVHD, chronic GVHD, secondary neutropenia, and reduced intensity conditioning containing alemtuzumab (Table 3). T-cell depletion of the graft was not associated with LA. There was no association

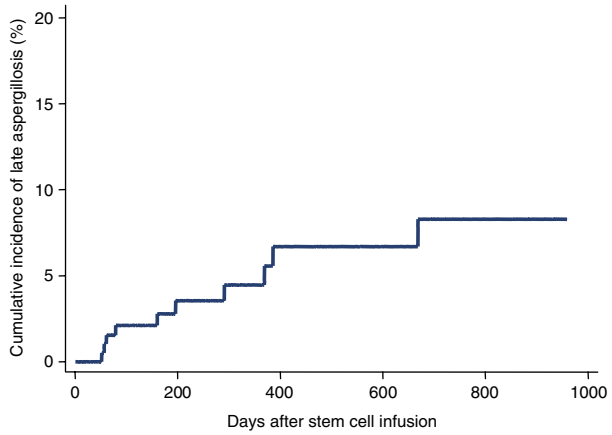

Fig. 1. Cumulative incidence of late aspergillosis in T-cell depleted hematopoietic stem cell transplantation.

of LA with age at the time of HSCT, gender, underlying disease, conditioning regimen, donor type, or stem cell source.

In multivariate analysis, acute GVHD (95% confidence interval [CI]: 1.19–14.4, $P = 0.03$) and conditioning regimen containing alemtuzumab (CI: 1.61–13.2, $P = 0.004$) remained significant risk factors for LA.

Fifteen of 16 cases of LA occurred in adult HSCT recipients. We conducted the univariate and multivariate analysis for adult HSCT recipients only. In this subgroup, risk factors associated with LA were the same as for the entire cohort. Owing to the very small number of pediatric cases, we could not perform the analysis for the pediatric patients only.

Univariate analysis of risk factors for late aspergillosis (LA)

Characteristic	LA (total, $n = 16$), n (%)	No LA (total $n = 376$), n (%)	OR	P
T-cell depletion	9 (56.3)	236 (62.8)	0.76	0.60
Conditioning				
Ablative				
TBI-containing	10 (62.5)	222 (59.0)	1.16	0.78
All chemotherapy	1 (6.3)	108 (28.7)	0.16	0.08
Non-ablative				
With alemtuzumab	4 (25.0)	24 (6.4)	4.89	0.01
Without alemtuzumab	1 (6.3)	22 (5.9)	1.07	0.94
Graft-versus-host disease				
Acute grade 2–4	7 (43.8)	50 (13.3)	5.07	0.002
Chronic	10 (62.5)	52 (13.8)	3.73	0.001
Secondary neutropenia	6 (37.5)	57 (15.2)	3.35	0.02

n, number; OR, odds ratio; TBI, total body irradiation.

Table 3

Attributable mortality of LA

Diagnosis was established antemortem in 14 of 16 (87.5%) patients. Autopsy was performed in 8 (50%) cases. In addition, 3 cases had lung biopsy within 2 weeks of their death. All-cause mortality at 30 and 90 days after diagnosis of LA was 56.3% and 69.9%, respectively.

Table 4 shows the immediate cause of death for the 16 cases of LA by type of conditioning regimen (T-cell depleted, unmodified, and reduced-intensity).

LA was the immediate cause of death in one of 10 (10%) cases of LA in the T-cell depleted group (case #5). Disseminated aspergillosis was diagnosed at autopsy. In addition, the patient had cryptococcal meningitis and cerebral toxoplasmosis detected at autopsy. Two patients died with sepsis (case #1 with concomitant veno-occlusive disease and case #3 with purulent pericarditis with tamponade). Four patients died of bacterial pneumonia (cases #6, #8, #9, #10). One patient had aspiration pneumonia following seizures (#6). Three patients (#8, #9, #10) had bacterial pneumonia diagnosed antemortem with lung biopsy within 2 weeks of death (no *Aspergillus* was detected in the biopsy specimen). The remaining 3 patients died of disseminated CMV infection (#2), disseminated toxoplasmosis (#4), and respiratory syncytial virus pneumonia (#7).

Two of 2 (100%) of LA cases in unmodified HSCT died of invasive aspergillosis detected in autopsy (#11, #12). Both cases had concomitant serious non-infectious complications of HSCT (acute GVHD #11, Epstein–Barr virus [EBV] lymphoma #12).

None of 4 cases in reduced-intensity conditioning died of aspergillosis (#13, #14, #15, #16). Aspergillosis may have contributed to death in a patient with concomitant bacterial sepsis, CMV infection, and adenoviral infection (#14).

Discussion

Invasive aspergillosis is considered a highly lethal complication of HSCT with case fatality rates approaching 80%. A shift in the timing of aspergillosis to a later period post HSCT has been observed over the last 10 years (2, 6). Shorter periods of pre-engraftment neutropenia may partially account for the decline in early aspergillosis. Longer survival due to overall improved supportive care may account for the increase in LA. Furthermore, the availability of voriconazole and the echinocandins as prophylaxis or treatment may be influencing the incidence and outcome of aspergillosis.

