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DNA Microarray-based Identification of Fungal Pathogens in Neutropenic Patients in Alexandria University Hospitals in a Twelve-month Interval

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Authors' contributions

This work was carried out in collaboration between all authors. Author DESM designed the study. Author YAERA performed the statistical analysis. Author DESM wrote the protocol. Authors DESM and AARG wrote the first draft of the manuscript. Authors NAS and SHF managed the analyses of the study. Author DESM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Systemic fungal infections are increasing, and they cause severe morbidity and high mortality rates among immuno-compromised patients. Conventional laboratory methods for identifying fungal pathogens, although continuously improving, are still time consuming. Therefore, they are usually inadequate for ensuring early targeted therapy, especially for uncommon or newly identified fungal species. Molecular detection and identification using polymerase chain reaction for the amplification of fungal DNA is being applied more frequently for the early diagnosis and identification of fungal pathogens.

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Materials and Methods: The current study included 31 neutropenic cancer patients who had fever, who were not responding to antibiotics and who attended the outpatient clinics or were inpatients in the Haematology Department of the Medical Research Institute hospital and Oncology Department of Alexandria Main University hospital during a 12-month period. Blood samples were collected from each patient for the diagnosis of invasive fungal infections (IFIs) by conventional blood culture and DNA microarray.
Results: Out Of the 24 neutropenic cancer patients tested by conventional blood culture, fungal growth was detected in 2 (8.3%) blood cultures (both for *Candida albicans*), and the blood cultures for the remaining cases were negative. Through DNA microarray, three patients (9.7%) were found to have no fungal infections, two (6.5%) with one fungal infection, six (19.4%) with infection with two fungal species and 20 (64.5%) with infection with more than two fungal species.
Conclusion: The present oligonucleotide array system allowed the rapid and reliable identification of a vast number of fungal pathogens. Advanced diagnostic methods may have led to an overall higher sensitivity of diagnosing IFI, but the actual incidence of IFI may not have changed.

Keywords: Neutropenic; systemic fungal infections; blood culture; DNA microarray.

1. INTRODUCTION

The effect of fungal infections on human health is not widely recognised, and deaths resulting from these infections are usually overlooked. The World Health Organization has no program on fungal infection, and most public health agencies, except the US Centers for Disease Control and Prevention, conduct little or no mycological surveillance [1].

Systemic fungal infections are increasing, and they cause severe morbidity and high mortality rates for immunocompromised patients, especially those with acute leukaemia after intensive chemotherapy or allogeneic stem cell transplantation. Besides its increasing incidence, the epidemiology of invasive fungal infections (IFIs) is changing. In fact, both uncommon and resistant fungal pathogens, e.g. *Zygomycetes*, *Candida krusei* and *Aspergillus terreus*, emerged during the last decade [2,3].

Unfortunately, a significant obstacle to the successful treatment of IFIs is the lack of sensitive and specific methods for their early diagnosis [4]. Establishing a definite diagnosis is particularly challenging, time consuming and difficult because of the lack of specific signs and symptoms until late in the disease process. Moreover, colonisation is difficult to distinguish from invasive disease. Therefore, IFIs are usually diagnosed late, thereby delaying the initiation of antifungal treatment and increasing mortality [5-8].

However, conventional laboratory methods for identifying fungal pathogens, although continuously improving, remain time consuming. Therefore, they are inadequate for ensuring early targeted therapy, especially for uncommon or newly identified fungal species [9]. Molecular detection and identification using polymerase chain reaction (PCR) to amplify fungal DNA is being applied more frequently for the early diagnosis and identification of fungal pathogens [10]. Most molecular procedures enable the identification of one or a few species at a time, and thus they will entail a high cost if all relevant species are considered [11,12]. An ideal approach is to overcome this limitation by applying DNA microarray technology, which may enable the discrimination of a wide range of pathogens in a single assay [9]. The advancement of molecular tools such as DNA microarray significantly contributes to the detection of pathogens, such as fungi, bacteria and parasites [13,14]. This study aimed to conduct the early diagnosis of IFIs by using a sensitive and specific DNA microarray that combines multiplex PCR and consecutive DNA chip hybridisation to detect fungal genomic DNA in serum samples.

