Investigating the presence of fungal agents in febrile neutropenic patients with hematological malignancies using different microbiological, serological, and molecular methods

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ABSTRACT

This study aimed to investigate fungal agents in febrile neutropenic patients with hematological malignancies. Direct microscopy and cultures were performed on clinical samples collected from febrile neutropenic episodes. The galactomannan (GM) antigen was tested using enzyme-linked immunosorbent assays, and *Aspergillus fumigatus* and *Candida albicans* deoxyribonucleic acid (DNA) assessed using real-time polymerase chain reaction (PCR) in consecutive serum samples. Of the 199 episodes investigated, 1.5% were classified as definite invasive aspergillosis (IA), 4.0% as IA with high probability, and 4.0% as IA with low probability. Additionally, candidaemia was detected in eight episodes (4.1%). The GM antigen was found negative for 86.4% of episodes, as one positive for 7.0% of episodes, as two or more consecutive positives for 5.5% of episodes, and as positive in any two serum samples in 1.0% of episodes. While no *C. albicans* DNA was detected in 98.5% of 199 episodes, one positive result was obtained in 1.0% of episodes, and two or more consecutive positives in 0.5% of episodes. *A. fumigatus* PCR results were found negative in 81.9% of episodes, as one positive in 16.1% of episodes, as positive in any two serum samples in 1.0% of episodes, and consecutively positive in 1.0% of episodes. GM antigen tests were found consecutively positive in all three patients diagnosed as having definite IA. These findings indicate that conventional, serological, and molecular methods should be used in combination to detect fungal agents in febrile neutropenic patients.

KEY WORDS: Febrile neutropenia; invasive fungal infection; culture; PCR; galactomannan

INTRODUCTION

Fungal infections are among the most important causes of mortality and morbidity in neutropenic patients. The risk of development of an invasive fungal infection (IFI) is 15–25% in high-risk patient groups, particularly those with a long history of neutropenia, organ damage, and prior fungal infection or colonization in hematological cancer or transplant cases [1]. Over 90% of fungal infections are caused by *Candida* and *Aspergillus* species; however, there has been an increase in fungal infections caused by rarer species, including *Trichosporon*, *Pseudellescheria*, *Fusarium*, and *Scedosporium* [2].

There is a high mortality rate in neutropenic patients with fungal infections, e.g. 50% with *Candida* infections [3], and 100% with *Aspergillus* infections [4]. Previous studies showed that early treatment of fungal infections leads to better prognosis in neutropenic patients. Therefore, early diagnosis and treatment of these infections are of crucial importance. To date, an established diagnostic method for early diagnosis of IFIs, with adequate sensitivity and specificity, is lacking [5]. Diagnosis is based on the fundamental principle of combining clinical and laboratory findings. Methods using direct microscopy and culture are gold standards and remain as important diagnostic tools. However, failure to adequately apply invasive diagnostic methods in certain cases, for example, in the absence of clinical findings and the presence of thrombocytopenia in patients with hematological malignancy, together with long waiting times before culture results are obtained, leads to significant delays in initiating treatment. Therefore, the availability of appropriate tests for early diagnosis is essential in order to guide treatment, particularly in critically ill patients. The serological detection of fungal antigens and the use of molecular methods to test for nucleic acids have led to

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important advances in diagnosis ability [6]. Among the most studied methods are the galactomannan (GM) antigen test, the 1,3-β-D-glucan (BDG) test, and molecular polymerase chain reaction (PCR) tests [5].

This study aimed (i) to investigate fungal agents in febrile neutropenic patients at high risk of IFIs, by using various methods: direct microscopy, culture, GM antigen test, and real-time PCR, and (ii) to compare the different methods used.

