Haematologica 2000; 85:745-752
original paper

Hematology & Medicine

Computed tomographic scan of the chest, latex agglutination test and plasma (1→3)-β-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: A prospective study of 215 patients

MASAHIRO KAMI,* YUJI TANAKA,° YOSHINOBU KANDA,° SEISHI OGAWA,* TOMOHIKO MASUMOTO,#
KUNI OHTOMO,# TOMOKO MATSUMURA,* TOSHIKI SAITO,* UTAKO MACHIDA,* TAKESHI KASHIMA,@
HISAMARU HIRAI*°

**Department of Hematology and Oncology, °Department of Cell Therapy and Transplantation Medicine, #Department of Radiology and @Department of Pathology, Faculty of Medicine, The University of Tokyo, Tokyo, Japan*

ABSTRACT

Background and Objectives. **Blood and radiologic tests are frequently used for diagnosis of invasive pulmonary aspergillosis, but it remains unknown which is more useful for its early diagnosis. The aim of the study was to compare usefulness of computed tomographic (CT) scan of chest, latex agglutination (LA) test and determination of plasma (1→3)-β-D-glucan (BDG) levels for early diagnosis of invasive pulmonary aspergillosis (IPA).**

Design and Methods. **We treated 215 consecutive patients who underwent cytotoxic chemotherapy. From initiation of chemotherapy until death or discharge, blood samples were taken weekly and subjected to LA and BDG tests. We performed chest CT scans when patients had any signs of pulmonary infection or an antibiotic-resistant fever.**

Results. **Of the 215 patients, 30 (14.0%) were diagnosed as having IPA. In sixteen cases the diagnosis was definite and in 14 it was suspected. In patient-based analysis, sensitivities of LA and BDG were 44% and 63%, respectively. Sensitivity tended to be lower in patients with IPA localized to the lung than those with disseminated invasive aspergillosis. Specificities were 93% and 74%, respectively. Either a halo or an air-crescent was observed in 7 of the 16 patients with IPA, and all of the IPA patients showed some abnormal signs on chest CT scans. On average, CT scan signs preceded a positive LA test by 7.1 days and a positive BDG assay by 11.5 days. In 6 of the 11 patients who became positive for either LA or BDG assay, CT scan signs preceded the positive results by more than seven days.**

Interpretations and conclusions. **Chest CT scan is more beneficial than the blood tests and X-ray for early diagnosis of IPA.**

Key words: invasive pulmonary aspergillosis, computed tomography, latex agglutination test, (1→3)-β-D-glucan, X-ray, hematologic malignancy, early diagnosis

Correspondence: Masahiro Kami, M.D., Department of Hematology, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo 105-8470, Japan. Phone: international +81-3-3588-1111 – Fax: international +81-3-3582-7068 – E-mail: kami-tky@umin.ac.jp

Invasive pulmonary aspergillosis (IPA) is a common fungal infection in patients undergoing cytotoxic chemotherapy for hematologic malignancies. The incidence of aspergillosis infections has increased dramatically during the last decade with the widespread use of aggressive chemotherapy and immunosuppressive agents. Despite improvement in its recognition, prophylaxis and treatment, it is still associated with high morbidity and high mortality. The mortality rate reaches 50 to 60% when IPA occurs during chemotherapy-induced neutropenia and could exceed 90% in the setting of bone marrow transplantation.[1] The survival of patients depends on early diagnosis and prompt initiation of therapeutic measures,[2] but microbiologic or histopathologic diagnoses are rarely established because blood cultures are rarely positive for *Aspergillus* species[3] and invasive procedures are required to obtain pathologic specimens. A critical problem is the difficulty in making an early diagnosis of IPA.

Reliable non-invasive diagnostic techniques have therefore been sought. Although the chest X-ray is a useful method for detecting IPA, the findings are usually non-specific and findings indicative of IPA are often absent, particularly in the case of patients with severe neutropenia.[4] Computed tomography (CT) of the chest has been advocated for the early diagnosis of IPA,[2] as this method allows better assessment of the pattern and distribution of known abnormalities than radiography, as well as the detection of infiltrates not apparent on chest radiography. The most characteristic CT finding in cases of IPA is a halo of ground-glass attenuation around focal nodules,[5] which corresponds pathologically to hemorrhage around a focus of pulmonary infarction.[6]

Detection of circulating *Aspergillus* or fungal antigens is another method for diagnosing IPA. Three methods are currently employed: the latex agglutination test (LA) test, enzyme-linked immunosorbent assay (EIA) and measurement of plasma (1-3)-β-D-glucan (BDG) concentration. The LA test and EIA, which detect circulating galactomannan antigens, are commercially available. Although EIA has a higher sensitivity than the LA test, it is not approved for diagnostic use in Japan and we used the LA test for IPA diagnosis in this study. BDG is a ubiquitous component of fungi[7] and determination of its plas-

ma concentration is another useful screening method detecting deep mycoses including IPA.[8] Because these blood tests detect circulating fungal components, their specificities for fungal infections are as high as 90%.[8-10] However, it remains unknown how early these assays become positive in IPA , which is the most important problem in managing these patients.

