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Evaluation of Mycobiota in Patients with Hematological Malignancies in One-Day Examination

Ocena mikrobioty chorych na nowotwory układu krwiotwórczego w badaniach „jednego dnia”

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Abstract

Background. Patients suffering from malignancies of the blood and lymphatic systems belong to a population with the highest risk of developing fungal infections.

Objectives. The aim of the study was an epidemiological evaluation of respiratory, digestive, and urinary tract mycobiota determined on the basis of sputum, feces, and urine cultures from patients who came on a specific day to a day unit of a hematological clinic.

Material and Methods. One-day examinations were performed five times from Nov. 2006 to May 2008. A total of 161 patients were examined, including 66 patients with acute myeloblastic leukemia (AML), 23 with acute lymphoblastic leukemia (ALL), 8 with chronic myelogenous leukemia (CML), 1 with myelofibrosis (MF), 5 with myelodysplastic syndrome (MDS), 6 with Hodgkin's disease (HD), 30 with non-Hodgkin's lymphoma (nHL), 13 with chronic lymphocytic leukemia (CLL), and 9 with multiple myeloma (MM).

Results. A total of 72/139 (51.8%), 82/127 (64.7%), and 12/140 (8.5%) positive samples were obtained from sputum, feces, and urine respectively. Sputum cultures revealed 93, fecal cultures 145, and urine cultures 15 fungal strains. Most commonly isolated were *Candida albicans* (113 strains) and non-*albicans Candida* species (91 strains). Analysis of the incidence of fungi in the sputum, feces, and urine in relation to diagnosis revealed that the mycobiota of patients with malignant lymphomas and chronic lymphocytic leukemia were more differentiated than those with acute leukemia. This did not concern patients undergoing mega-dose chemotherapy and those staying in laminar flow rooms, who had only a few positive materials infected with single strains of *C. albicans*.

Conclusions. The prevalence of *Candida* species in the mycobiota of patients with hematological malignancies, with the high participation of non-*albicans Candida* species, should be treated as a potential factor increasing the risk of invasive fungal infection. This finding points to the necessity of implementing antifungal prophylaxis in patients with malignant lymphomas and chronic lymphocytic leukemia treated with purine analogues and/or monoclonal antibodies (Adv Clin Exp Med 2009, 18, 1, 55–62).

Key words: mycobiota, fungi, hematological and lymphatic system malignancies.

Streszczenie

Wprowadzenie. Chorzy na choroby rozrostowe krwi i układu chłonnego należą do populacji obciążonej największym ryzykiem rozwoju grzybiczy.

Cel pracy. Charakterystyka epidemiologiczna chorych, przebywających w danym dniu na oddziale hematologicznym z uwzględnieniem mikrobioty układu oddechowego, pokarmowego, moczowo-płciowego, określonej na podstawie wyników hodowli płwociny, kału i moczu.

Materiał i metody. Badania „jednego dnia” wykonano pięciokrotnie (XI 2006, VIII 2007, XI 2007, IV 2008 i V 2008). Zbadano łącznie 161 chorych, w tym m.in. 66 chorych na ostrą białaczkę szpikową (AML), 23 – ostrą białaczkę limfoblastyczną (ALL), 8 – przewlekłą białaczkę szpikową (CML), 1 – ostre mielofibrozę (MF), 5 – zespół mielodysplastyczny (MDS), 6 – chorobę Hodgkina (HD), 30 chłoniaki nieziarnicze (nHL), 13 – przewlekłą białaczkę limfatyczną (CLL) i 9 na szpiczaka mnogiego (MM).

Wyniki. Uzyskano łącznie 72/139 (51,8%), 82/127 (64,7%) i 12/140 (8,5%) dodatnich materiałów odpowiednio z płwociny, stolca i z moczu. W płwocinie wyhodowano 93, w kale 145 i moczu 15 szczepów grzybów. Najczęściej hodowano grzyby z rodzaju *Candida alb.* (113 szczepów) i *Candida non alb.* (91 szczepów). Analiza występowania szczepów grzybów zarówno w płwocinie, jak i w moczu i stolcu w zależności od rozpoznania wykazała, że u chorych na chłoniaki złośliwe i przewlekłą białaczkę limfatyczną częściej występowały różne szczepy grzybów w porównaniu z chorymi na ostre białaczki. U chorych poddanych megachemioterapii, przebywających w pokojach z wymuszonym przepływem sterylnego powietrza (*laminar flow*) tylko pojedyncze materiały były dodatnie i były to pojedyncze szczepy *Candida alb.*