MATERIALS AND METHODS

Patients and samples

This study was approved by the Ethics Committee of the Faculty of Medicine at Gaziantep University under Resolution No. 13.03.2012/129. A total of 101 patients who were admitted with the diagnosis of febrile neutropenia to the Hematology Clinic for Internal Medicine at Şahinbey Research and Application Hospital of Gaziantep University were enrolled in the study. The patients were recruited between April 2012 and July 2013. A majority of 64 patients (63.4%) were female, and 37 (36.6%) were male; the mean age of the study population was 44.6 ± 16.8 years. Criteria for study inclusion were adult patients with a hematological malignancy, a neutrophil count of ≤ 500/mm³, febrile status with temperature of ≥ 38.3°C measured either orally or in the axilla, and the use of chemotherapeutic drugs. All patients received antifungal prophylaxis, according to the recommended guidelines from the European Conference on Infections in Leukemia (ECIL)-3 [7]. A total of 49 patients (48.5%) had acute myelocytic leukemia, 13 (12.9%) acute lymphocytic leukemia, 10 (9.9%) myeloma, eight (7.9%) diffuse large B-cell lymphoma, three (3.0%) myelodysplastic syndrome, three (3.0%) a lymphoma of the central nervous system, three (3.0%) aplastic anemia, three (3.0%) mantle cell lymphoma, two (2.0%) follicular lymphoma, two (2.0%) peripheral T-cell lymphoma, one (1.0%) anaplastic large cell lymphoma, one (1.0%) Hodgkin's lymphoma, one (1.0%) chronic lymphocytic leukemia, one (1.0%) angioimmunoblastic lymphoma, and one (1.0%) Burkitt's lymphoma. In addition, only 42 out of the 101 patients (41.6%) underwent peripheral blood stem cell transplantation (PBSCT). Of those 42 patients, 20 patients (47.6%) were administered allogeneic PBSCT from human leucocyte antigen (HLA)-identical siblings (47.6%), 21 patients (50%) autologous PBSCT, and one patient (2.4%) autologous plus allogeneic PBSCT. For each patient, a minimum of one and a maximum of five neutropenic episodes were recorded and investigated for IFIs, as follows: one episode in 101 patients, two episodes in 61 patients, three episodes in 29 patients, four episodes in seven patients, and five episodes in one patient.

All clinical samples were collected for subsequent laboratory analyses, including the GM antigen test, and *Aspergillus fumigatus* and *Candida albicans* PCR tests, from

194 neutropenic episodes for blood culture, 61 episodes for urine culture, 34 episodes for sputum culture, 12 episodes for catheter culture, two episodes for tracheal aspirate (TRA), and one episode for bronchoalveolar lavage (BAL), throat swab, wound swab, brain biopsy material, and pleural material cultures. The number of clinical samples sent for culture were as follows: 443 blood samples, 229 urine samples, 40 sputum samples, 20 catheter samples, two TRA samples, a BAL sample, a throat swab sample, a wound swab sample, a brain biopsy material, and a pleural sample.

A total of 813 serum samples from 199 episodes in 101 febrile neutropenic patients were screened for the GM antigen, with an average of five serum samples analyzed on two consecutive occasions within a week from each episode, using the enzyme-linked immunosorbent assay (ELISA) method. *A. fumigatus* DNA was investigated in 818 plasma samples and *C. albicans* DNA in 749 plasma samples from 199 episodes with the method of real-time PCR.

The study patients were grouped, according to the European Organization for Research and Treatment of Cancer (EORTC) criteria [8].

Direct microscopic examination and culture

Samples collected from the respiratory tract, wound swabs, and brain biopsy were analyzed using direct microscopy. All samples were treated with 10% potassium hydroxide solution and examined for fungal infection under a light microscope (Olympus, Tokyo, Japan), using objectives ×20 to ×40.