The present study was undertaken to determine the diagnostic usefulness of CT scans, the LA test and the BDG assay in the early course of IPA.

Design and Methods

Patients and conditions of hospitalization

We prospectively examined 215 patients with hematologic malignancies from January 1997 to August 1999. All patients undergoing bone marrow transplantation and some patients receiving high-dose chemotherapy were cared for in protective isolation in a room equipped with high-efficacy particulate air filtration throughout their period of neutropenia. All the patients received either fluconazole (200 mg or 400 mg/day) or itraconazole (200 mg/day) as antifungal prophylaxis. We managed neutropenic fever as described in the report by Pizzo *et al.*[11] Axillary temperature was measured three times a day. At the time of the first febrile episode, we empirically started both a β lactam antibiotic and an aminoglycoside. Intravenous administration of amphotericin B (AMPH-B) was added when the fever persisted for more than five to seven days. Chest X-rays were taken during febrile periods at least once a week.

Diagnostic criteria for IPA

We diagnosed patients as definitely having IPA when there was histologic evidence of tissue invasion by branched septate hyphae together with a lack of response to antibacterial agents and culture findings positive for *Aspergillus* species from the sputum or lung. We diagnosed patients as having suspected IPA when suggestive CT signs, either a halo or an air-crescent, and a persistent fever unresponsive to broad-spectrum antibiotics were present without histopathologic evidence of IPA. When histologic evidence of tissue invasion by branched septate hyphae was identified at biopsy or autopsy, but *Aspergillus* species were not cultured from the lesions, we diagnosed the patients as having suspected IPA. We excluded patients who satisfied the criteria of suspected IPA but for whom there was clinical or histopathologic evidence of pulmonary diseases other than IPA. The other patients were defined as control patients.

Comparison of each diagnostic test was performed between patients with definite IPA and control patients. We show the results of patients with suspected IPA, but patients with suspected IPA were excluded from evaluation of these tests because, by definition, it was unknown whether IPA really existed in these patients. Definite invasive aspergillosis was divided into two groups: disseminated invasive aspergillosis and localized invasive aspergillosis. Infection by *Aspergillus* species with multiple, non-contiguous organ involvement was defined as disseminated invasive aspergillosis. Invasive aspergillosis localized to a single organ including the lung was defined as localized invasive aspergillosis.

Clinical specimens

From the time of initiation of chemotherapy until death or discharge, blood samples were taken weekly and subjected to the LA test (Pastorex Aspergillus, Sanofi, Diagnostic Pasteur, Paris, France), and plasma BDG concentration (Fungi-Tec, Seikagaku Corporation, Tokyo, Japan) was determined.[7] The cut-off level of the BDG measurement was 20 pg/mL.[8]

Cultures from the throat, urine, feces, blood, and sputum were performed when antibiotic-resistant fever developed. They were plated on Sabouraud's glucose agar slants (30C°), and cultured for a week. Bronchoalveolar lavage (BAL) is not usually performed in our institution if patients have either thrombocytopenia or neutropenia. Thus, microbiological diagnosis of aspergillosis is established based upon the results of cultures from sputum or autopsy samples.

CT criteria for IPA diagnosis

We performed chest CT scans using a Phillip-Tomoscan 350-Scanner, when patients had any signs of pulmonary infection or an antibiotic-resistant fever. When we could not exclude the possibility of IPA after the first CT scan in cases with persistent antibiotic-resistant fever, CT scans were repeated. When IPA diagnosis was established, CT scans were performed serially, on average, once a month. Scans were obtained at 1.0-cm intervals using 1.0 cm collimation. Consecutive 10-mm thick sections were obtained from the entire chest with additional 2-mm thin sections through any suspected fungal lesions. No contrast medium was administered. CT images were viewed at the lung (W, 1,200 HU; C, 800 HU) and mediastinal (W, 500 HU; C, 0 HU) windows.

The CT signs of a halo or air-crescent have been identified as indicators of IPA.[5,12] The halo sign is highly indicative of IPA and occurs in an early stage of the disease.[12,13] The air crescent sign is a later sign that appears with bone marrow recovery. This sign is not pathognomonic of IPA, but in leukemic patients it is highly suggestive of disease caused by filamentous fungi.[13]

Both the chest radiographs and the CT scans were reviewed and interpreted by radiologists who did not know the clinical courses of the patients.