2. MATERIALS AND METHODS

The current study included 31 neutropenic cancer patients who had fever, who did not respond to antibiotics and who attended the outpatient clinics or were inpatients in the Haematology Department of the Medical Research Institute hospital and Oncology Department of Alexandria Main University hospital) during a 12-month period. Full written consent was requested from patients enrolled in the study.

Selecting the cases to meet the goal of the study was difficult because a fixed strategy involving the subsequent empirical administration of antibiotics and/or antifungal drugs was in place for managing neutropenic cancer patients. Accordingly, all patients were pyrexial and received antibiotics. Nine patients (29%) received antifungal drugs for one day, and three patients (9.7%) received antifungal drugs for two to three days but remained feverish.

In the data collection process, a pre-designed questionnaire sheet was completed for every patient. This questionnaire included personal data (name, age, sex, residence and occupation) and clinical data [present and past history, diagnosis, duration of chemotherapy, antifungal drugs and/or antibiotics, corticosteroids, duration of neutropenia, laboratory investigations (e.g. complete blood count) and chest X-ray].

2.1 Conventional Blood Culture [15]

A total of 5 ml of the blood sample was inoculated into biphasic blood culture bottles containing Sabouraud's dextrose agar (SDA) and Sabouraud's dextrose (SD) broth with chloramphenicol 0.5 gm/L. The bottles were incubated aerobically at 37°C and were inspected and inverted upside down every day. The bottles were considered negative if no growth appeared after four weeks.

2.2 DNA Microarray [16]

DNA was extracted from serum using the QIAamp Viral DNA Mini kit (QIAGEN, Valencia, CA, USA) by following the manufacturer's instructions and stored at −20°C until use. The amplification and hybridisation of extracted DNA to detect fungus infection were conducted using Fungi 2.1 DNA microarray chip (Chipron GmbH, Berlin, Germany). The cycling conditions were set to 3 min at 95°C (initial denaturation), 30 s at 94°C, 45 s at 56°C, 45 s at 72°C (for 40 cycles), 3 min at 72°C for final extension and cooling at 4°C. A Step One cycler (Applied Biosystems) was used. An aliquot of 7 µL of the PCR products was run on a 2% agarose gel. In the second step, DNA was spotted manually on LCD chips, and hybridisation was performed according to the instruction manual.

According to the manufacturer, the array can discriminate among 25 different fungal species or species clusters. A combination of a transmission light scanner and image analysis software supplied by the manufacturer was used for scanning and final analysis.

2.3 Statistical Analysis Method [17]

Statistical analysis was conducted by using the IBM SPSS statistics program v. 21. The categorical variables were described by frequency and percentage. Chi-square test was used to examine the significant association between two categorical variables. Fisher's exact test and the Monte Carlo test were used if more than 20% of the excluded cell counts were < 5 at a 0.05 level of significance.

The kappa measure of agreement was conducted to detect the statistical significant agreement between two techniques (e.g. blood culture and microarray) at a 0.05 level of significance. The McNemar test was performed to detect the statistical significant difference in the proportion of positive and negative results between blood culture and microarray.

3. RESULTS

The study group included 19 (61.3%) females and 12 (38.7%) males. Their age range was 1–60 years. Among the patients, 25.8% were in the age groups of 10–20 years and 40–50 years. Out of the 31 neutropenic cancer patients, those with haematological malignancies accounted for 67.7% (12 leukaemia patients, 8 lymphoma patients and 1 multiple myeloma patient), with bone and soft tissue malignancies for 12.9%, with gastrointestinal tumours for 6.5%, with brain sarcoma for 6.5%, with renal tumours for 3.2% and with breast cancer for 3.2% (Fig. 1).

All the 31 patients were found to be feverish for more than five days. Seven (22.6%) patients had oral thrush, six patients (19.4%) had cough, two (6.5%) female patients had vaginitis and one patient (3.2%) had dysuria. An overlap was found in the clinical symptoms, as the patients could suffer from more than one symptom.

Blood culture was conducted on only for 24 of the 31 neuropenic cancer cases. Fungal growth was detected in two (8.3%) blood cultures (both for *Candida albicans*). The remaining 22 (91.7%) cases were found to be negative.