All clinical samples, except blood culture samples, were cultured on 5% sheep blood agar (bioMérieux, Marcy-l'Etoile, France) and eosin–methylene blue (EMB) agar (bioMérieux, Marcy-l'Etoile, France) media, as well as two Sabouraud dextrose agar (SDA) (bioMérieux, Marcy-l'Etoile, France) media. A colony count of 10^4 cfu/mL in urine cultures was considered as significant growth [9], and colonies obtained from all cultures were Gram-stained. Gram-positive cream-colored and yellow colonies, which also produced a distinctive yeast smell, were further examined microscopically with the germ tube (germination pipe) test on Cornmeal-Tween 80 agar medium (Oxoid, Hampshire, England), and were also identified, according to their biochemical properties, using the Vitek 2 automated system (bioMérieux, Durham, St. Louis, USA). *C. albicans* ATCC 14053 and *C. lusitaniae* ATCC 34449 strains were used as positive identification controls.

Blood culture

Blood cultures demonstrating growth in the BacTAlert 3D (bioMérieux, Durham, St. Louis, USA) automated blood culture system on 5% sheep blood agar (bioMérieux, Marcy-l'Etoile, France) and EMB agar (bioMérieux, Marcy-l'Etoile,

France) media were incubated for 24–48 hours. A passage was performed from cultures producing Gram-positive yeast cells detected on the SDA medium. Cream-colored and yellow colonies with a distinctive yeast smell at the end of incubation period were identified, using the conventional as well as the Vitek 2 (bioMérieux, Durham, St.Louis, USA) automated systems.

Serological tests

Serum samples were assessed for the presence of the GM antigen by ELISA using the Platelia *Aspergillus* kit (BioRad, Marnes-la-Coquette, France), according to the manufacturer's instructions.

A GM index of < 0.5 was considered to be negative, whereas a GM index of ≥ 0.5 was considered positive.

Molecular methods

The MagNA Pure LC kit (Roche Diagnostics, Mannheim, Germany) was used for nucleic acid isolation in all plasma samples collected, according to the manufacturer's instructions. The isolated deoxyribonucleic acid (DNA) samples were then analyzed by real-time PCR, in order to detect *A. fumigatus* and *C. albicans* DNA, using the LightMix kit (Tib Molbiol, Berlin, Germany) in a Light Cycler 2.0 apparatus (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions.

The LightMix kit detects the fungal 18S ribonucleic acid (RNA) region that shows the presence of fungal DNA in a nucleic acid extraction. A LightMix Kit *C. albicans* (Tib Molbiol, Berlin, Germany) has a 490 bp long fragment of the *C. albicans* genome was amplified with specific primers and detected with probes labelled with LightCycler Red 640 (Tib Molbiol, Berlin, Germany). LightMix Kit *A. fumigatus* (Tib Molbiol, Berlin, Germany) has a 504 bp long fragment of the *Aspergillus fumigatus* genome and was amplified with primers, and detected and labelled similarly. The kits also include control DNAs of *C. albicans* and *A. fumigatus* equivalent to 10^5 targets. Approximately 10 *C. albicans* and *A. fumigatus* DNA copies were assessed, using LightCycler 1.×/2.0 Apparatus and LightCycler FastStart DNA Master HybProbe (Roche Diagnostics, Mannheim, Germany). The positive control used was provided in the manufacturer's kit, whereas water was used as the negative control. The resulting fluorescent curves were evaluated with LightCycler software version 4.1 (Roche Diagnostics, Mannheim, Germany).

Histopathological assessments

Results of histopathological assessments of brain biopsy samples performed on three patients were retrieved from the patient files.

Statistical analyses

Correlation among the categorical variables was tested using the chi-square analysis. Specificity values and 95% confidence intervals (CIs) were calculated for the various methods used and compared with the gold standard, which is to culture for *Candidas* and also galactomannan antigen for *A. fumigatus* according to EORTC criteria [8]. Furthermore, the agreement between methods was checked using the kappa statistic. Frequency, percentage, and mean ± standard deviation (SD) values were provided as descriptive statistics. The SPSS for Windows version 11.5 package program (SPSS Inc., Chicago, Illinois, USA) was used for statistical analyses, and $P < 0.05$ was considered statistically significant. All statistical evaluations made use of the number of neutropenic episodes in patients, and not the number of patients or samples.

