Galactomannan Does Not Precede Major Signs on a Pulmonary Computerized Tomographic Scan Suggestive of Invasive Aspergillosis in Patients with Hematological Malignancies

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Background. Detection of serum galactomannan (GM) antigen and presence of the halo sign on a pulmonary computerized tomographic (CT) scan have a high specificity but a low sensitivity to diagnose invasive aspergillosis (IA) in patients at risk for this disease. To our knowledge, the relationship between the time at which pulmonary infiltrates are detected by CT and the time at which GM antigens are detected by enzyme immunoassay (EIA) has not been studied.

Methods. In a prospective study, tests for detection of GM were performed twice weekly for patients with hematological malignancies who had undergone hematopoetic stem cell transplantation (HSCT) or had received induction and/or consolidation chemotherapy. A pulmonary CT scan was performed once weekly. Infiltrates were defined as either major or minor signs. IA was classified as proven, probable, or possible, in accordance with the definition stated by the European Organization for Research and Treatment of Cancer–Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group.

Results. We analyzed 161 episodes of infection in 107 patients (65 allogeneic HSCT recipients, 30 autologous HSCT recipients, and 66 induction and/or consolidation chemotherapy recipients). A total of 109 episodes with no IA, 32 episodes with possible IA, and 20 episodes with probable or proven IA were identified. Minor pulmonary signs were detected by CT in 70 episodes (43%), and major pulmonary signs were detected by CT in 11 episodes (7%). Univariate and multivariate analyses revealed no significant association between detection of GM by EIA and detection of abnormal pulmonary signs by CT. A significant association was found between GM levels and receipt of piperacillin-tazobactam. GM test results were not positive before major signs were seen on CT images. Only 7 (10%) of 70 patients with minor pulmonary signs had positive GM test results before detection of the greatest pathologic change by CT.

Conclusions. We show that detection of GM by EIA does not precede detection of major lesions by pulmonary CT. In the clinical setting, the decision to administer mold-active treatment should based on detection of new pulmonary infiltrates on CT performed early during infection, rather than on results of EIA for detection of GM.

Invasive mold infections are increasing among patients with hematological malignancies [1–5]. Incidences of invasive aspergillosis (IA) as high as 12% have been reported, with mortality rates of up to 80% [6–8]. The gold standards for the diagnosis of IA are positive results of culture of a sample obtained from a sterile site or detection of hyphae by histopathologic or cytopathologic examination of a biopsy specimen [9]. Sequential thoracic CT scanning is a standard method used in the diagnosis of IA. Early IA lesions are small, round, dense areas that are typically located in the lung periphery and increase in size over time. A halo sign on a CT scan has been reported to be the first reliable sign of infection, with a high specificity (93%) but a low sensitivity (33%) [10]. Cavitation or air-crescent formation occur later in the course of the disease and are usually noted after bone marrow recovery [11–13].

Received 11 March 2005; accepted 2 June 2005; electronically published 12 September 2005.

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Clinical Infectious Diseases 2005; 41:1143–9

1058-4838/2005/4108-0010$15.00

Recently, serial testing for detection of galactomannan (GM) antigen has been proposed as a screening tool for the diagnosis of IA [14–16]. Whereas initial studies showed high sensitivities and specificities for the GM test [17, 18], subsequent prospective studies primarily involving populations of patients with hematological malignancy showed specificities of 85%–99% but a broad range of sensitivities (29%–94%) [10, 19–25].

To standardize IA diagnoses for clinical research, a rating system that classifies cases as possible, probable, and proven on the basis of clinical, radiological, and microbiological criteria (including results of GM tests [26]) was established by a panel of experts from the European Organization for Research and Treatment of Cancer–Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC-MSG) [26]. However, the rating system has not been evaluated as a guide for daily clinical practice. Currently, patients at risk of developing IA who do not respond to broad-spectrum antibiotic therapy and who have thoracic CT scans that show a new infiltrate are treated empirically with a mold-active drug [27, 28].

One study that evaluated the times to detection of GM by ELISA and of pulmonary infiltrates by CT scanning showed that IA could be diagnosed by detection of circulating GM a mean duration of 8 days before diagnosis by other means [10]. However, this study was limited to patients who had recently undergone allogeneic stem cell transplantation, and CT scans were only performed for patients with a clinical suspicion of invasive fungal infection. The purpose of our cohort study was to analyze the time of onset of pulmonary lesions on CT images obtained weekly and the time of detection of GM in serial EIA of serum samples from all patients admitted to the hematological ward. In particular, we were interested in whether elevated GM values preceded minor or major lesions observed on CT scans of patients at risk for IA.