As recommended by the manufacturers, when the arrays were used for the first time, the extracted DNAs from different clinical specimens

were amplified and analysed by agarose gel electrophoresis to show that the primer mixes were specific for the rRNA region (ITS) regions from a broad range of fungal genera. Moreover, genomic DNA extracted from *C. albicans* and *Escherichia coli* was included in the PCR as a positive and the negative control, respectively. In addition, sterile distilled water was included in each PCR run as a negative control.

A representative example of the fungal DNA amplification efficiencies is shown in Fig. 2. A specific amplification band of the 290 base pair (bp) DNA fragment by PCR with the primer mix was obtained. These results showed that only a single band was amplified from sera and that the primer mix used was appropriate to amplify the ITS region of fungi.

All of the 31 patients were neutropenic and treated with antibiotics. Among them, 30 (96.8%) patients were undergoing chemotherapy and hospitalised, 29 (93.5%) received intravenous fluids, 6 (19.4%) were under steroid therapy, and none received radiotherapy. An overlap was found among the risk factors. No statistical significant difference was found between any of the study risk factors and the microarray results (*P* = 1, 1, 0.6, respectively)

Regarding the results of the testing sera of the 31 neutropenic cancer patients by DNA microarray, positive results were obtained for 28 cases (90.3%) and negative fungal DNA results for 3 cases (9.7%). No statistical significant association was found between the results of the blood culture and those of the DNA microarray assay (*kappa*=0.01, *P*=0.6). This non-significant difference could be due to either the low sensitivity of the blood culture technique used or the small number of cases in this study.

Of the 31 neutropenic cancer patients, 19 (61.3%) were not treated with antifungal drugs and 12 (38.7%) were treated with antifungal drugs. Among those who underwent treatment, eight (25.8%) cases were given fluconazole and four (12.9%) cases with amphotericin-B. No statistical significant relation was found between the antifungal drug therapy and the microarray results in the study group (*P*=0.32). Thus, the antifungal prophylaxis policy might need to be changed according to the fungal species detected.

Out of the 31 patients tested by microarray, 2 cases (6.5%) were infected with one fungal infection, 6 (19.4%) were infected with two fungal species and 20 (64.5%) were infected with more than two fungal species. Only three (9.7%) cases had no fungal infections (negative microarray). These results showed statistical significance (*P*= 0) (Fig. 3).

3.1 Clinical Correlation

As Fisher's exact p value was 0.53, no statistical significant relation was found between the microarray results and the type of malignancy. No statistical significant relation was found between the microarray results and certain clinical symptoms, as most of them had a positive microarray. Similarly, no statistically significant difference was observed between any of the study risk factors and the microarray results.

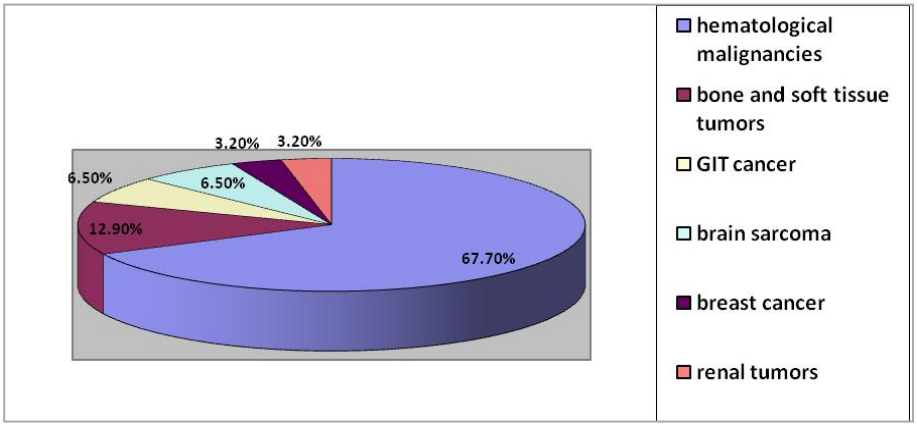

Fig. 1. Distribution of the 31 neutropenic cancer patients according to clinical diagnosis