RESULTS

Of a total of 199 neutropenic episodes investigated, according to the classification by the EORTC/Mycoses Study Group, definite invasive aspergillosis (IA) was detected in three (1.5%) episodes by histopathological observation of hyphal structures with septae in brain biopsy samples (data not shown); eight (4.0%) neutropenic episodes were identified as high-probability IA, and eight (4.0%) as low-probability IA, whereas the remaining 180 (90.5%) episodes were not found at risk of IA. Moreover, candidaemia was detected in eight episodes (4.1%).

Direct microscopic examination and culture

Direct microscopy of clinical samples revealed no fungal structures. The distribution of culture results from the clinical samples, according to the number of neutropenic episodes, is shown in Table 1.

A total of 443 blood cultures, collected from 194 febrile neutropenic episodes in 101 patients, were analyzed. No growth occurred in blood cultures from 124 (63.9%), out of 194, episodes, whereas bacterial growth was detected from 62 (32%) episodes and the growth of various *Candida* species from seven (3.6%) episodes. Of the *Candida* species that grew, four were identified as *C. glabrata*, and one was identified as *C. parapsilosis*, one as *C. albicans*, and one as *C. tropicalis*. On the other hand, mixed infection with both *C. parapsilosis* and a bacterial agent was detected from one (0.5%) neutropenic episode.

Furthermore, a total of 229 urine samples collected from 61 episodes were analysed. Of the *Candida* species that grew, two were identified as *C. glabrata*, and one as *C. albicans*.

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Additionally, a total of 40 sputum cultures from 34 neutropenic episodes were assessed. No growth was detected from 25 (73.5%), out of 34, episodes, whereas bacterial growth was obtained from seven (20.6%) episodes. In addition, growth of *Aspergillus* species was observed from two episodes (5.9%), although growth was found in only a single culture, with no further *Aspergillus* growth obtained in subsequent sputum cultures from the patients.

Moreover, a total of 20 catheter cultures were analyzed from 12 episodes. No growth occurred in six (50%), of the 12, episodes, while bacterial growth was found from three episodes (25%) and *Candida* species from two episodes (16.6%), although both *Candida* species and a bacterial agent grew concurrently from one of these two episodes (8.3%). The *Candida* species were identified as *C. albicans* from two episodes, and as *C. tropicalis* from one episode.

GM antigen findings

The GM antigen was found negative for 86.4% of the episodes, as one positive for 7.0% of them, two or more consecutive positives for 5.5% of them, and positive in any two sera in 1.0% of them. The distribution of the GM results obtained with a GM index of ≥ 0.5 is shown in Table 2.

PCR findings

A total of 749 plasma samples to assess for *C. albicans* DNA, and 818 plasma samples to check for *A. fumigatus* DNA, were analysed from 199 neutropenic episodes in 101 febrile neutropenic patients, twice a week from each episode. While no *C. albicans* DNA was determined in 98.5% of 199 episodes, one positive was determined in 1.0% of them and two or more consecutively positives were determined in 0.5% of them. *A. fumigatus* PCR results were found negative in 81.9% of the episodes, as one positive in 16.1% of them, positive in any two sera in 1.0% of them, and consecutively positive in 1.0% of them. *C. albicans* and *A. fumigatus* PCR results from all episodes are presented in Table 3.

Comparison of culture and PCR results

Blood cultures from 194 episodes were analysed, and *C. albicans* PCRs were carried out simultaneously from 192 of the 194 episodes. A comparison between the blood culture results and *C. albicans* PCR results is shown in Table 4. In addition, the specificity and negative predictive value (NPV) of the PCR were 98.9% (95% CI 96.2–99.9) and 96.8% (95% CI 93.2–98.8), respectively. Of the *Candida* species that grew in blood cultures, four were identified as *C. glabrata* and two as *C. parapsilosis*, whereas one was identified as *C. albicans* and one as *C. tropicalis*. It was noted that the *C. albicans* strain that grew in the blood culture was also detected with *C. albicans* PCR.