Analysis of risk factors for IPA

Risk factors for IPA were determined by comparing patients with definite IPA and control patients. Risk factors included age, sex, primary disease and its status (refractory or sensitive to cytotoxic chemotherapy), use of laminar air flow, duration of neutropenia, use of corticosteroids, treatment of hematologic malignancy. Neutropenia was defined as a number of peripheral neutrophils below 500/μL. Steroid administration for more than 14 days was regarded as steroid use.

In vitro analysis of LA, EIA and BDG

We investigated the sensitivity of each test *in vitro* in detecting circulating *Aspergillus* components. *Aspergillus fumigatus* was cultured in Sabouraud medium at 37°C for three days. Then, 100 μL of the culture medium were diluted with 900 μL of blood obtained from a healthy donor, and 100 μL of the

sample were serially diluted with the blood in the ratio of 1:10. All of these samples were subjected to the LA test, EIA (Platelia Aspergillus, Diagnostic Pasteur, France), and BDG assay. The cut-off levels in the BDG assay and in the EIA were 20 pg/mL.[8] and an optical density (OD) value of 0.372, respectively.[14]

Criteria for IPA, non-IPA and false-positive samples

Samples from non-IPA patients were regarded as non-IPA samples. Those from IPA patients were included in the non-IPA samples when they were taken either before the first antibiotic-resistant febrile episode or after the improvement of IPA: disappearance of fever and normalization of chest CT scans with or without scar formation. The other samples from IPA patients were defined as IPA samples.

Positive samples taken from non-IPA patients or from IPA patients before the first antibiotic-resistant febrile episode were regarded as false-positive samples.

Statistics

Univariate analysis using the chi-squared test and the Mann-Whitney U test were performed with patients' parameters to evaluate the risk of IPA. Then multivariate analysis of the risk factors with multiple logistic regression analysis were added for the proper variables. Values of $p < 0.05$ were considered to be statistically significant.

Results

Patients' characteristics

We examined 215 consecutive patients with hematologic malignancies, including acute myeloblastic leukemia (n=70), acute lymphoblastic leukemia (n=49), chronic myelocytic leukemia (n=27), chronic lymphocytic leukemia (n=1), non-Hodgkin's lymphoma (n=46), Hodgkin's disease (n=3), myelodysplastic syndrome (n=11) and others (n=8). The median age was 46 (16-83 years). Sixty-four patients received hematopoietic stem-cell transplantation. Seventy-five patients were isolated in a room equipped with laminar air flow. Ninety-five patients had a refractory hematologic disease.

Diagnosis of IPA and other fungal infections

Of the 215 patients, 30 (13.9%) were diagnosed as having IPA. For sixteen of those the diagnosis was definite while in 14 patients it was suspected. Twenty-two of the 30 IPA patients (73%) died during hospitalization and the other eight patients recovered with the recovery of neutrophils and intensive antifungal treatment. The death occurred due to IPA (n=9), Aspergillus tracheobronchitis (n=1), CNS aspergillosis (n=4), leukemic progression (n=3), and concomitant bacterial pneumonia (n=3). Eight of the 16 definite IPA patients had disseminated invasive aspergillosis, and the other eight patients had invasive aspergillosis localized to the lung. Involved organs identified at autopsy included the lung (n=16), CNS (n=5), gastrointestinal tract (n=4), heart (n=3), liver (n=3), spleen (n=3), thyroid gland (n=2), and adrenal gland (n=1). Other fungal infections included invasive candidiasis (n=4) and zygomycosis (n=1). Invasive candidiasis was caused by *Candida tropicalis* (n=2), *Candida glabrata* (n=1), and *Candida parapsilosis* (n=1).

Risk factors for IPA

Comparisons were performed between patients with definite IPA and control patients (Table 1). Univariate analysis revealed that primary disease (acute leukemia) (p=0.0060), status of the primary hematologic malignancy (p<0.0001) and duration of neutropenia (p<0.0001) were significant risk factors (Table 1). We added logistic regression analysis for further evaluation. Status of the primary hematologic malignancy (odds ratio, 2.071, 95% confidence interval, 1.269 to 3.380; p=0.0036), acute leukemia (odds ratio, 1.832, 95% confidence interval, 1.0368 to 3.237; p=0.0371) and shortening of neutropenia (per day) (hazard ratio, 0.9521, 95% confidence interval, 0.9180 to 0.9870; p=0.0081) remained significant risk factors.

In vitro analysis of LA and BDG assay

In vitro study results showed that the BDG assay is the most sensitive of the three assays for detecting the circulating *Aspergillus* antigens.The BDG assay was found to be approximately 10 and 100 times more sensitive than EIA and the LA test, respectively (Table 2).