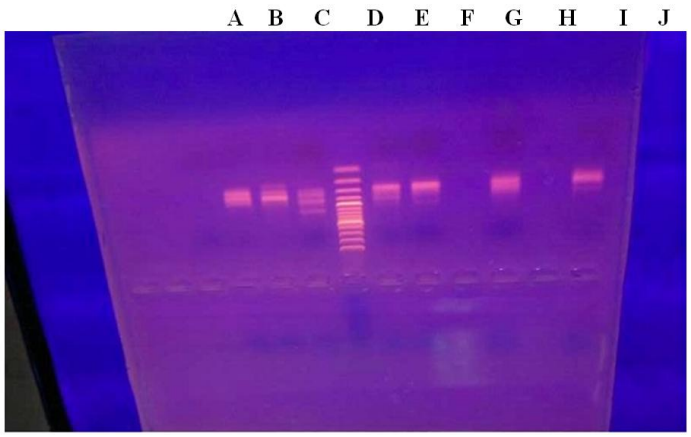

Fig. 2. Agarose gel electrophoresis results. Lanes from A–C, E, F and H show the PCR amplicon (generated by the primer mix) with a molecular size of 290 bp. Lane D shows the DNA ladder (100 bp). Lanes G and I represent the negative controls from *E. coli* and distilled water. Lane J shows the amplified product from the positive control of *C. albicans*

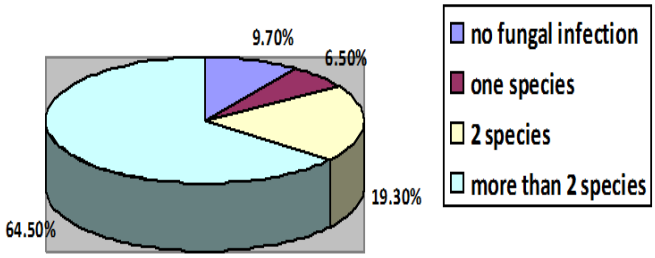

Fig. 3. Pattern of isolation of fungal species causing infection among 31 neutropenic cancer patients by DNA microarray

Table 1. Fungal species detected in the 28 neutropenic cancer patients positive for fungal infection by DNA microarray

Microarray results *	No	%
Candida albicans	25	89.3%
Candida krusei	19	67.9%
Candida parapsilosis	12	42.9%
Candida lusitaniae	6	21.4%
Candida tropicalis	5	17.9%
Candida guilliemondii	6	21.4%
Candida glabrata	3	10.7%
Candida kefyr	1	3.6%
Candida lambica	1	3.6%
Candida dubliniensis	1	3.6%
Aspergillus fumigates	2	7.1%
Aspergillus niger	3	10.7%
Aspergillus terreus	1	3.6%
Aspergillus A.nidul.vers .nidul.versi i	1	3.6%
Rhizopus	2	7.1%
Mucor	1	3.6%
Cryptococcous neoformans	1	3.6%

**An overlap was found between the species of fungi isolated from positive cases; 26 (92.9%) of the patients had mixed infection with more than one species*

3.2 Follow -up

The 31 neutropenic cancer patients included in the current study were followed up after sampling up to one year. Among them, 10 patients recovered from malignancy, and 13 remained under medication. Eight patients died: two died because of febrile neutropenia (within 30 days of a febrile neutropenia attack) and six died because of causes other than febrile neutropenia (after 30 days from a febrile neutropenia attack).

4. DISCUSSION

The early initiation of an effective antifungal therapy and the reversal of predisposing risk factors remain the standard treatment for systemic fungal infections. The diagnosis of fungal infection by blood culture in immuno-compromised febrile high-risk patients is usually unsuccessful because of the low yields of colony forming unit (CFU) [18,19]. Thus, the current work aimed to conduct an early and rapid

diagnosis of IFIs in neutropenic cancer patients who had fever and did not respond to antibiotics by using a sensitive and specific DNA microarray combining multiplex PCR and consecutive DNA chip hybridisation to detect fungal genomic DNA in serum samples.