PATIENTS AND METHODS

We performed a prospective, single-cohort study that included all adult patients who were admitted to the hematology ward at the University Hospital in Basel, Switzerland, between May 2002 and September 2003 to undergo autologous or allogeneic hematopoetic stem cell transplantation or to receive treatment with induction and/or consolidation chemotherapy.

Monitoring of patients. Patients were investigated daily for clinical symptoms of infection. Routine laboratory tests were performed each day. At the onset of fever (defined as a temperature of >38.5°C) during the neutropenic stage, blood samples were obtained for culture, and broad-spectrum antibiotic treatment that accorded with published guidelines [29] was started. If a patient remained febrile for >72 h, an antifungal agent (i.e., amphotericin B) was added to the treatment regimen. When infiltrates were detectable by CT, bronchoscopy with bronchoalveolar lavage was performed. In the absence of recovery of a specific pathogen, empirical voriconazole therapy for *Aspergillus* infection was started.

Table 1. Demographic and clinical characteristics of 107 patients who presented to the hematology ward with hematological malignancies between May 2002 and September 2003.

Variable	Value
Sex	
Male	66 (62)
Female	41 (38)
Age at study entry, median years (range)	48 (16–78)
Duration of hospitalization, median days (range)	29 (6–88)
Underlying disease	
Acute myeloid leukemia	32 (29.9)
Acute lymphatic leukemia	21 (19.6)
Non-Hodgkin lymphoma	20 (18.7)
Myelodysplasia	13 (12.2)
Chronic myeloid leukemia	8 (7.5)
Multiple myeloma	6 (5.6)
Hodgkin lymphoma	3 (2.8)
Chronic lymphatic leukemia	3 (2.8)
Multiple sclerosis	1 (0.9)
No. of infection episodes	161
Therapy during hospitalization	
Allogeneic stem cell transplantation	65 (40)
Autologous stem cell transplantation	30 (19)
High-dose chemotherapy	66 (41)
Neutropenia	
Experienced a neutropenic episode during study	139 (86)
Duration of neutropenia, days	
Median (range)	10 (0–56)
Total for all patients	1973
Total no. of galactomannan measurements	1418

NOTE. Data are no. (%) of patients, unless otherwise indicated.

Tests for detection of GM in serum were performed twice weekly by use of the GM EIA (Platelia Aspergillus; BioRad Laboratories) [18]. A total of 1418 GM tests were performed. GM test results were interpreted as positive when an optical density (OD) index of ⩾0.5 was reached during 2 consecutive measurements [30].

A pulmonary CT scan for each patient was performed routinely on a weekly basis or if clinically indicated. CT scans were assessed a second time by a radiologist who was not involved in the primary evaluation. Changes observed on CT images were categorized as major signs (halos, crescent-signs, or cavernous lesions) or as minor signs (all other infiltrates).

At the time of hospital discharge or death, episodes of pulmonary infection were classified in terms of evidence for invasive *Aspergillus* species. Cases of IA were classified according to the EORTC/MSG definitions as proven, probable, or possible; patients without IA were classified as having no fungal infection [26].

Data on patient outcomes were collected from hematological databases and categorized according to whether the patient had survived without IA until the end of September 2003 or had died. For patients who died, we noted whether an autopsy was performed, and, if so, data regarding the presence of IA were recorded.

Supportive-care measures. Each patient was hospitalized in a single room in a hospital unit that was supplied with high-efficiency particulate air filters. Antimicrobial prophylaxis during hospitalization consisted of trimethoprim-sulfamethoxazole (160 and 800 mg, respectively, 3 times weekly) and fluconazole (400 mg once per week); patients infected with herpes simplex virus also received either valacyclovir (two 500-mg doses twice daily) or acyclovir (5 mg/kg twice daily).

Statistical analysis. Statistical analyses were performed with SAS, version 8.2 (SAS Institute). Two different modeling processes—univariate and multivariate—were adopted for data analysis. We developed a univariate model with PROC GENMOD (SAS Institute) and used a binomial distribution with a logit link. To allow for multiple observations associated with the same patient, the model was recalculated, incorporating the measurements that were made more than once to produce the multivariate model (i.e., a generalized estimating equations [GEE] model that allowed correlation between repeated measurements performed on the same patient). We used an unstructured correlation/covariance matrix to permit direct parameterization in terms of variances and covariances. The primary explanatory covariate of interest (CT finding) was added to the model on both occasions. Collett's procedure was used to build the model, although no covariates other than the use of piperacillin-tazobactam (P-T) were found to be statistically significant for inclusion ($P > .05$). Potential confounders (age and sex) were then entered into the model. Fisher's exact test was used to compare patients who were and patients who were not treated with P-T. P values of .01 were considered to indicate statistical significance.