Wnioski: Wyniki badań wskazują na utrzymywanie się w mikrobiocie chorych na nowotwory układu krwiotwórczego grzybów z rodzaju *Candida*, z dużym odsetkiem rodzajów *Candida non albicans*. U chorych na chłoniaki złośliwe i przewlekłą białaczkę limfocytową leczonych analogami puryn i/lub przeciwciałami monoklonalnymi jest wskazane stosowanie profilaktyki przeciugrzybiczej, podobnie jak u chorych na ostre białaczki (*Adv Clin Exp Med* 2009, 18, 1, 55–62).

Słowa kluczowe: mikrobiota, grzyby, choroby rozrostowe krwi i układu chłonnego.

In the past 25 years an increasing participation of fungal pathogens in the etiopathology of infections has been observed [1]. According to Baddley and Clark, this is a consequence of the generalized administration of antibiotics and the increasing population of people undergoing immunosuppression [1, 2]. Patients suffering from hematological malignancies, both in the phase of cytostatic treatment, which induces remission of the disease, as well as in those on mega-dose chemotherapy and those who have had hematopoietic cell transplant, constitute a population with the highest risk of developing fungal infection [2, 3]. In patients treated with standard doses of cytostatic agents, the most important factors facilitating the spread of fungal infection include neutropenia along with the compromised immunological status as a result of the disease [4]. Patients undergoing transplantation, regardless of its kind, are affected by profound and long-lasting disturbances of specific and non-specific immunological mechanisms.

Periodic evaluation of the mycobiota of patients with hematological malignancies at different stages of therapy allows one to diagnose or predict fungal infection; moreover, they enable an evaluation of the efficacy of prophylactic administration of antifungal drugs. The one-day method was found useful in fully evaluating the risk of fungal infection, especially with non-albicans *Candida* species and mold infections. These are prospective studies which provide insight into the mycobiota of all patients hospitalized on a given day. They enable a determination of the degree of colonization and also provide information on all the species and strains of fungi in individual patients. The one-day examination method was employed to evaluate the incidence of septic conditions in Poland and anemia in malignant diseases in France [5, 6]. Mycological studies using this method were first performed by the authors in November 2002 and in November 2004 [7].

The aim of the study was to provide an epidemiological description of patients with blood

and lymphatic system malignancies who were staying on a particular day in a hematological ward. The respiratory, digestive, and urinary tract mycobiota were investigated by means of sputum, feces, and urine cultures.

Material and Methods

The investigations involved patients of the Department of Hematology, Blood Neoplasms, and Bone Marrow Transplantation, Wrocław Medical University, who were hospitalized on Nov. 27, 2006 (examination A), August 31, 2007 (examination B), Nov. 29, 2007 (examination C), April 09, 2008 (examination D), and May 29, 2008 (examination E). The diagnoses of the patients included in the study are shown in Table 1.

Examination A comprised 30 patients, 11 women and 19 men aged from 22 to 68 years; 25 were hospitalized in the medical ward in 2- to 4-bed rooms and 5 were in separate single-bed rooms. Examination B involved 35 patients, 17 women and 18 men aged from 18 to 70 years; the patients were hospitalized in 2- to 4-bed rooms and 4 were in separate single-bed rooms (3 after PBPC transplantation, 1 before mega-dose chemotherapy). Examination C included 34 patients, 15 women and 19 men aged 19–74 years; 4 were hospitalized in the transplant ward and the remaining patients in the medical ward. Examination D involved 29 patients, 11 women and 18 men aged 20–83 years; all the patients were in the medical ward in 3-, 4-, and 5-bed rooms. Examination E included 33 patients, 12 women and 21 men aged 18–74 years; 5 patients were in the transplant ward, 2 of whom had had mega-dose chemotherapy and hematopoietic cell transplant. Twenty-four of the 161 patients developed agranulocytosis (granulocyte count below 500/μl). These were 6, 5, 3, 4, and 6 patients in examinations A, B, C, D, and E, respectively.

