Increased Bone Marrow Iron Stores Is an Independent Risk Factor for Invasive Aspergillosis in Patients With High-Risk Hematologic Malignancies and Recipients of Allogeneic Hematopoietic Stem Cell Transplantation

Dimitrios P. Kontoyiannis, MD[1]
Georgios Chamilos, MD[1]
Russell E. Lewis, PharmD[1]
Sergio Giralt, MD[2]
Jorge Cortes, MD[2]
Issam I. Raad, MD[1]
John T. Manning, MD[3]
Xin Han, MD[3]

[1] Department of Infectious Diseases, Infection Control, and Employee Health, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[2] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[3] Department of Leukemia, Pathology, and Laboratory Medicine, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

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Address for reprints: Dimitrios P. Kontoyiannis, MD, Department of Infectious Diseases, Infection Control, and Employee Health, Unit 402, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030; Fax: (713) 745-6839; E-mail: dkontoyi@mdanderson.org

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BACKGROUND. Invasive aspergillosis (IA) is a leading cause of death in patients with leukemia and those who have undergone hematopoietic stem cell transplantation. Laboratory studies have demonstrated that iron is essential for *Aspergillus* growth and virulence.

METHODS. In the current study, the authors retrospectively evaluated the bone marrow iron stores (BMIS) in patients with leukemia as well as recipients of allogeneic hematopoietic stem cell transplantation with IA (n = 33) and those without fungal infections (n = 33). The first available bone marrow biopsy specimens prior to the IA diagnosis or date of hospitalization (control group) were assessed in a blinded fashion using a standardized scoring system (0–4). Both groups were comparable with regard to clinical characteristics and classic risk factors for IA.

RESULTS. The majority of patients with IA (70%) were found to have increased BMIS (score $\geq$3) compared with the control patients (16%) ($P < .0001$). Increased BMIS was found to be an independent risk factor for IA on multivariate analysis ($P < .0001$).

CONCLUSIONS. The prospective validation of BMIS for risk stratification in patients with leukemia or those who undergo allogeneic hematopoietic stem cell transplantation is needed. ***Cancer* 2007;110:1303–6.** © *2007 American Cancer Society.*

KEYWORDS: aspergillosis, iron, bone marrow, leukemia, risk factors.

Invasive aspergillosis (IA) is a leading cause of death in patients with leukemia and recipients of hematopoietic stem cell transplantation (HSCT).[1,2] Classic risk factors for IA in such patients include profound and prolonged neutropenia, active malignancy, chronic corticosteroid use, cytomegalovirus (CMV) reactivation, graft-versus-host disease (GVHD), and other systemic immunosuppression (eg, tumor necrosis factor blockade).[1,2] Patients often have multiple, interrelated risk factors for IA.[2] However, IA does not develop in all patients who are at high risk for it, suggesting an incomplete understanding of its pathogenesis and risk factors. Although differences in environmental exposure to *Aspergillus* conidia might be an explanation, other host-specific immune factors such as differences in polymorphisms of innate immunity genes[3] may identify new subgroups of patients at highest risk. Alternatively, other undefined host factors linked with increased *Aspergillus* virulence may play a role. Consequently, searching for novel factors for IA risk stratification is an important area that could lead to targeted prophylactic strategies and improve pre-emptive therapeutic strategies.

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Recent laboratory studies demonstrated that iron acquisition is essential for the increased growth and virulence of *Aspergillus*.[4] In addition, small case series implied that IA is associated with iron overload in patients with hematologic malignancies.[5–7] Because patients with leukemia or myelodysplastic syndrome (MDS) and recipients of allogeneic HSCT receive frequent blood transfusions, the possibility of iron overload as a risk factor for IA should be evaluated further. Thus, in the current study, we sought to determine whether iron overload as measured using bone marrow iron stores (BMIS) is an independent risk factor for IA in patients with leukemia or allogeneic HSCT recipients.