Table 2. Correlation between DNA microarray and blood culture result, type of antifungal therapy and follow-up of patients

Case	DNA microarray results	Blood culture results	Antifungal	Patients follow up
1	●*Candida krusei* & ●*Candida parapsilosis* & ●*Candida albicans*	-	-	♦
2	●*C. albicans* & ●*C. guilliemondii* & ●*C. krusei* &●*C. parapsilosis*	+	F	♦♦
3	●*C. albicans* & ●*C. tropicalis*	+	A	♦♦
4	●*C. albicans* & ●*C. tropicalis*	-	F	#
5	●*C. albicans* &● *C. dubliniensis*	-	-	♦
6	●*C. albicans* & ●*C. lambica*	-	-	♦
7	●*C. parapsilosis* & ● *C. krusei*	-	A	♦♦
8	●*C. krusei* & ● *C. tropicalis* & ●*C. albicans* &● *C. glabrata*	-	-	#
9	●*C. krusei* & ● *C. guilliemondii* & ●*C. albicans* &●*C. parapsilosis*	-	-	♦
10	●*C. krusei* &● *C. albicans* &●*C. glabrata* &● *C. kefyr*	-	-	♦♦
11	●*C. krusei* & ●*C .albicans* & ●*C. parapsilosis* &● *C. lusitaniae*	-	F	#
12	●*C. krusei* & ●*C. albicans* &● *A. niger c.02* & ●*A. niger c.03*	-	-	♦
13	●*C .krusei* & ●*C. tropicalis* & ●*C. albicans* & ●*C. lusitaniae*	-	-	##
14	●*C. albicans* & ●*C. tropicalis* & ●*C. parapsilosis* &● *C. guilliemondii*	-	F	♦♦
15	●*C. albicans* &●*C. parapsilosis* & ●*C. krusei* & ●*C. lusitaniae*	-	-	♦♦
16	*Negative*	-	A	#
17	●*A. nidul.versi.01* & ●*A. fumigates.01* & ●*A. niger c.02* &● *C. krusei*	-	F	♦♦
18	●*C. albicans* &● *C .krusei* &● *C. guilliemondii* &●*C. lusitaniae*	-	-	♦
19	●*C. albicans* &● *Rhizopus.03* & ●*Cryp.* & ●*C. parapsilosis*	-	-	♦♦
20	●*Rhizopus.03*	-	-	♦♦
21	*Negative*	-	-	♦♦
22	*Negative*	-	F	♦
23	●*Mucor* & ●*C. glabrata* & ●*A. terreus* & ●*C. albicans*	-	A	##
24	●*C. albicans* & ●*C .krusei* &● *C. guilliemondii*	-	-	♦♦
25	●*C. albicans* &● *C. krusei* &● *C. parapsilosis*&●*A. fumigatus.02*	-	-	#
26	●*C. albicans*	-	-	♦
27	●*C. albicans* & ● *C. krusei* &● *C. parapsilosis*	-	F	♦♦
28	●*C. albicans* & ●*C. krusei*	-	-	#
29	●*C. albicans* & ● *C. krusei* &● *C. guilliemondii*	-	-	♦♦
30	●*C. albicans* & ●*C. krusei* &● *C. parapsilosis* &● *C. lusitaniae*	-	F	♦
31	●*C. albicans* & ●*C. krusei* & ●*C. parapsilosis* & ●*C. lusitaniae*	-	-	♦

●●● The dots represent the intensity of the blue precipitate formed by the enzyme substrate provided as a 'stain' on the slides of the microarray as a positive result

●High intensity of colour, ●Low intensity of colour, ● Moderate intensity of colour. F Cases received diflucan (fluconazole) as empirical antifungal therapy, and A Cases received fingizone (AmB) as empirical antifungal therapy

The duration of antifungal drug intake ranged from 1–3 days in all cases receiving antifungal drug therapy

 # Cases died after 30 days of febrile neutropenia; ## Cases died within 30 days of febrile neutropenia ♦ Cases recovered from cancer ♦♦ Cases under medication

Pyrexia is the principal sign of infection in neutropenic patients and is usually the only evidence of infection. It is commonly the sole finding, and no specific pattern or degree of fever has been clearly associated with a specific infection in immunocompromised patients. Furthermore, patients who are profoundly immunosuppressed can have serious infections in the absence of pyrexia, which can be muted by immunosuppressive drugs that may be part of the therapeutic regimen, especially steroids and non-steroidal anti-inflammatory agents [20,21].