In the group of 66 patients with AML, 7 were in the remission induction phase (epirubicin +

Table 1. Diagnoses of the patients included in the study**Tabela 1.** Liczba chorych w kolejnych badaniach w zależności od rozpoznania

Diagnoses (Rozpoznanie)	Consecutive examinations, n = number of patients (Kolejne badania, n = liczba chorych)					
	A	B	C	D	E	Totals
AML	11	18	15	12	10	66
ALL	3	4	7	4	5	23
CML	4	4	0	0	0	8
MF	1	0	0	0	0	1
MDS	0	0	1	3	1	5
HD	3	0	2	0	1	6
NHL	5	5	8	5	7	30
CLL	2	2	0	3	6	13
MM	1	2	1	2	3	9
Total (Suma)	30	35	34	29	33	161

AML – acute myeloblastic leukemia, ALL – acute lymphoblastic leukemia, CML – chronic myelogenous leukemia, MF – myelofibrosis, MDS – myelodysplastic syndrome, HD – Hodgkin's disease, nHL – non-Hodgkin's lymphoma, CLL – chronic lymphocytic leukemia, MM – multiple myeloma.

AML – ostra białaczka szpikowa, ALL – ostra białaczka limfoblastyczna, CML – przewlekła białaczka szpikowa, MF – ostemielofibroza, MDS – zespół mieloblastyczny, HD – choroba Hodgkina, nHL – chłoniaki nieziarnicze, CLL – przewlekła białaczka limfatyczna, MM – szpiczak mnogi.

+ cytosine arabinoside), 29 received remission consolidation therapy (mitoxantron + cytosine arabinoside, in high doses [HAM], or high-dose cytosine arabinoside [HD Ara-C]), and the remaining patients were on remission sustaining therapy. Of the 23 ALL patients, 12 received remission-inducing therapy (prednison, vincristin, epirubicine,

L-asparaginase) and 11 consolidation therapy (cytrophosphamide, cytosine arabinoside, or methotrexate and etoposide). In the group of 13 CLL patients, purine analogues and alemtuzumab (anti-CD52 monoclonal antibody) were administered to 7 (53.9%) and 2 (15.3%) patients, respectively. In the group of 30 nHL patients, rituximab (anti-CD20 monoclonal antibody) was administered to 3 patients (10.0%). Seventy-eight percent of the of patients suffering from acute leukemia and 38% of those with lymphoproliferative malignancy were administered prophylactic ketoconazole or fluconazole. Invasive fungal infection (IFI) was not detected; however 4 patients showed clinical signs of probable mycoses (FUO and/or X-ray of lung) and were treated with voriconazole.

One day before the assessment the patients were informed that the aim of the study was to determine the presence and kind of fungi infecting them. Participation in the study was voluntary and the patients gave their written consent. The patients were given three sterile containers and were asked to collect sputum after rinsing their mouth with boiled water, midstream urine, and feces samples by means of a glass rod attached to one container. The material was incubated on Sabouraud's culture medium with chloramphenicol and Chromagar. The isolated fungi were identified on the basis of their morphology and biochemical properties with the ID32C test (BioMerieux).

Results

There were 87/93 containers collected in examination A: 30 sputum, 27 feces, and 30 urine samples (Table 2). The cultures of the patients' material revealed the presence of fungi in 37/87 samples, including 18/30 sputum samples, 17/27 feces, and 2/30 urine samples. A total of 49 species

Table 2. Investigated material in five one-day examinations**Tabela 2.** Badany materiał w 5 próbach jednego dnia

Consecutive examination (Kolejne badania)	No. of patients (Liczba pacjentów)	No. of samples tested/ No. (%) of positive results (Liczba badanych materiałów/ liczba dodatnich hodowli (%))			No. of strains isolated from (Liczba izolatów uzyskanych z)			
		sputum	feces	urine	sputum	feces	urine	total
A	30	30/18 (60%)	27/17 (62.9)	30/2 (6.6)	23	24	2	49
B	35	27/12 (44)	26/13 (50)	31/3 (9.6)	15	19	3	37
C	34	28/17 (60.7)	31/23 (74)	32/6 (18.75)	22	39	8	70
D	29	28/10 (35.7)	28/19 (67.8)	29/1 (3.4)	15	33	1	49
E	33	26/15 (57.6)	15/10 (66.6)	18/0 (0)	18	27	0	45
A+B+C+D+E	161	139/72 (51.8)	127/82 (64.5)	140/12 (8.5)	93	142	14	249