Table 1. Characteristics of patients with definite invasive pulmonary aspergillosis and control patients

Factors		*Patients with definite invasive aspergillosis*	*Control patients*	*p value*
Number		16	185	
Age (range), yrs		54.1 (16-81)	45.1 (16-83)	0.0631
Sex	male/female	13/3	122/63	0.2739
Primary disease	acute leukemia/others[#]	15/1	110/75	0.0060*
Status of primary disease	remission/refractory	1/15	113/72	<0.0001*
Protective isolation	yes/no	4/12	67/118	0.4272
Duration of neutropenia (days)		36.1 (3-82)	19.5 (3-55)	<0.0001*
Steroid use	yes/no	2/14	21/164	0.8899
Treatment for hematologic malignancy	BMT/conventional chemotherapy	3/13	57/128	0.4019
Outcome	dead/alive	16/0	42/143	<0.0001*

*[#]Acute leukemia includes acute myeloblastic leukemia, acute lymphoblastic leukemia and chronic myelocytic leukemia in blastic crisis or accelerated phase. Others include non-Hodgkin's lymphoma, multiple myeloma, myelodysplastic syndrome, and chronic myelocytic leukemia in chronic phase. *Statistically significant.*

Table 2. Sensitivity of LA, ELISA and BDG for detecting the circulating *Aspergillus* antigens.

Diluted samples#	*LA*	*ELISA (optical density)**	*BDG (pg/mL)**
1	+	over	14690
2	+	2.856	1504
3	-	1.585	114
4	-	0.303	49.9
5	-	0.164	19.3
6	-	0.149	17.3
7	-	0.179	12.7

*Abbreviations; LA, latex agglutination test, ELISA, enzyme-linked immunosorbent assay and BDG, (1→3)-beta-D-glucan measurement; #samples were serially diluted from sample 1 to sample 7 with the blood obtained from a healthy volunteer. *The cut-off values of ELISA and BDG are 0.372 and 20 pg/mL, respectively.*

LA test

Seven of the 16 definite IPA patients, 5 of the 14 suspected IPA patients and 12 of the 185 control patients became positive in the LA test. One of the eight patients with localized invasive aspergillosis and six of the eight patients with disseminated invasive aspergillosis became positive in the LA test. In patient-based analysis, the sensitivity and specificity of the LA test were 44% and 94%, respectively. The sensitivity was 75% in the case of patients with disseminated invasive aspergillosis, but it decreased to 13% in the case of those with localized invasive aspergillosis. The sensitivity tended to be lower in the case of patients with localized *Aspergillus* infection than in those with disseminated disease (p=0.0629).

In *sample-based* analysis, 24 of the 179 definite IPA samples, 8 of the 180 suspected IPA samples and 17 of the 1,770 control samples became positive in the LA test, respectively. Five of 93 samples obtained from patients with localized invasive aspergillosis and 19 of 83 samples obtained from patients with disseminated invasive aspergillosis became positive in the LA test. In sample-based analysis, the sensitivity and specificity of the LA test were 13% and 99%, respectively. The sensitivity was 23% in the case of samples from patients with disseminated invasive aspergillosis, but it decreased to 5% in patients with localized infection. The sensitivity was lower in samples from patients with localized *Aspergillus* infection than in those from patients with disseminated infection, and the difference was statistically significant (p=0.0010).

BDG assay

Ten of the 16 patients with definite IPA , eight of the 14 suspected cases and 44 of the 185 control patients became positive for BDG. Three of the eight patients with localized invasive aspergillosis and seven of the eight patients with disseminated invasive aspergillosis became positive for BDG. In patient-based analysis, the sensitivity and specificity of the BDG assay were 63% and 76%, respectively. The sensitivity was 88% in the case of patients with disseminated invasive aspergillosis, but it decreased to 38% in those with localized invasive aspergillosis. The sensitivity was lower in patients with localized *Aspergillus* infection than in those with disseminated disease, and this difference was statistically significant (p=0.0406).

In *sample-based* analysis, 29 of the 178 definite IPA samples, 33 of the 210 suspected IPA samples and 117 of the 1,877 non-IPA samples were positive for BDG. Three of 99 samples obtained from patients with localized invasive aspergillosis and 26 of 79 samples obtained from patients with disseminated invasive aspergillosis became positive for BDG. In sample-based analysis, the sensitivity and the specificity of the BDG assay were 16% and 94%, respectively. The sensitivity was 33% in samples from patients with disseminated invasive aspergillosis, but decreased to 3% in those from patients with localized infection. The sensitivity was lower in samples from patients with localized *Aspergillus* infection than in those from patients with disseminated infection; this difference was statistically significant (p<0.0001).

Conventional X-ray

Eleven of the 16 patients with definite IPA and 9 of the 14 case of suspected IPA showed a variety of pulmonary signs on chest x-ray; these included ill-defined infiltrates (n=11), non-cavitary nodules (n=5) and pleural effusion (n=11). The lung lesions were equally distributed among all of the lobes and showed no predilection for a perihilar, medial, or peripheral location on frontal radiographs. At diagnosis, neither the air-crescent sign nor cavity formation was observed in any of the IPA patients. On average, abnormal findings on the conventional X-ray were documented 18.0 and 13.8 days after the onset of fever in cases of patients with definite IPA and in those with suspected IPA, respectively.