Moreover, a total of 20 catheter cultures from 12 episodes were assessed. *Candida* species grew from three episodes (25%), with *C. albicans* identified from two episodes and *C. tropicalis* from one episode. On comparing *C. albicans* PCR results with the catheter culture results, the specificity and NPV of the PCR were 100% (95% CI 66.4–100) and 81.8% (95% CI 48.2–97.7), respectively. The comparison between the catheter culture results and *C. albicans* PCR results is presented in Table 5.

The growth of *Candida* species was observed from three of 61 episodes for which urine cultures were analysed. *C. glabrata* was identified from two episodes, and *C. albicans* identified from one episode. It was noted that *C. albicans* had also grown in the blood and catheter cultures of the patient in whose urine culture *C. albicans* had grown. When *C. albicans* PCR results were compared with the urine culture results, the specificity and NPV of PCR were 98.3% (95% CI 90.8–99.9) and 96.6% (95% CI 88.3–99.6), respectively. It was observed that the *C. albicans* strain that had grown in the urine culture was also detected on *C. albicans* PCR.

TABLE 1. Distribution of culture results for patient samples analysed, according to the number of episodes

Sample	No growth observed, *n* (%)	Bacterial growth, *n* (%)	Bacterial and *Candida* growth, *n* (%)	*Candida* growth, *n* (%)	*Aspergillus* growth, *n* (%)	Total number of neutropenic episodes, *n* (%)
Blood	124 (63.9)	62 (32.0)	1 (0.5)	7 (3.6)	0	194 (100)
Urine	54 (88.5)	4 (6.6)	0	3 (4.9)	0	61 (100)
Sputum	25 (73.5)	7 (20.6)	0	0	2 (5.9)	34 (100)
Catheter	6 (50.0)	3 (25.0)	1 (8.3)	2 (16.6)	0	12 (100)
Other[a]	7 (100)	0	0	0	0	7 (100)

[a]Two tracheal aspirate samples, one bronchoalveolar lavage sample, one wound swab sample, one throat swab sample, one pleural sample, and one brain biopsy sample

TABLE 2. Distribution of galactomannan results obtained with index values of ≥0.5

Galactomannan result	All negative, *n* (%)	One positive, *n* (%)	Two or more consecutive positives, *n* (%)	Any two positives, *n* (%)	Total, *n* (%)
Galactomannan index ≥0.5	172 (86.4)	14 (7.0)	11 (5.5)	2 (1.0)	199 (100)

Comparison of *A. fumigatus* PCR and GM results

A comparison of the results obtained with a GM index of ≥ 0.5 and *A. fumigatus* PCR results is provided in Table 6.

On comparing *A. fumigatus* PCR and GM results, with a single positive value considered as significant, the specificity and NPV of PCR were 84.3% (95% CI 78.0–89.4) and 88.4% (95% CI 82.5–92.9), respectively. When the two methods

TABLE 3. *C. albicans* and *A. fumigatus* PCR results from all neutropzc episodes

Samples	*C. albicans* PCR		*A. fumigatus* PCR	
	N	%	n	%
All negative	196	98.5	163	81.9
One positive	2	1.0	32	16.0
Any two positives	0	0	2	1.0
Two consecutive positives	1	0.5	2	1.0
Total	199	100	199	100

PCR, polymerase chain reaction

TABLE 4. Comparison between blood culture results and *C. albicans* PCR results

Samples	Blood culture-negative, *n* (%)	Blood culture-positive, *n* (%)	Total, *n* (%)
PCR-negative	182 (98.9)	7 (87.5)	189 (98.4)
PCR-positive	2 (1.1)	1 (12.5)	3 (1.6)
Total	184 (100)	8 (100)	192 (100)

PCR, polymerase chain reaction

TABLE 5. Comparison between catheter culture results and *C. albicans* PCR results

Samples	Culture-negative, *n* (%)	Culture-positive, *n* (%)	Total, *n* (%)
PCR-negative	9 (100)	2 (66.7)	11 (91.7)
PCR-positive	0 (0)	1 (33.3)	1 (8.3)
Total	9 (100)	3 (100)	12 (100)

PCR, polymerase chain reaction.

were evaluated for agreement, poor agreement was observed between the tests (kappa = 0.124, *p* = 0.077).