of fungi were cultured: 23 in sputum, 24 in feces, and 2 in urine the specimens. The most commonly isolated fungal species were *Candida* (45/49 strains), including 28 *C. albicans* and 17 non-*albicans Candida* strains. Of other fungal species, 4/53 were isolated exclusively from feces (2 *Geotrichum*, 1 *Trichosporon asahii*, and 1 *Saccharomyces cerevisiae*) (Table 3). The same fungal species were cultured simultaneously from two materials in 10 patients.

There were 84/105 containers collected in examination B: 27 sputum, 26 feces, and 31 urine specimens. Culture revealed the presence of fungi in 12/27 sputum specimens, 13/26 feces specimens, and 3/31 urine specimens. The total of 37 fungal species were isolated, including 15 in sputum, 19 in feces, and 3 in urine specimens. Most

commonly isolated fungi belonged to the *Candida* species, with a slight prevailing non-*albicans Candida* species (15 samples) of *C. albicans* (14 samples). Moreover, culture revealed the presence of *Aspergillus sp.* (1 sputum, 1 feces), *Geotrichum sp.* (1 sputum, 2 feces), *Trichosporon sp.* (1 feces, 1 urine), and *Saccharomyces cerevisiae* (1 sputum). One person revealed the presence of *C. glabrata* in three materials and in another three patients fungi were present in two materials (*Aspergillus sp.*, *C. krusei*, *Geotrichum sp.*).

In examination C, 91/102 containers were collected: 28 sputum, 31 feces, and 32 urine specimens. Culture revealed the presence of fungi in 46/91 samples, including 17/28 sputum samples, 23/31 feces samples, and 6/32 urine samples. In total, 70 fungal species were cultured: 22 in spu-

Table 3. Fungal species isolated in the material from patients in five one-day examinations

Tabela 3. Grzyby wyizolowane z materiału uzyskanego w 5 próbach “jednego dnia”

Fungi (Grzyby)	No. of isolates obtained from (Liczba izolatów uzyskanych z)															
	A			B			C			D			E			Total
	S	F	U	S	F	U	S	F	U	S	F	U	S	F	U	
<i>C. albicans</i>	15	11	2	6	7	1	13	15	1	8	10	0	14	10	0	113
<i>C. glabrata</i>	2	2	0	1	3	1	2	6	3	3	4	0	2	3	0	32
<i>C. krusei</i>	0	2	0	3	3	1	2	4	1	3	3	0	2	2	0	25
<i>C. dubliniensis</i>	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	5
<i>C. inconspicua</i>	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	3
<i>C. tropicalis</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	3
<i>C. lambica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
<i>C. spp.</i>	3	0	0	2	2	0	3	1	3	0	3	0	0	3	0	19
<i>Trichosporon asahii</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Trichosporon sp.</i>	0	0	0	0	1	1	1	4	0	0	0	0	0	1	0	8
<i>Geotrichum sp.</i>	0	2	0	1	2	0	0	5	0	0	0	0	0	1	0	11
<i>Rhodothorula</i>	0	0	0	0	0	0	0	1	0	0	6	0	0	1	0	8
<i>S. cerevisiae</i>	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	3
<i>Acremonium sp.</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
<i>Aspergillus niger</i>	0	0	0	0	0	0	0	0		0	0	1	0	0	0	1
<i>Aspergillus sp.</i>	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2
<i>Cladosporium sp</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Fusarium sp.</i>	0	0	0	0	0	0	0	2	0	0	1	0	0	1	0	4
<i>Mucor sp</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Penicillium sp</i>	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	3
<i>Scopulariopsis</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Mycelia sterali</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	3
Total	23	24	2	15	19	3	22	39	8	15	33	1	18	27	0	249

A, B, C, D, E – individual one-day examinations.

S – sputum, F – feces, U – urine.

A, B, C, D, E – pojedyncze próby “jednego dnia”.

S – płwocina, F – kał, U – mocz.