CT scan

A total of 291 CT scans were taken in 107 patients (16 patients with definite IPA, 14 with suspected IPA and 77 control patients). In 32 of the 107 patients, abnormal signs suggesting the presence of IPA were observed on CT scans. All of the 16 definite IPA patients developed abnormal CT signs including halo (n=6), air-crescent sign (n=2), multiple nodular consolidations (n=15), wedge-shaped consolidation (n=1), and thickening of the tracheobronchial wall (n=1) (Table 3). Of the 16 patients with definite IPA, seven developed halo and/or air-crescent signs, which are specific for IPA. By definition, all the patients with suspected IPA showed either the halo or air-crescent sign (Table 3). However, halo signs were observed in two control patients, in which IPA was discarded because lymphoma cells were histopathologically identified as involving the lung. When halo and air-crescent signs are defined as indicators of IPA, the sensitivity and specificity of the chest CT scan were 44% and 98%, respectively. However, all the IPA patients showed some abnormal signs on the chest CT scan and multiple nodular consolidations were observed in 15 of the 16 patients with definite IPA (94%).

Microbiological examination

Aspergillus species were cultured from all the patients with definite IPA. *Aspergillus* species were cultured from sputum (n=6), BAL (n=1), and autopsy specimens of

Table 3. Time interval between onset of fever and the day when each test became positive.

3a. Patients with definitive invasive pulmonary aspergillosis

Pt.#	Diagnosis	Outcome	CT# (day)	X-ray# (day)	LA# (day)	BDG# (day)	Culture day#	Culture source	CT findings halo	CT findings air-crescent	CT findings multiple nodular consolidations
1	definite	dead	5	6	19	19	32	sputum	-	-	+
2	definite	dead	31	31	34	30	autopsy	lung	+	-	+
3	definite	dead	29	29	9	negative	autopsy	lung	-	-	+
4	definite	dead	18	normal	negative	53	24	sputum	-	-	+
5	definite	dead	11	normal	negative	negative	autopsy	lung	+	-	+
6	definite	dead	9	16	16	16	autopsy	lung	-	-	+
7	definite	dead	12	normal	negative	negative	16	sputum	-	-	+
8	definite	dead	20	20	32	25	autopsy	lung	-	+	+
9	definite	dead	3	10	10	negative	5	sputum	-	-	+
10	definite	dead	5	20	12	5	25	sputum	+	-	+
11	definite	dead	7	10	negative	18	49	sputum	-	-	+
12	definite	dead	14	14	negative	negative	autopsy	lung	+	-	+
13	definite	dead	9	23	43	49	autopsy	lung	+	+	+
14	definite	dead	8	normal	negative	negative	autopsy	lung	-	-	+
15	definite	dead	7	normal	7	0	12	biopsy lung	-	-	-
16	definite	dead	12	19	negative	negative	autopsy	lung	+	-	+
average ± SD				12.5±8.2	18.0±7.8	20.2±12.9	23.8±17.9	23.3±14.5			

Table 3.B. Patients with suspected invasive pulmonary aspergillosis

Pt.#	Diagnosis	Outcome	CT# (day)	X-ray# (day)	LA# (day)	BDG# (day)	Microbiological examination day#	Microbiological examination source	CT findings halo	CT findings air-crescent	CT findings multiple nodular consolidations
17	probable	improved	9	normal	negative	negative	negative		+	+	-
18	probable	improved	16	16	69	27	negative		-	+	+
19	probable	improved	12	normal	negative	19	negative		+	-	+
20	probable	dead	10	10	negative	18	negative		+	-	+
21	probable	improved	6	normal	negative	14	negative		-	+	+
22	probable	dead	7	11	negative	negative	negative		+	-	-
23	probable	improved	8	8	8	13	42	sputum	+	-	+
24	probable	dead	11	14	negative	negative	negative		-	+	+
25	probable	dead	7	16	negative	negative	negative		+	-	+
26	probable	dead	9	normal	negative	negative	negative		+	-	+
27	probable	improved	19	15	negative	19	negative		-	+	+
28	probable	dead	8	16	15	15	21	sputum	+	-	+
29	probable	improved	9	normal	17	negative	negative		+	+	+
30	probable	improved	11	18	22	15	negative		-	+	-
average ± SD				10.1±3.6	13.8±3.3	26.2±24.4	17.5±4.5	31.5±14.8			

#Time interval between the onset of fever and the day when each test became positive. Abbreviations; ND = not detected, CT = computed tomography, LA = aspergillus latex agglutination test, BDG = (1→3)-beta-D-glucan and SD = standard deviation.

the lung (n=9). Invasive aspergillosis was diagnosed during life (n=7) and at autopsy (n=9).