MATERIALS AND METHODS

The medical records of 33 hospitalized patients with hematologic malignancies who had IA (study group) and 33 high-risk hospitalized patients without any fungal infections (contemporaneous control group) who were selected from a cohort of patients in a published case-control study from our institution (between September 2002 and March 2004)[8] and for whom bone marrow biopsy slides were available were reviewed retrospectively. IA was defined according to published guidelines.[9] Both groups were matched with regard to date of hospitalization. The groups were compared in terms of demographics and clinical characteristics (type and status of malignancy, receipt of HSCT, neutropenia, GVHD, significant cumulative corticosteroid dose, comorbidities, and CMV infection). The first available bone marrow biopsy specimens with regard to IA diagnosis (study group) or the day of hospitalization (control group) were assessed in a blinded fashion by an experienced hematopathologist (X. H.). Pearl blue iron stains were performed with bone marrow aspirate smears from archived pathologic materials. BMIS were calculated using a standardized scoring system (0–4).[10] Briefly, scores of 0, 1+, and 2+ indicate absent, minimal, and moderate amounts of stainable iron within the bone marrow particles, respectively, whereas scores of 3+ and 4+ indicate the presence of moderately and markedly increased stainable iron stores, respectively. Increased BMIS were defined by a score of $\geq$3. Severe neutropenia was defined as $\leq$100 polymorphonuclear cells/mm^3 for $>$ 10 days. Significant corticosteroid use was defined as the use of $>$ 600 mg of prednisone equivalent within 1 month from the diagnosis of IA.

The Fisher exact test and Mann-Whitney *U* test were used for comparisons of categoric and continuous variables, respectively. Patient risk factors that were independently associated with the development of IA were determined using forward stepwise regression analysis. All variables with a P value $\leq$. 2 on univariate analysis were screened for inclusion in the model. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were reported for all statistically significant variables at $P \leq .05$. All analyses were performed using the Stata Rel 8.0 software program (StataCorp, College Station, TX).

RESULTS

All but 1 of the patients in the study group had pulmonary IA; nearly half (16 patients; 48%) had IA because of *Aspergillus fumigatus*, and 27 patients (82%) had breakthrough IA after the prior use of antifungals. The 12-week mortality rate in the study group was 70% (19 of 27 patients). The median day of BMIS evaluation with respect to IA diagnosis was comparable in both groups (Table 1) ($P = .3$). Both groups were comparable with regard to demographics and clinical characteristics including age, sex, malignancy type and status, receipt of HSCT, neutropenia, GVHD, receipt of a significant cumulative corticosteroid dose, and CMV infection.[1,2] However, the median Acute Physiological and Chronic Health Evaluation (APACHE) II score was found to be higher in the study group (13; range, 5–22) compared with the control group (12; range, 5–22) ($P = .04$). The majority of the patients with IA (23 of 33 patients [70%]) had increased BMIS scores ($\geq$3) compared with the control patients (6 of 33 patients [18%]) ($P < .0001$) (Fig. 1). On multivariate analysis, an increased BMIS score (OR of 12.3; 95% CI, 3.4–44.9 [$P < .0001$]) and an APACHE II score $>$ 11 (OR of 5.16; 95% CI, 1.3–20.5 [$P = .01$]) were found to be independently associated with an increased risk of IA. Because the higher median APACHE II score in the IA group compared with the control group could likely reflect differences in the net state of immunosuppression in these patients rather than being a risk factor for IA per se, we repeated multivariate analysis after excluding the APACHE score and found that increased BMIS was the only significant variable associated with IA (OR of 12.4; 95% CI, 3.9–38.9 [$P < .0001$]).