Table 4. The number of positive samples in five one-day examinations in relation to diagnosis and place of hospitalization**Tabela 4.** Liczba dodatnich hodowli w 5 badaniach „jednego dnia” w zależności od diagnozy i miejsca hospitalizacji

Diagnosis, n = number of patients (Rozpoznanie, n = liczba chorych)	No. of positive samples and percentage of patients (%) in relation to diagnosis and location (Liczba dodatnich hodowli (n) i procent pacjentów w zależności od rozpoznania i miejsca hospitalizacji)		
	sputum	feces	urine
Patients hospitalized in a medical ward, n (%) (Pacjenci hospitalizowani na oddziale hematologicznym)			
AML 60	20 (33.3)	24 (40)	9 (15)
ALL 23	9 (39.1)	11 (47.9)	0
NHL 26	13 (50)	13 (50)	3 (11.5)
CLL 13	6 (46.1)	4 (30.7)	2 (15.3)
Patients hospitalized in a transplant ward (n) (Pacjenci hospitalizowani na oddziale przeszczepowym)			
AML 6	0	0	0
NHL 4	1	1	0
HD 4	1	0	1
MM 4	2	1	0

tum, 39 in feces, and 9 in urine. The most prevalent in the investigated material were *Candida* species, of which 29 were *C. albicans* and 25 non-*albicans Candida* strains. Moreover, 15 strains of other fungi were isolated. Thirteen patients revealed the presence of fungi in two materials (8 *C. albicans*, 2 *C. glabrata*, 2 *C. krusei*, and *Trichosporon sp.*). Most commonly the materials were sputum and feces (12 subjects) as well as urine and feces (1 person).

In examination D, 85/87 materials were collected: 28/29 sputum, 28/29 feces, and 29/29 urine samples. Culture of the materials revealed the presence of fungi in 30/87 samples: 10/28 subjects in sputum, 19/28 in feces, and 1/29 in urine. A total of 49 fungal species were cultured including 15 species isolated in sputum, 33 in feces, and 1 in urine. The most commonly isolated fungal species were *C. albicans* (18 strains) and non-*albicans Candida* (16 strains). The remaining 15 species were 6 *Rhodotorula sp.* (feces), 3 *Penicillium* (1 sputum, 2 feces), and single strains isolated only from feces samples (*Fusarium*, *Scopulariopsis*, *Cladosporium*, *Mucor*, and an unidentified mold) and 1 *Aspergillus niger* in a urine sample. Eight subjects had the

same kind of fungus in two materials (sputum and feces: 2 *C. glabrata*, 4 *C. albicans*, 1 *C. krusei*). *C. albicans*, *C. krusei*, and *Penicillium* were isolated from the sputum and feces specimens of 1 subject.

In examination E, 59 containers with samples were collected of the 99 distributed: 26 sputum, 15 feces, and 18 urine samples. Culture revealed the presence of fungi in 15/26 sputum samples and in 10/15 feces samples; all the urine samples were sterile. Altogether, 45 fungal species were isolated: 18 in sputum and 27 in feces. Most commonly they belonged to *C. albicans* (24 strains, 14 sputum, 10 feces) and non-*albicans Candida* strains (15 strains, 4 sputum, 11 feces). Other fungi were isolated exclusively from feces: 1 *Geotrichum sp.*, 1 *Rhodotorula sp.*, 1 *Trichosporon sp.*, 1 *Fusarium sp.*, and 2 molds. Moreover, the same strain was isolated from two materials in 5 subjects: 4 *C. albicans* and 1 *C. glabrata*.

Discussion

The use of novel therapeutic procedures, including aggressive anticancer therapy as well as organ and hematopoietic cell transplant, has sig-

nificantly increased the risk of fungal infections [8]. Thus it has become necessary to improve diagnostics and antifungal therapy. Analysis of mycobiota performed in the form of one-day examination provides information on the prevalence and profile of fungal species in particular materials. The numbers of patients involved in the successive examinations, the percentages of patients with specific diagnoses, as well as the percentages of patients with agranulocytosis were similar on all the test days. The examinations were performed in various seasons and under variable atmospheric conditions. The highest number of fungal species was isolated in the examinations of November 2007 (examination C) and November 2006 (examination A), which may suggest a role of climatic conditions. Moreover, examinations A and C revealed the highest numbers of mixed infections, caused by 2–3 fungal species, which were isolated most commonly from the sputum and feces, in one patient also from urine. The observed changes in the prevalence of colonization and the composition of the mycobiota may have been caused by seasonal nutritional changes or an increased exposure to viral infections. Patients who have undergone mega-dose chemotherapy and initial sterilization therapy including antifungal agents who were staying in laminar flow rooms had only single-positive materials and only single *C. albicans* strains were isolated.