In contrast, a microbiological identification was established in two of the 14 suspected cases of IPA. In these two patients, *Aspergillus fumigatus* was cultured from the sputum during life. The other 12 patients had no microbiological evidence of invasive aspergillosis.

Time interval between onset of fever and the day when each test became positive

In patients with definite IPA, CT scans, X-ray, LA, BDG and culture findings became positive 15.1 (range 3 to 31), 18.0 (range 6 to 31), 20.2 (7 to 43), 23.8 (range 0 to 53) and 23.3 (5 to 49) days after the onset of fever, respectively (Table 3). Positive findings in CT scans preceded those in the LA test by 7.1

(range -20 to 34) days, those in the BDG assay by 11.5 (range -7 to 40) days, those on conventional X-rays by 4.7 (range 0 to 14) days, and those in culture by 15.1 (range 2 to 42) (Table 3). In 6 of the 11 patients who became positive for either LA or BDG, positive findings in CT scans preceded them by more than seven days.

In patients with suspected IPA, CT scans, X-ray, LA, BDG and culture findings became positive 10.1 (range 6 to 19), 13.8 (range 8 to 18), 26.2 (8 to 69), 17.5 (range 13 to 27) and 31.5 (range 21 to 42) days after the onset of fever, respectively (Table 3). Positive findings in CT scans preceded those in the LA test by 16.6 (range 0 to 53) days, those in the BDG assay by 6.8 (range 0 to 11) days, those on conventional X-rays by 2.0 (range -4 to 9) days, and those in the fungal culture by 23.5 (range 13 to 34) days. In 6 of the 9 patients who became positive for either LA or BDG, positive findings in CT scans preceded the assay positivity by more than seven days.

False-positive results of the blood tests

We encountered 17 samples false-positive for LA from 12 control patients. During neutropenia, we examined 871 samples for LA, of which 13 became positive (1.5%). In contrast, we examined 899 samples for LA during periods with no neutropenia, of which 4 became positive (0.4%). False-positives occurred more frequently during neutropenia than during periods with no neutropenia, and the difference was statistically significant (p=0.0280).

We encountered 117 samples false-positive for BDG assay from 44 control patients, of which 27 samples were taken from four patients with invasive candidiasis. The other 90 samples were taken from 40 control patients without evidence of fungal infection. Sixty-five of the 90 samples false-positive for BDG (72%) were taken during neutropenia. During neutropenia, we examined 865 samples for BDG assay, of which 65 became positive (7.5%). In contrast, we examined 949 samples for BDG during a period with no neutropenia, of which 25 became positive (2.6%). False-positives occurred more frequently during neutropenia than during a period with no neutropenia, and this difference was statistically significant (p<0.0001).

Discussion

Early studies showed the usefulness of blood tests[10,15] and CT scans[2,16] for diagnosis of IPA, but a comparison of these tests has not been performed. At present, we do not know which is better or quicker at diagnosing IPA. This is the first prospective study to compare these tests. In this study, chest CT scans became positive in all the patients with definite IPA while the other tests sometimes remained negative, implying that chest CT scans are more effective at detecting IPA lesions than blood tests or conventional X-rays. The blood tests and chest X-rays are useful for a limited number of patients, probably those with advanced IPA. Sensitive blood tests may help us to confirm a diagnosis of suspected IPA, but they have some limitations compared to CT scans. Although many fungal infections are not recognized antemortem and are only discovered at autopsy, we succeeded in making a correct diagnosis of IPA in all of the patients subsequently proven to have IPA at autopsy in this study. We suppose that the chest CT scan contributed to making the IPA diagnosis. We recommend an early CT scan if patients have antibiotic-resistant fever after cytotoxic chemotherapy. We will describe results of comparison of the blood tests and chest CT scans in the following paragraphs.

Firstly, the blood tests may have lower sensitivities than CT scans. In patient-based analysis, the sensitivities of the LA test and the BDG assay were 44% and 63%, respectively. Although the sensitivity of the BDG assay was higher than that of the LA test, the finding that 149 of the 178 IPA samples were negative for BDG cannot be neglected. In sample-based analysis, the sensitivities of the LA test and the BDG assay decreased to 13 and 16%, respectively. This indicates that repeated blood tests are necessary to make a diagnosis of IPA even when a sensitive blood test is utilized. Interestingly, the sensitivities of the blood tests were influenced by the disease distribution of invasive aspergillosis. While the sensitivities of the LA test and the BDG assay were 75% and 63%, respectively, in patients with disseminated invasive aspergillosis, these values decreased to 13% and 38% in patients with localized invasive aspergillosis. When the lesion was localized to the lung, it seemed to be difficult to detect the lesion by means of blood tests, and blood tests may not be suitable for early diagnosis of IPA.