In the current study, out of the 24 neutropenic cancer patients tested by SDA and SD broth conventional biphasic blood culture, fungal growth was detected in only two (8.3%) blood cultures (both for *C. albicans*), and the remaining cases were negative. The high negativity of the blood culture in this study can be attributed to several factors: the type of blood culture system used (which was not the optimal system for detecting fungal infections but was the most inexpensive and most readily available system), the inadequacy of the volume of blood sample due to the deteriorated clinical condition, the young age of a large number of the patients and the high incidence of phlebitis secondary to the repeated infusions and chemotherapy received in these patients. Thus, an insufficient number of organisms were inoculated into the blood culture bottles. The other important factors are the blood culture depending on the presence of viable fungal elements in the blood sample and the transient nature of fungaemia.

In recent years, numerous DNA-based methods have been developed to identify a variety of medically important fungi [20,22]. The internal transcribed spacer (ITS) region of the fungal ribosomal RNA is one of the most commonly used targets. The ITS region exists in multiple copies in the fungal genome and shows high interspecies sequence divergence and low intraspecies sequence variation [20]. Fungal identification methods based on ITS include restriction fragment length polymorphism analysis, sequence analysis, multiplex PCR, real-time PCR, probe hybridisation and DNA array [23-27].

A common challenge in the studies on DNA microarray-based species detection is the differentiation between specific and non-specific signals. As cross-hybridisation among different fungal genera was observed in several studies, designing more than one probe per species is important [28,29].

Two aspects of the specificity of the microarray detection system used in the current study have two aspects according to the manufacturer. First, the risk of misidentification of any fungal species with another due to sequence similarities is minimised by the design of species–specific probes (more than one probe per species), which can distinguish between these species. Second, the great difference between the level of fluorescence of the perfect match and the cross-hybridisation can help to identify the possible misidentified cases. The targets amplified are highly conserved sequences that have been thoroughly evaluated for their specificity. The primer binding sites are located in highly conserved regions. DNA from species of other fungal genera is also be amplified but cannot be hybridised to the capture probes of the array.

Fungal DNA microarray (Fungi 2.1, Chipron GmbH) was used to detect and identify DNA from 29 clinically relevant fungal species/ genera, mainly *Candida* and *Aspergillus*. The hybridisation results of the specimens in the array in our study could be interpreted because the signal-to-background ratio was sufficient (samples should have intensity > 3000 to be considered positive and not as a background).

Sakai et al. [30] designed species- and/or genus-specific probes within the ITS region and tested the specificity of selected sequences using 355 reference strains. Genus-specific probes were designed for some fungi (*Alternaria* sp., *Rhizomucor* sp., *Mucor* sp. and *Trichosporon* sp.). They successfully designed 319 probes of species/genus-specific oligonucleotides at a range of 13–21 bp with a poly-T anchor at the 5′ or 3′ end for identifying 42 species from 24 genera of fungal pathogens. Three to twelve different specific capture probes were designed and spotted on the array slides for each fungal species/genus to ensure hybridisation reaction for proper identification. Among the 319 probes, six universal probes for fungi were designed. Thus, the array could detect any fungi other than the objective fungi without a specific signal.

In the current study, microarray identified 28 positive cases (90.3%) and 3 negative cases (9.7%). The positive cases were 2 (6.5%) cases with one fungal infection, 6 cases (19.4%) infected with two fungal species and 20 cases

(64.5%) infected with more than two fungal species. The two cases that were positive for *C. albicans* by blood culture were positive for mixed fungal infection by microarray. The first case had mixed infection with *C. albicans, C. guilliemondii, C. krusei and C. parapsilosis*, and the second case was infected with *C. albicans and C. tropicalis*.