The analysis of the prevalence of fungal species in sputum as well as in urine and feces in relation to the diagnosis demonstrated that patients with malignant lymphoma and chronic lymphatic leukemia were more often infected with various fungal species compared with subjects with acute leukemia, despite the fact that the latter more often developed agranulocytosis (Table 4). This may have resulted from the use of the purine analogues administered to patients with lymphoma and lymphocytic leukemia, which cause profound immunosuppression without neutropenia. The use of monoclonal antibodies, such as rituximab and alemtuzumab, may also predispose to fungal infections in this group of patients [9]. On the other hand, antifungal prophylaxis was administered to 76% of the leukemia patients and to only 38% of the patients with the remaining hematological malignancies. Prophylactic administration of fluconazole may affect the distribution of various *Candida* species in patients with hematological malignancies. This group more often develops non-albicans *Candida* infection, especially with strains characterized by a low susceptibility to fluconazole, such as *C. krusei* (naturally resistant), *C. glabrata*, *C. inconspicua*, *C. norvegensis*, and others [10, 11]. In the study by Pagano, non-albi-

cans *Candida* spp. were responsible for 57% of all episodes of invasive fungal infection, although the prophylactic use of itraconazole was associated with an increased prevalence of *C. albicans* infection [12]. In the group of patients of the present study receiving prophylactic ketoconazole, non-albicans *Candida* strains were isolated from 33% of the patients and *C. albicans* in 41%. The incidence of *C. albicans* was much higher in the patients who did not receive any antifungal agents (*C. albicans* was isolated from at least one of the investigated materials in 70–75% of patients). Among the non-albicans *Candida* species, *C. glabrata* and *C. krusei* were the most common.

It is interesting to note that in the examination in November 2004, *C. glabrata* was not isolated from any of the patients, while in 2002 and in 2006–2008 the species constituted over 10% of all isolates. This observation confirms the variability of mycobiota in hospitalized patients and proves the necessity of regular monitoring.

One-day examinations performed repeatedly since the year 2002 have proved the persisting risk of fungal infections caused by *Candida* spp. [7], which are the most common pathogens responsible for fungal infections in patients with blood and lymphatic system malignancies [16–18]. It should be emphasized that, apart from *Candida* species, other fungal strains, such as *Geotrichum*, *Trichosporon*, *Rhodotorula*, and *Saccharomyces*, were isolated from the patients and they constituted 12% of all the isolates. All the above species are notorious for causing infections in hematological patients, and special significance has been attributed to *Trichosporon* which, due to a natural resistance to echinocandins and frequently decreased susceptibility to amphotericin B, may evoke breakthrough infections [19].

Apart from *Candida*, another fungal pathogen commonly isolated from hematological patients is *Aspergillus* [1–4, 12]. In the present study, *Aspergillus* was isolated from three patients. Single patients were infected with other molds, such as *Fusarium*, *Acremonium*, *Scopulariopsis*, and *Mucor*, which are also known to cause severe invasive infections. The development of invasive fungemia may be preceded by previous colonization of the respiratory tract, less commonly of the alimentary canal; for this reason all the patients from whom mold had been isolated were closely scrutinized. Until now, none of the patients developed invasive fungemia.

As shown in a study by an Italian group, *Zygomycetes* were the second most common reason for mold infection in patients with hematological malignancies, which may be associated with the use of voriconazole as antifungal prophylaxis

[13–15]. In the present study group, *Mucor sp.* was isolated from alimentary mycobiota in only one patient who had not been administered voriconazole.

The authors concluded that the findings of this study point to the presence of *Candida* species, with a high prevalence of non-albicans *Candida*, in the mycobiota of patients with hematological

malignancies. Patients suffering from malignant lymphoma and chronic lymphocytic leukemia treated with purine analogues should be administered antifungal prophylaxis, as should patients suffering from acute leukemia. One-day examination may be useful in diagnosing mold infections, for example *Aspergillus*, even before clinical symptoms of aspergillosis appear.

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