Secondly, there was a time lag between diagnosis by CT and diagnosis on the basis of blood test results. In our study, positive findings in CT scans preceded those in the LA test and the BDG assay by 7.1 and 11.5 days, respectively, on average. Such a time lag may be critical for neutropenic patients. Because the reticuloendothelial system rapidly clears circulating fungal components such as galactomannan antigen,[17] a large amount of antigen is required for its presence to be detected using the LA test or the BDG assay. To supply such a large amount of fungal antigen into the peripheral blood, there needs to be an infection focus. In the majority of patients with invasive aspergillosis, the lung is the main organ affected by *Aspergillus* species which may continuously release their antigens into the blood. Thus, chest CT scan will become positive before the LA test or the BDG assay, both of which may be markers of advanced IPA.

Thirdly, CT scans are more economical than blood tests for IPA diagnosis. In Japan, a BDG assay and a LA test cost $33 and $25, respectively, and it takes $150 to perform a CT scan. When BDG and LA assays are ordered once or twice a week during neutropenia, the total costs will be almost equivalent. Considering the wide difference in the ability to detect early changes indicative of IPA, early CT scans are more cost-effective for IPA diagnosis than the blood tests.

Lastly, the blood tests occasionally produce false-positive results especially during the neutropenic period, reducing the specificity of these tests. Sulahian *et al.* reported comparative outcomes indicating that false-positive results were obtained for 31 of 169 (19%) BMT patients without clinical signs of aspergillosis. Samples taken within the first month after BMT were occasionally positive for *Aspergillus* antigens by EIA, giving a specificity of 81% in the case of these patients.[18]

Because we identified *Aspergillus*-specific DNA by the polymerase chain reaction method in some samples false-positive for LA or BDG (data not shown), it is likely that some fungi including *Aspergillus* species, or their components did actually exist in the blood samples obtained from the control patients. It is quite interesting that a majority of the false-positive results occurred during neutropenia. This may suggest the presence of a transient fungemia or *Aspergillus* antigenemia following cytotoxic chemotherapy and spontaneous clearance from the blood stream with the recovery of the immune system. At present, the clinical significance of such asymptomatic antigenemia remains unknown. However, it is difficult to interpret the positive results of the blood tests in the case of patients without characteristic CT signs, because IPA develops in patients with prolonged neutropenia.

This study showed that chest CT scans were more effective in detecting early pulmonary changes indicative of IPA than the blood tests and we therefore recommend the early use of CT scans to check for small pulmonary lesions when neutropenic patients develop antibiotic-resistant fever. However, CT scans have some problems to be discussed. One limitation of chest CT scans remains the rare cases in which extrapulmonary organs such as the palate or gastrointestinal tract are the portals of *Aspergillus*. In such cases, the chest lesions detected by CT scan may be a manifestation of disseminated invasive aspergillosis.

The most significant limitation is that findings of the CT scans are not limited to invasive aspergillosis. Both halo and air-crescent signs have been reported in a variety of other diseases, for example tuberculosis, cytomegalovirus infection, herpes simplex infection, Legionella pneumonia, metastasis of cancers and mucormycosis.[19] Furthermore, the most common findings in patients with IPA, specifically multiple nodular consolidations, may be non-specific. These findings do not always indicate the presence of Aspergillus infection in highly immunocompromised patients. Diagnosis of suspected IPA based upon the CT findings should be confirmed by other examinations.

Fungal culture is the standard for confirming IPA diagnosis. However, sensitivity of *Aspergillus* culture is not high, and it takes one to two weeks to culture *Aspergillus* species from the sputum or lesions. In this study, Aspergillus was cultured during life in seven of the 16 patients with definite IPA, and there was a 15.1 day interval between the CT diagnosis and the microbiological diagnosis. The conventional fungal culture is not helpful for antemortem confirmation of IPA diagnosis. We suppose that the antigen detection tests may be promising for it. Of the 16 IPA patients, seven and ten patients tested positive for LA and BDG, respectively in this study. Using the combination of the conventional fungal culture and the antigen detection methods, antemortem diagnosis of *Aspergillus* infection was confirmed in 12 of the 16 cases of definite IPA. When cultures are negative or cannot be performed in life and the diagnosis of IPA is only suspected on the basis of CT findings, serologic data can represent an important confirmatory diagnostic tool.

In conclusion, the chest CT scan is the most sensitive method for diagnosing IPA, and we recommend early chest CT scans to detect minimal changes of IPA. Based upon these findings, aggressive anti-fungal therapy should be initiated. The blood tests may be useful as an adjunct to confirm the IPA diagnosis, when the IPA diagnosis is made without microbiological or histopathologic evidence of IPA. Considering the sensitivities of the blood tests, frequent sampling is quite important.