In a study on 110 episodes of yeast fungaemia, Yamamoto et al. [31] identified 112 yeast isolates, with 2 of the 110 episodes showing coinfection with two *Candida* isolates. Among these yeast isolates, 11 (9.8%) were non-*Candida* yeasts, and 101 (90.2%) were *Candida* species. The 11 non-*Candida* isolates comprised *Cryptococcus neoformans* (6 isolates, 5.4%), *Trichosporon asahii* (4 isolates, 3.6%) and *Kodamaea ohmeri* (1 isolate, 0.9%). Among the 101 *Candida* isolates, the most common was *C. albicans* (46 isolates, 41.1%), followed by *C. parapsilosis* (22 isolates, 19.6%), *C. glabrata* (15 isolates, 13.4%), *C. tropicalis* (9 isolates, 8.0%) and *C. guilliermondii* (5 isolates, 4.5%). *C. krusei*, *C. lusitaniae* and *C. famata* were isolated in one episode each.

Host factors play a major role in defining patients with possible or probable IFIs according to the European Organisation for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG). The EORTC/MSG criteria describe five host factors: prolonged neutropenia (< 500 neutrophils/mm^3 for > 10 days), allogeneic stem cell transplantation, prolonged use of corticosteroids (minimum dose of 0.3 mg/kg/day of prednisone equivalent for >3 weeks), T cell immunosuppressants and inherited immune deficiency. The fulfilment of at least one of these host factors is required for the category of both possible (host factor plus clinical criterion) and probable IFI (host factor plus clinical plus mycological criterion). However, a too strict definition of host criteria may lead to an underestimation of the burden of IFI, as cases of IFI may be overlooked in the probable category [32-34]. Studies evaluating these host factors are rare, and thus definitions are mostly based on expert opinion [33]. No statistical significant relation was found between any of our study risk factors and the microarray results as in our study. DNA microarray was positive in 28 patients (90.3%) receiving antibiotics, 27 patients (90%) both undergoing chemotherapy and hospitalised, 26 patients (89.7%) receiving IV fluids and 4 patients (66.7%) being treated by steroids.

In a study conducted by Biswal et al. [35] in India, the study population included 120 patients who were given induction chemotherapy. The factors that significantly increased the risk of infection were neutropenia in 95.5% of the cases, presence of an intravenous line in 67.9%, mucosal injury in 80.4%, a history of bone marrow puncture in 89.3% and blood transfusions in 56.3%.

The 30-day crude mortality was defined as death from any cause within 30 days after the onset of febrile neutropenia. IFI-related mortality was defined as death during treatment of a probable or proven IFI with a reversible underlying disease (progression or failure to improve) in the absence of any other condition considered to have caused death [36]. The 31 neutropenic cancer patients included in the current study were followed up for one year. Among them, 10 patients recovered from malignancy, and 13 remained under medication. Eight patients died, with two of them expiring due to febrile neutropenia (within 30 days of a febrile neutropenia attack) and the remaining six dying because of causes other than febrile neutropenia (after 30 days from a febrile neutropenia attack).

The turnaround time of blood culture is too long at a median of four days compared with the total of 135 min required for the microarray technique (90 min for PCR and 45 min for hybridisation). A shortcoming of the DNA microarray method is the cost of $200 per test, whereas the blood culture test only costs $20 per test. However, if the DNA microarray method became a more common test, then the cost will be significantly reduced.

The conflicting results on the numbers and types of fungal infections could be explained by the high incidence of empirical antifungal therapy that results in the reduced incidence of proven/probable IFI. Advanced diagnostic methods may lead to an overall higher sensitivity of diagnosing IFI, but the actual incidence of IFIs may not be changed.

5. CONCLUSIONS

The current oligonucleotide array system enables the rapid and reliable identification of a vast number of fungal pathogens. Moreover, it is more sensitive than conventional methods in detecting multiple species of fungaemia. Therefore, the DNA microarray is a promising tool for the rapid detection of

microorganisms in febrile patients with neutropenia.

Microarray assay can be concluded to help in diagnosing IFIs in the early stages of infection preceding clinical manifestations. It enables the early initiation of antifungal drugs, lowers mortality and enhances clinical recovery.

However, further studies are needed to evaluate the host factors predisposing IFIs and to set definitions for both categories of possible and probable IFIs. Patients should be cared for in an environment that minimises the risk of cross-infection among patients, hospital staff and visitors, preferably in isolation rooms in accordance with the infection control policy. Treatments should start immediately after the collection of samples for blood culture and must not be delayed. If defervescence cannot be achieved by antifungal treatment, the diagnostic tests should be repeated regularly even after initiating the antifungal treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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