At our institution we use T-cell depletion for GVHD prophylaxis in more than 60% of HSCT. T-cell depletion has

Cause of death of cases of late aspergillosis

Case #	Age, sex	HSCT type	Certainty	Time to Dx (days)[1]	Cause of death	Autopsy	Time to death (days)[2]	GVHD[3]	Secondary neutropenia
1	43, M	TCD	Definite	92	Sepsis, veno-occlusive disease	Limited	3	No	No
2	24, M	TCD	Probable	97	Disseminated CMV	Yes	18	No	Yes
3	50, M	TCD	Definite	101	Sepsis/tamponade		100	Chronic	No
4	40, F	TCD	Definite	119	Disseminated toxoplasmosis	Yes	0	No	No
5	32, M	TCD	Definite	200	Aspergillosis	Yes	7	Chronic	No
6	15, M	TCD	Probable	236	Bact. pneumonia		2	Chronic	No
7	36, F	TCD	Probable	311	Viral pneumonia		19	Chronic	No
8	47, F	TCD	Probable	409	Bact. pneumonia		0	Chronic	Yes
9	43, F	TCD	Probable	426	Bact. pneumonia	Yes	120	Chronic	Yes
10	23, F	TCD	Probable	677	Bact. pneumonia		115	Chronic	No
11	53, M	Unmodified	Definite	127	Aspergillosis	Yes	16	Acute	No
12	33, M	Unmodified	Definite	128	Aspergillosis, EBV lymphoma	Yes	36	No	Yes
13	53, M	Reduced intensity	Definite	68	Bact. pneumonia		288	Chronic	
14	35, M	Reduced intensity	Definite	84	Sepsis, aspergillosis		21	No	Yes
15	55, M	Reduced intensity	Definite	208	Sepsis, malignancy	Yes	55	Chronic	No
16	63, M	Reduced intensity	Definite	251	Alive			Chronic	No

[1]Time from stem cell infusion to LA diagnosis (Dx).
[2]Time from diagnosis of LA to death.
[3]Active graft-versus-host disease (GVHD) at time of diagnosis of LA.
M, male; F, female; HSCT, hematopoietic stem cell transplantation; TCD, T-cell depletion; CMV, cytomegalovirus; Bact., bacterial; EBV, Epstein–Barr virus.

Table 4

been associated with prolonged and profound defect in T-cell mediated immunity and increased incidence of infections, particularly viral infections (7, 8). The importance of T cells for defense against fungal infections has been demonstrated recently in animal models and clinical studies (2).

In our cohort, the majority of aspergillosis occurred in the late post-transplant period. When we compared the incidence of LA among the unmodified, T-cell depleted, or reduced-intensity HSCT, we did not find any significant difference (2.2%, 4%, and 6.8%, respectively). Similar rates of aspergillosis have been reported by others. A retrospective study comparing serious infections among unmodified, T-cell depleted, and cord blood HSCT did not find significant difference in the incidence of fungal infections among the three groups during a 2-year follow up (3). The majority of our reduced-intensity HSCT patients received alemtuzumab, which has been associated with increased rates of viral and fungal infections by others (8, 9).

We focused our attention to T-cell depleted HSCT since the majority of LA cases occurred in these patients. The 2-year cumulative incidence of LA was 7%. Interestingly, the median number of post-transplant days to the diagnosis of LA tended to be greater in T-cell depleted HSCT, with approximately 50% of cases diagnosed later than 6 months after HSCT.

In terms of radiographic appearance, the majority of patients presented with nodules or mass on computerized chest tomography. However, the 'halo sign' – a hallmark of early aspergillosis – was not observed in any of our cases. This is in agreement with recent reports that suggest that as aspergillosis evolves, radiographic presentation may include non-specific findings such as consolidation or interstitial infiltrates (10, 11). In terms of microbiology *A. fumigatus* was the most common isolate (69%). *A. terreus* accounted for 15% of the isolates. The majority of LA cases had received mold-active prophylaxis within 1 month of diagnosis. This brings up several interesting points. First, LA occurred almost exclusively in the high-risk patients who met criteria for mold-active prophylaxis. Second, it is plausible that prophylaxis may have contributed to the indolent course of aspergillosis.

LA in our cohort was associated with substantial mortality. Approximately two-thirds of cases died within 3 months of diagnosis. This number is consistent with previous studies that report case fatality rates of up to 80% for aspergillosis in general. We asked the question of whether

LA directly caused death in our patients. We show that the majority of patients did not die as a consequence of aspergillosis but from concomitant complications of HSCT. Among T-cell depleted HSCT patients, the attributable mortality from *Aspergillus* was only 10%. The most common causes of death were other infections (bacterial 6, viral 2, toxoplasmosis 1). Among those with reduced-intensity HSCT, *Aspergillus* possibly contributed to death in 1 of 4 (25%) patients. There were only 2 cases of LA in unmodified HSCT. Both cases had documented invasive aspergillosis at autopsy. Of note, 1 patient also had EBV lymphoma and the other had grade III GVHD. Both complications are associated with extremely high mortality.

In summary, LA in the era of the highly active antifungals may be a marker of profound immunosuppression. In the majority of cases, LA was indolent. The overall 30-day mortality of LA was in excess of 50%. However, the vast majority of patients did not die of aspergillosis. We postulate that LA may be a distinct entity within the general term of invasive aspergillosis.

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