Contributions and Acknowledgments

MK, YT, YK and SO designed the study, MK was responsible for the data management and prepared the manuscript. MK and TS contributed to paper writing. TM performed LA and BDG analyses and interpreted the data. TM and KO examined the CT findings. MK, YT, TM, TS, SO UM and HH collaborated in patient care and data analysis. KK performed the pathologic examinations. MK contributed the execution of the study and statistical analysis.

The criteria for authors' name order are: 1st name: principal investigator and writer; 2nd to 4th names: contribution in study design; 5th and 6th names: examination of the CT findings; 7th to 9th names: clinical work; 10th name: examination of the pathologic specimens, 11th name: head of the Department in which the study was performed.

We thank Miss Suzuki (Sanofi Fujirebio Diagnostic Inc.) for assistance with the EIA and the LA assay, and Mr. Tanaka and Dr. Ban Ph.D. (BML Corporation) for assistance with the (1-3)-beta-D-glucan assay.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received January 4, 2000; accepted March 21, 2000.

Potential implications for clinical practice

- The sensitivities of the latex agglutination test and the beta D glucan assay in diagnosing invasive pulmonary aspergillosis were 44% and 63%, respectively. They were lower than that of chest computed tomography (CT).
- There was a time lag between diagnosis by CT and diagnosis on the basis of blood test results.
- Chest CT scan is faster and more sensitive than blood tests and X-rays for diagnosing IPA. Blood tests play a confirmatory role in an IPA diagnosis based upon CT findings and clinical symptoms.

References

1. Denning DW, Stevens DA. Antifungal and surgical treatment of invasive aspergillosis: review of 2,121 published cases. Rev Infect Dis 1990; 12:1147-201.
2. Caillot D, Casasnovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. J Clin Oncol 1997; 15:139-47.
3. Duthie R, Denning DW. Aspergillus fungemia: report of two cases and review. Clin Infect Dis 1995; 20:598-605.

4. Klein DL, Gamsu G. Thoracic manifestations of aspergillosis. Am J Roentgenol 1980; 134:543-52.
5. Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. Radiology 1985; 157:611-4.
6. Hruban RH, Meziane MA, Zerhouni EA, Wheeler PS, Dumler JS, Hutchins GM. Radiologic-pathologic correlation of the CT halo sign in invasive pulmonary aspergillosis. J Comput Assist Tomo 1987; 11:534-6.
7. Obayashi T, Yoshida M, Tamura H, Aketagawa J, Tanaka S, Kawai T. Determination of plasma (1-->3)-beta-D-glucan: a new diagnostic aid to deep mycosis. J Med Vet Mycol 1992; 30:275-80.
8. Obayashi T, Yoshida M, Mori T, et al. Plasma (1-->3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet 1995; 345:17-20.
9. Hopwood V, Johnson EM, Cornish JM, Foot AB, Evans EG, Warnock DW. Use of the Pastorex aspergillus antigen latex agglutination test for the diagnosis of invasive aspergillosis. J Clin Pathol 1995; 48:210-3.
10. Verweij PE, Stynen D, Rijs AJ, de Pauw BE, Hoogkamp-Korstanje JA, Meis JF. Sandwich enzyme-linked immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J Clin Microbiol 1995; 33:1912-4.
11. Pizzo PA. Management of fever in patients with cancer and treatment-induced neutropenia. N Engl J Med 1993; 328:1323-32.
12. Denning DW, Evans EG, Kibbler CC, et al. Guidelines for the investigation of invasive fungal infections in haematological malignancy and solid organ transplantation. British Society for Medical Mycology. Eur J Clin Microbiol Infect Dis 1997; 16:424-36.
13. Mori M, Galvin JR, Barloon TJ, Gingrich RD, Stanford W. Fungal pulmonary infections after bone marrow transplantation: evaluation with radiography and CT. Radiology 1991; 178:721-6.
14. Stynen D, Goris A, Sarfati J, Latge JP. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. J Clin Microbiol 1995; 33:497-500.
15. Machetti M, Feasi M, Mordini N, et al. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. Bone Marrow Transplant 1998; 21:917-21.
16. Heussel CP, Kauczor HU, Heussel GE, et al. Pneumonia in febrile neutropenic patients and in bone marrow and blood stem-cell transplant recipients: use of high-resolution computed tomography. J Clin Oncol 1999; 17:796-805.
17. Bennett JE, Friedman MM, Dupont B. Receptor-mediated clearance of Aspergillus galactomannan. J Infect Dis 1987; 155:1005-10.
18. Sulahian A, Tabouret M, Ribaud P, et al. Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of invasive aspergillosis. Eur J Clin Microbiol Infect Dis 1996; 15:139-45.
19. Libshitz HI, Pagani JJ. Aspergillosis and mucormycosis: two types of opportunistic fungal pneumonia. Radiology 1981; 140:301-6.