In addition, on comparing any positive results from *A. fumigatus* PCRs with consecutive positive GM results, the specificity and NPV of the PCR were 83.87% (95% CI 77.78–88.85) and 95.12% (95% CI 90.61–97.87), respectively, and poor agreement was found between the two tests (kappa = 0.125, *p* = 0.041).

Comparison of histopathological results with GM and *A. fumigatus* PCR results

No fungal growth was detected in the culture of a brain biopsy sample of one of three patients, although hyphal structures with septae were detected histopathologically in the brain biopsy sample; no culture had been carried out on brain biopsy samples from the other two patients. Consecutive positive GM results were obtained in all three patients (100%). *A. fumigatus* PCR results were negative in two (66.7%) of these patients, whereas consecutive positive PCR results were obtained in one patient (33.3%). The sensitivity and specificity of the tests could not be calculated and compared, as histopathological examinations, although the gold standard, could not be performed for all study patients.

DISCUSSION

Superficial and deep opportunistic fungal infections are frequent in neutropenic oncology patients and can be caused by any fungus, with the commonest fungi being from the *Candida* genus, followed by *Aspergillus* [10-12]. While the commonest agent is *C. albicans* in candidaemia, the incidence of non-*albicans* species is also increasing [13-16].

In a meta-analysis performed for Invasive Candidiasis (IC) [17], PCR sensitivity and specificity were reported as

TABLE 6. Comparison between results achieved with galactomannan index values ≥0.5 and A. fumigatus PCR results

Samples	GM (index ≥0.5)				Total
	All negative	One positive	Any two positives	Two or more consecutive positives	
PCR					
All negative					
n	145	11	1	7	164
%	84.3	78.6	50.0	63.6	82.4
One positive					
n	26	2	1	3	32
%	15.1	14.3	50.0	27.3	16.1
Any two positives					
n	1	1	0	0	2
%	0.6	7.1	0	0	1.0
Two or more consecutive positives					
n	0	0	0	1	1
%	0	0	0	9.1	0.5
Total					
n	172	14	2	11	199
%	100	100	100	100	100

PCR, polymerase chain reaction

100% in patients with candidaemia, whereas PCR sensitivity and specificity were 95% and 92%, respectively, in suspected cases of IC. In contrast, in cases of proven and highly probable IC, blood cultures and PCR had a positivity rate of 38% and 85%, respectively. In a study by Nguyen *et al.* [18] investigating the diagnostic values of real-time PCR, BDG, and blood cultures in IC cases, PCR was reported to be more sensitive than the other diagnostic methods. In this study, it was stated that identical *Candida* species were detected in 82% of patients by PCR and blood cultures and sensitivity was 98% when blood cultures and PCR were used in combination. In a study carried out using real-time PCR [19], it was reported that DNA amplification was 100% specific for *Candida* species. In our study, analysis for *C. albicans* DNA was found as one positive in 1% of episodes, and consecutively positive in 0.5% of episodes. The real-time PCR kit which we used for nucleic acid amplification can only detect *C. albicans* DNA. It was noted that the *C. albicans* strain that grew in the blood culture was also detected with *C. albicans* PCR. Also non-*albicans Candida* species can often be the infecting agents in patients with candidaemia. Therefore, it is important to select a PCR kit for detecting also non-albicans *Candida* spp.