RESULTS

Patient population. A total of 161 episodes of infection occurred in 107 patients admitted to the hematology ward, of whom 66 (62%) were male and 41 (38%) were female. Patient characteristics and underlying diseases are summarized in table 1.

Analysis of thoracic CT findings, results of tests for detection of GM, and clinical findings. In 80 (50%) of the 161 episodes, no evidence of infiltrates was detectable on the CT scans at any time during hospitalization (table 2). CT scanning revealed minor signs in 70 episodes (43%) and major pathologic signs in 11 episodes (7%) (usually in the form of a halo around a nodular infiltrate). A total of 109 episodes (68%) were defined as no IA, 32 episodes (20%) were defined as possible IA, and 20 episodes (12%) were defined as probable or proven IA, according to EORTC/MSG criteria.

For the 2-week period before and the 2-week period after the dates when the greatest pathological changes were observed on CT scans, test results for detection of GM were positive on 2 consecutive occasions in 57 cases (35%). The percentage of positive GM test results (i.e., 2 consecutive OD index measurements of $\geqslant 0.5$) was highest in the groups of patients classified as having probable or proven IA and lowest in the group of patients defined as having no IA.

On the dates when CT revealed the greatest amount of pathological change, 58 patients (36%) were already receiving mold-active therapy. The percentage of patients treated with a mold-active agent on these dates was highest among those who were classified as having a higher likelihood for IA.

A total of 66 (62%) of the 107 patients were alive at the end of the study. Of these 66 patients, 45 showed no evidence of IA, 13 showed evidence of possible IA, and 8 patients showed evidence of probable or proven IA during hospitalization.

Thirty-two (30%) of the patients died. The mortality rate was highest in the patient group classified as having possible IA (46% [11 of 24 patients]), followed by the group with prob-

Table 2. Pulmonary CT findings, galactomannan values, treatment, and outcome for 161 episodes of infection in 107 patients who presented to the hematology ward with hematological malignancies between May 2002 and September 2003.

	Laboratory and clinical findings, proportion (%) of episodes						Outcome, proportion (%) of patients					
	Pulmonary CT scan finding			Positive GM test result[a]	Treatment							
Infection classification	No infiltrate	Minor sign	Major sign		Mold active	P-T	Survived	Died	IA in autopsy	No IA in autopsy	No autopsy done	No info available
All	80/161 (50)	70/161 (43)	11/161 (7)	57/161 (35)	58/161 (36)	28/161 (17)	66/107 (62)	32/107 (30)	3/107 (3)	8/107 (7)	21/107 (20)	9/107 (8)
No IA	76/109 (70)	33/109 (30)	0 (0)	20/109 (18)	19/109 (17)	7/109 (6)	45/70 (64)	16/70 (23)	0 (0)	4/70 (6)	12/70 (17)	9/70 (13)
Possible IA	3/32 (9)	26/32 (81)	3/32 (10)	21/32 (65)	21/32 (65)	10/32 (31)	13/24 (54)	11/24 (46)	2/24 (8)	3/24 (13)	6/24 (25)	0 (0)
Probable or proven IA	1/20 (5)	11/20 (55)	8/20 (40)	16/20 (80)	18/20 (90)	11/20 (55)	8/13 (62)	5/13 (38)	1/13 (7.5)	1/13 (7.5)	3/13 (23)	0 (0)

NOTE. GM, galactomannan antigenemia; IA, invasive aspergillosis (includes possible, probable, and proven cases, as defined by the European Organization for Research and Treatment of Cancer–Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [26]); P-T, piperacillin-tazobactam.

[a] See Patients and Methods for the definition of GM test results.

Table 3. Comparison of galactomannan (GM) test results and pulmonary CT findings for patients with hematological malignancies who were or were not receiving piperacillin-tazobactam (P-T) at the time that the greatest pathological change was detected by CT.

Pulmonary CT finding	No. of episodes, by patient group				
	All patients (n = 161)	GM positive, P-T use (n = 15)	GM negative, P-T use (n = 13)	GM positive, no P-T use (n = 26)	GM negative, no P-T use (n = 107)
No infiltrates	80	1	1	21	57
Minor signs	70	10	10	4[a]	46[a]
Major signs	11	4	2	1[a]	4[a]

NOTE. See Patients and Methods for the definition of GM test results.