DISCUSSION

Because the immunopathogenesis of IA remains largely unexplored, much remains to be learned regarding novel risk factors and better risk stratification in the complex patient population with hematologic malignancies. Previous studies found iron overload in 15% to 20% of patients with leukemia

TABLE 1
Characteristics of Patients in the Control and Study Groups and Results of Univariate and Multivariate Analyses

			Univariate analysis		Multivariate analysis	
Characteristic	**Control patients**	**Patients with IA**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Median day of BMIS evaluation (range)*	−8 (−160 to −180)	−9 (−344 to −85)	–	.3000	–	–
Median age (range), y	50 (2–78)	56 (25–78)	–	.3500	–	–
Male sex	18/33 (55%)	20/33 (61%)	–	.8000	–	–
MDS or AML†	11/33 (33%)	15/33 (45%)	–	.4500	–	–
Active malignancy	17/33 (52%)	24/33 (73%)	–	.1700	–	–
Neutropenia (< 500 cells/m^3) at diagnosis	12/33 (36%)	14/33 (42%)	–	.6200	–	–
Severe neutropenia (< 100 cells/mm^3 for > 10 days)‡	6/33 (18%)	7/33 (21%)	–	.7500	–	–
Lymphopenia (< 500 cells/mm^3)‡	17/33 (52%)	21/33 (64%)	–	.4500	–	–
Monocytopenia (< 10 cells/mm^3)‡	12/33 (36%)	14/33 (42%)	–	.8000	–	–
Significant corticosteroid use§	8/33 (24%)	14/33 (42%)	–	.1900	–	–
Allogeneic HSCT‖	8/33 (24%)	14/33 (42%)	–	.1900	–	–
GVHD	2/8 (25%)	8/14 (57%)	–	.2000	–	–
CMV reactivation	3/33 (9%)	9/33 (27%)	–	.1800	–	–
History of diabetes mellitus	4/33 (12%)	7/33 (21%)	–	.5100	–	–
APACHE II score > 11‡	17/33 (52%)	26/33 (79%)	3.5 (1.2–10.3)	.0300	5.16 (1.3–20.5)	.0100
Median APACHE II score (range)¶	12 (5–22)	13 (5–22)	–	.0400	–	–
Malnutrition (albumin level ≤ 3 mg/dL)‡	17/33 (52%)	22/33 (67%)	–	.3100	–	–
Increased BMIS score (≥3)#	6/33 (18%)	23/33 (70%)	10.4 (3.2–32.8)	< .0001	12.3 (3.4–44.9)	< .0001

IA indicates invasive aspergillosis; OR, odds ratio; 95% CI, 95% confidence interval; BMIS, bone marrow iron stores; MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia; HSCT, hematopoietic stem cell transplantation; GVHD, graft-versus-host disease; CMV, cytomegalovirus; APACHE, Acute Physiological and Chronic Health Evaluation.

* The days of BMIS evaluation were assessed with respect to the day of diagnosis of IA (study group) or the day of hospitalization (control group). There were no statistically significant differences noted with regard to the date of hospitalization between the patients in the study and those in the control group (data not shown).

† Of the 33 patients with hematologic malignancies and IA, 10 had AML, 9 had non-Hodgkin lymphoma, 5 had MDS, 2 had acute lymphoblastic leukemia, 2 had chronic lymphocytic leukemia, 2 had chronic myelogenous leukemia, 2 had Hodgkin disease, and 1 had multiple myeloma. Of the 33 control patients with hematologic malignancies, 10 had non-Hodgkin lymphoma, 9 had AML, 4 had acute lymphoblastic leukemia, 4 had chronic lymphocytic leukemia, 2 had MDS, 2 had multiple myeloma, and 2 had chronic myelogenous leukemia.

‡ At the time of diagnosis.

§ Prednisone equivalent (> 600 mg) administered during the month before the diagnosis of IA was made.

‖ Four of 8 allogeneic HSCT recipients in the control group (50%) and 7 of 14 allogeneic HSCT recipients in the study group (50%) received transplants from mismatched related or matched unrelated donors ($P = .65$).