In general, candiduria very rarely causes candidaemia in immune-compromised patients [20]. In our study, the growth of *C. albicans* was detected in blood and catheter cultures of a patient whose urine culture also showed growth of *C. albicans*. As a result *C. albicans* DNA was found on this patient. Growth of *C. albicans* in the urine culture of one of our patients and the positive *C. albicans*-specific PCR result of the same patient suggests that *Candida* growth in urine cultures might be significant in neutropenic patients, and that it merits careful evaluation.

IA is a serious disease, which in the majority of cases can be fatal unless diagnosed and treated early [21]. Although culture is the gold standard for a definitive diagnosis, it is difficult and time-consuming to carry out [22]. Also in our study, the number of samples collected, such as BALs or TRAs, was quite limited, due to the difficulty in obtaining invasive respiratory tract samples from our study patients. GM antigen, BDG, and PCR tests are the most widely studied methods [23]. Studies using the platelia GM enzyme immunoassay (EIA) test yielded positive results, and the sensitivity and specificity varied between 29% and 99% in many prospective studies [24]. In a meta-analysis of 27 studies by Pteiffer *et al.* [25], the sensitivity and specificity of the GM test were found to be 71% and 89%, respectively. In our study, the GM antigen test was obtained as one positive during 7.03% of episodes, as two or more consecutive positives during 5.52% of episodes, and was positive in any two serum samples in 1% of episodes GM antigen tests were found to be consecutively positive in all three patients with a confirmed diagnosis of IA.

Although it is difficult to differentiate fungal colonization from an infection in immunosuppressed patients, it is recommended to screen for antigens twice weekly in serum samples of at-risk patients, as colonization can rapidly turn into an infection, with a tendency to become widespread in the body [26]. However, in their recent study, Duarte *et al.* [27] reported that, in patients receiving posaconazole prophylaxis, the GM results might be misleading, with markedly high false-positive rates and decreased PPV rates, thus failing to show true positive results. The low pretest risk of IA, in the context of effective antifungal prophylaxis, renders serum GM surveillance of asymptomatic patients unreliable, as all results would be either negative or false positive. However, the test remains useful in the diagnosis of patients with a clinical suspicion of invasive fungal disease, thus calling for a more efficient co-utilisation of effective antifungal prophylaxis and GM testing in this clinical setting.

Indeed, molecular methods are important alternatives to conventional methods, as they are sensitive and safe and can yield fast results [28]. In a study by Silva *et al.* [29], IA was investigated, using PCR, in 1311 consecutive serum samples from 172 neutropenic patients, and by screening for the GM antigen in 806 consecutive serum samples from 169 neutropenic patients. The authors reported the sensitivity and specificity to be 75.0% and 91.9%, respectively, for PCR and 87.5% and 93.1%, respectively, for GM. Thus, it was suggested that both tests were important in the diagnosis of IA in two or more consecutive serum samples. Other similar studies also recommended the combined use of GM and PCR tests, with emphasis on increased sensitivity [30-32]. In our study, the specificity and NPV were 84.3% and 88.4%, respectively, when we compared *A. fumigatus* PCR and GM results, with a single positive value considered significant. Furthermore, when we compared any positivity in *A. fumigatus* PCR with the consecutive positives in GM, the specificity and NPV of the PCR were 83.9% and 95.1%, respectively. GM is an immunogenic antigen, and its presence in *A. fumigatus* and other *Aspergillus* species (*A. flavus*, *A. niger*, *A. versicolor*, *A. terreus*, *A. nidulans*, and *A. oryzae*) has been shown [33]. In our study, we believe that the real-time PCR kit used influenced the sensitivity of the test, as it was specific only to *A. fumigatus*.

CONCLUSION

The use of conventional diagnostic methods, such as direct microscopy and culture, as well as serological and molecular methods alone is inadequate in the diagnosis of fungal infections in patients with immunodeficiency. These methods should be used in combination. The limitations of our study is including routine patient's samples which have been set to

routine mediums. Our study just has created for evaluating of using methods that determines fungal agents. Therefore we've not added bacterial agents to discussion.

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DECLARATION OF INTERESTS

The authors declare no conflict of interest.

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