[a] $P = .391$, by Fisher's exact test.

able or proven IA (38% [5 of 13 patients]) and the group without IA (23% [16 of 45 patients]).

Autopsies were performed for 11 of these patients. IA was found to be present in 3 patients; 2 had been classified as having possible IA, and 1 had been classified as having probable IA. The intervals between clinical diagnosis of IA and autopsy for these 3 patients were 12 and 14 weeks for the patients considered to have possible IA and 5 days for the patient considered to have probable IA. Autopsy was performed for 5 of 11 patients who had been classified as having possible IA during their hospitalization and died before the end of the study. In 2 of these patients, IA was proven histologically; IA was not found in the other 3 patients.

Temporal sequence of positive results of GM tests and pathologic signs on CT scans. To investigate the relationship between detection of GM and the greatest pathological change on CT scans, we analyzed 81 episodes to compare the courses of GM test results with CT images revealing pathologic findings. Of 70 episodes in which minor signs were observed, GM test results were negative during the entire infection episode in 36 cases (51%); in 6 cases (9%), GM test results were positive at the time that the greatest pathological change was revealed by CT. In the remaining 28 episodes, a positive GM test result was observed in 7 cases (10%) before a lesion appeared on a CT scan, and a positive GM test results appeared in 21 cases (30%) only after radiological signs had appeared on CT scans.

Of interest, 3 (27%) of 11 patients had major signs on pulmonary CT scans and no positive GM test results throughout the infection episode. All 3 patients were treated with antifungals, and regression of pulmonary nodules was observed. In 1 patient, the lesion was surgically removed, and unspecific alveolitis was histologically diagnosed. Culture results remained negative. The outcome for all 3 patients was favorable at the end of the study. For 5 patients (45%), GM test results were positive at the same time that pathologic signs were evident on CT scans, and for 2 patients (18%), GM test results were positive only after the lesions had already appeared on CT scans. For only 1 of these 11 patients, the OD value was $\geq$0.5 before major pulmonary signs were detected by CT. However, this patient was rehospitalized just 1 week after an episode in which the first major signs appeared on CT scans, and thereafter, OD values increased to >0.5.

Correlation of galactomannan antigenemia, P-T treatment, and pulmonary infiltrates. Elevated levels of circulating GM antigens resulting from the administration of P-T have been recently described in the literature [31–33]. In 28 episodes of infection (17%), antibiotic treatment with P-T was administered at the time that the greatest pathological changes were noted on CT scans. Table 3 organizes patients according to positive or negative GM test results and receipt of P-T. Analysis of patients who did not receive P-T revealed that pulmonary findings for the group with positive GM test results were not significantly different from those for the group with negative GM test results ($P = .391$, by Fisher's exact test). Moreover, no significant difference among patients not treated with P-T was observed regarding the presence or absence of any pulmonary infiltrate ($P = .70$, by Fisher's exact test).

Factors revealed by univariate and multivariate analyses to be associated with positive GM test results. Univariate and multivariate analyses were performed to evaluate the association

Table 4. Univariate and multivariate models of factors associated with a positive galactomannan test result.

Variable	Univariate OR (95% CI)	Multivariate OR (95% CI)
CT finding		
Minor sign vs. no infiltrate	1.12 (0.52–2.39)	1.09 (0.54–2.19)
Major sign vs. no infiltrate	2.54 (0.60–10.86)	2.47 (0.57–10.65)
P-T during CT[a]	3.60 (1.39–9.34)	3.70 (1.47–9.34)
Age	0.98 (0.96–1.01)	…
Sex (male vs. female)	0.81 (0.40–1.66)	…

NOTE. See Patients and Methods for the definition of GM test results. Testing for detection of GM was performed within 2 weeks of pulmonary CT scanning.

[a] Treatment with P-T at the time the greatest pathological change was detected by CT.

between an OD of ⩾0.5 and pathologic findings observed on thoracic CT scans. In the univariate and multivariate analyses adjusted for age and sex (in univariate analysis only) and P-T use, major and minor signs on CT scans were not significantly associated with positive GM test results (table 4). The association between the detection of GM and the use of P-T was statistically significant. Age and sex did not substantially influence estimates of the association between CT findings and GM test results in the multivariate analyses (i.e., these characteristics were not considered to be confounders) and were therefore not included in the final multivariate model.