¶ Increased BMIS (≥3) was found to be the only independent factor associated with IA ($P < .0001$; OR of 12.4 [95% CI, 3.9–38.9]) on stepwise logistic regression analysis performed after excluding the APACHE II score from the model.

A total of 23, 8, 5, and 1 control patients and 5, 5, 16, and 18 patients with IA had an iron score on bone marrow biopsy analysis with a BMIS score of +1, +2, +3, and +4, respectively.

and recipients of HSCT.[11,12] Several factors may have accounted for these increased iron stores, including ineffective erythropoiesis, the frequent administration of blood products, and expression of inactive variants of iron-binding proteins.[5] Several in vitro and clinical studies have linked iron overload with an increased risk of bacterial[13,14,15] and invasive fungal infections, especially Zygomycetes infections.[5–7,16] Fungi scavenge iron and overcome iron withholding by the host in several ways, such as the use of iron-binding proteins (siderophores).[4] Therefore, a net increase in host iron stores would increase iron availability and enhance fungal growth.

To our knowledge, of the few studies associating IA with iron overload, the majority were limited by small patient numbers, heterogeneity, and a lack of a control group to account for the contribution of "classical" IA risk factors. Furthermore, some studies

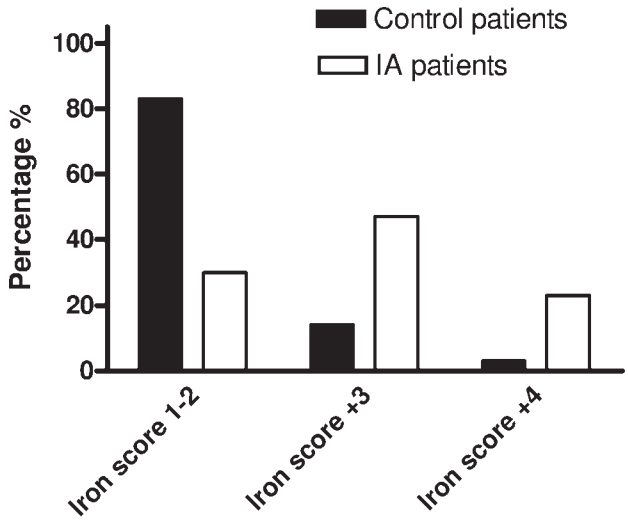

FIGURE 1. Distribution of patients with normal (1 or 2) or increased (≥3) bone marrow iron stores scores. IA indicates invasive aspergillosis.

used surrogate markers for iron overload that are either cumbersome, such as the calculation of liver iron stores or, as in the case of serum ferritin levels, unreliable because of the effects of inflammation and liver injury commonly observed in high-risk hematology patients.[5,7] However, Strasser et al. demonstrated a strong correlation between bone marrow iron staining and biochemical bone marrow and liver iron stores.[17] Although interobserver variability may exist in the assessment of BMIS, the semiquantitative grading system used by most hematopathologists to assess these stores is highly reproducible and validated for the diagnosis of moderate/severe iron overload.[18]

To our knowledge, Miceli et al. were the first to describe an association between pretransplantation increased BMIS and severe infections in 367 autologous HSCT recipients with multiple myeloma.[15] They found no association between increased BMIS and fungal infections, including IA, possibly because of the low prevalence of IA in the patient population studied.

Compared with previous studies regarding the association between iron overload and IA, the current study had the advantage of including a contemporaneous control group to avoid selection bias and adjust for confounding variables. Nonetheless, limitations of our study include its retrospective nature and the absence of data regarding the date of diagnosis of the underlying malignancy, the type and number of chemotherapeutic regimens, prior blood cell transfusions, other surrogate markers of iron overload such as ferritin, and free iron blood levels. Importantly, we cannot rule out the possibility that other differences among the groups could have influenced the validity of the current study findings. Therefore, a causal relation between increased BMIS and the occurrence of IA cannot be inferred from our study.