DISCUSSION

The EORTC/MSG criteria for establishing the diagnosis of possible, probable, or proven IA include the appearance of new infiltrates on CT scans, positive results of an *Aspergillus* galactomannan antigen test, and clinical signs of infection [26]. In the present study, we investigated the relationship between temporal onset of pulmonary lesions on CT scans performed weekly and EIA performed twice weekly for detection of GM. We did not find that elevated ODs reliably preceded the appearance of nodular lesions with or without an accompanying halo on CT scans. For patients treated with P-T, we could confirm an association between this agent and serum EIAs with elevated ODs. We cannot deduce the sensitivity and specificity of the GM test on the basis of our results, because we included all patients at high-risk for IA who were consecutively admitted to the hematological ward (which reflects the daily clinical situation in our unit), rather than only patients with only probable or proven cases of IA.

Major pulmonary signs on CT images have been reported to be the earliest specific sign of infection in neutropenic patients [12, 13, 34]. We found major signs in 8 (40%) of 20 infection episodes classified as probable or proven IA but in only in 3 (10%) of 32 episodes classified as possible IA. Minor signs were most frequently associated with episodes classified as possible IA (26 [81%] of 32 cases), followed by episodes defined as probable or proven IA (11 [55%] of 20 cases). Unfortunately, minor signs on pulmonary CT scans can be caused by many pathologic conditions, including those without infectious causes, and such signs have a low specificity (6.6%), with a sensitivity of 93% and positive and negative predictive values of 50% [10].

Neither univariate nor multivariate analyses showed an association between the time when the greatest pathological change was observed on a CT scan and an elevated EIA OD, even at a low cutoff value of 0.5. Of note, 3 (27%) of 11 patients for whom a halo sign was present on a CT scan had a negative result of a GM test (defined as an OD of <0.5) during the entire episode of infection. Of the 70 patients with minor abnormal CT findings, 36 (51%) had negative GM test results throughout their episodes. The appearance of minor signs on CT scans was preceded by elevated EIA ODs in only 7 episodes (10%). For patients with major signs on CT scans, we found no elevated EIA ODs preceding observance of the halo sign, which has been reported to be an early specific sign of IA during the neutropenic stage [12].

Reasons for variation in circulating levels of GM might include administration of antifungal compounds or a low burden of fungi [35–37]. In experimental animal models, it was shown that antifungal treatment decreased or delayed GM detection in serum [38, 39]. In several studies, antifungal prophylaxis affected the sensitivity of the antigen test [23, 40]. In our study population, patients received fluconazole prophylaxis, and treatment was switched to either amphotericin B or voriconazole if a new pulmonary infiltrate appeared on the CT scan. Overall, 58 patients (36%) were treated with a mold-active agent at the time the greatest pathological changes were evident on CT scans, which could, at least in part, explain the poor association between elevated EIA ODs and radiological signs.

False-positive results of GM tests have been described for patients who received P-T and/or other β-lactams [31–33, 41]. In our study, 20 of the 109 patients who were clinically classified as having no IA had 2 consecutive EIA ODs of ⩾0.5. P-T therapy was administered to 14 of these 20 patients, which could explain the false-positive test results. In univariate and multivariate analyses adjusted for age and sex, concomitant treatment with P-T was significantly associated with an elevated EIA OD. One might argue that the large proportion of patients receiving P-T in our study is a limitation of the results. However, in an analysis limited to patients who did not receive P-T, we did not find a significant difference in pulmonary infiltrate findings between the group with positive GM test results and the group with negative GM test results.

A possible limitation of our study is that number of episodes of probable or proven IA among our patients was small (20 cases [12%]). However, this number is comparable to findings reported in other published studies [23]. Another limitation is that the number of autopsies performed to confirm cases of proven IA was limited, because autopsy was not performed for all patients who died. On the other hand, if there is a long interval between death and autopsy, the examination does not necessarily give correct information about a prior fungal infection.

One of the strengths of our study is the large number of patients at risk for IA who had a long neutropenic phase. Another strength of the study is that we performed CT scans and measured GM levels in serum samples at regularly defined intervals and were therefore able to study the correlation between GM levels and radiological signs on the CT scans longitudinally and prospectively.

Our study shows that the first sign of possible IA or probable

or proven IA is the observation of a new infiltrate on a CT scan performed early during the disease course, followed by a positive GM test result, which is needed to classify patients according to the EORTC/MSG definitions. We could not observe an association between an elevated EIA OD and either minor or major pulmonary signs on a CT image. In a hospital setting where repeated performance of CT scans is possible, clinicians should add drugs with activity against molds to the broad-spectrum antibiotic regimen for selected high-risk patients as soon as a pulmonary nodular lesion is detected on a CT scan.

Acknowledgments

Financial support. Stiftung Forschung Infektionskrankheiten, Basel, Switzerland.

Potential conflicts of interest. All authors: no conflicts.

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