Prospective studies are needed to further elucidate the role of iron overload in the pathogenesis of IA in patients with hematologic malignancies and recipients of HSCT. Evaluating the role of promising new iron chelators such as deferasirox[19] as prophylactic agents in high-risk patients with increased BMIS or as adjunctive therapy with antifungals in patients with established IA could be an interesting research direction.

REFERENCES

1. Kontoyiannis DP, Bodey GP. Invasive aspergillosis in 2002: an update. *Eur J Clin Microbiol Infect Dis*. 2002;21:161–172.
2. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic transplant recipients: changes in epidemiology and risk factors. *Blood*. 2002;100:4358–4366.
3. Bochud PY, Chien JW, Janer M, Marr KA, Aderem A, Boeckh M. Donor's toll-like receptor 4 haplotypes increase the incidence of invasive mold infections in hematopoietic stem cell transplant recipients. Presented at 46th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, California, September 27–30, 2006. Abstract #B-1329.
4. Hissen AH, Wan AN, Warwas ML, Pinto LJ, Moore MM. The *Aspergillus fumigatus* siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. *Infect Immun*. 2005;73:5493–5503.
5. Karp JE, Merz WG. Association of reduced total iron binding capacity and fungal infections in leukemic granulocytopenic patients. *J Clin Oncol*. 1986;4:216–220.
6. Altes A, Remacha AF, Sarda P, et al. Frequent severe liver iron overload after stem cell transplantation and its possible association with invasive aspergillosis. *Bone Marrow Transplant*. 2004;34:505–509.
7. Iglesias-Osma C, Gonzalez-Villaron L, San Miguel JF, Caballero MD, Vazquez L, de Castro S. Iron metabolism and fungal infections in patients with haematological malignancies. *J Clin Pathol*. 1995;48:223–225.
8. Kontoyiannis DP, Lionakis MS, Lewis RE, et al. Zygomycosis in the era of *Aspergillus*-active antifungal therapy in a tertiary care cancer center: a case-control observational study of 27 recent cases. *J Infect Dis*. 2005;191:1350–1360.
9. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis*. 2002;34: 7–14.
10. Takkunen H. Iron deficiency in the Finnish adult population. *Scand J Haematol*. 1976;25:1–91.
11. Barton JC, Bertoli LF. Transfusion iron overload in adults with acute leukemia: manifestations and therapy. *Am J Med Sci*. 2000;319:73–78.
12. Altes A, Remacha AF, Sureda A, et al. Iron overload might increase transplant-related mortality in haematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2002; 29:987–989.
13. Weinberg ED. Iron, infection, and neoplasia. *Clin Physiol Biochem*. 1986;4:50–60.
14. Crichton R.Iron and infection. In: Inorganic Biochemistry of Iron Metabolism: From Molecular Mechanisms to Clinical Consequences. Volume 2. West Sussex, UK: John Wiley & Sons, Ltd, 2001:259–284.
15. Miceli MH, Dong L, Grazziutti ML, et al. Iron overload is a major risk factor for severe infection after autologous stem cell transplantation: a study of 367 myeloma patients. *Bone Marrow Transplant*. 2006;37:857–864.
16. Maertens J, Demuynck H, Verbeken EK, et al. Mucormycosis in allogeneic bone marrow transplant recipients: report of five cases and review of the role of iron overload in the pathogenesis. *Bone Marrow Transplant*. 1999;24:307–312.
17. Strasser SI, Kowdley KV, Sale GE, McDonald GB. Iron overload in bone marrow transplant recipients. *Bone Marrow Transplant*. 1998;22:167–173.
18. Burns ER, Goldberg SN, Lawrence C, Wenz B. Clinical utility of serum tests for iron deficiency in hospitalized patients. *Am J Clin Pathol*. 1990;93:240–245.
19. Fischer R. The oral chelator deferasirox-a new perspective for patients with iron overload. *Haematologica*. 2006;91: 91873–91880.