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Mycoses in the transplanted patient

M. O. DICTAR*, E. MAIOLO[2], B. ALEXANDER[3], N. JACOB[2] & M. T. VERÓN*,§
Instituto A. Fleming, Buenos Aires, Argentina; [2] Hospital de Agudos 'Dr. C. Argerich', Gobierno de la Ciudad de Buenos Aires (GCBA), Argentina; [3] Duke University Medical Center, Durham, NC, USA; §Clínica Privada Independencia, Buenos Aires, Argentina

The incidence of invasive fungal infection (IFI) has increased considerably over the past 20 years, and transplant recipients are at especially high risk for fungal infections owing to their overall immunosuppressed condition. Organ transplantation procedures were incorporated as a therapeutic option for many patients who lacked the normal functions of organs such as the heart, liver, kidney, lung, pancreas and small bowel. The prevalence of IFI in solid organ transplant (SOTR) patients ranges from 5 to 50% in kidney and liver transplants, respectively. In bone marrow transplant (BMT) patients, IFI are major causes of morbidity and mortality due to the protracted neutropenic period and graft-versus-host disease. *Candida* spp. and *Aspergillus* spp. account for > 80% of fungal episodes in both SOTR and BMT. The development of new immunosuppressive agents, new prophylaxis strategies (as pre-emptive therapy) and the improvement in surgical techniques led to increase survival of transplant recipients. In this session, a clear and concise update of the recent advances in the laboratory diagnosis of candidiasis and aspergillosis in this kind of patients was presented. However, we still need to establish more rapid, sensitive and specific methods for IFI diagnosis. Representatives of the 'Subcomision de Infecciones en el Paciente Neutropenico y Transplantado (SIPNYT)' de la Sociedad Argentina de Infectología (SADI), presented the results of an unusual multicenter study both retrospective and descriptive studies of IFI in SOTR and BMT patients in Argentina. In addition, a study of IFI in 1861 SOTR patients from four centers and the analysis of IFI in 2066 BMT patients from all 12 BMT centers from Argentina was presented. From these studies it can be concluded that 'all transplant recipients are not the same' and that they should be stratified according to their different risk degrees in order to determine the best prophylaxis and treatment strategies.

Keywords bone marrow transplant, immunocompromised patient, mycoses, solid organ transplant

The mycology laboratory in management of invasive fungal infections

In general, the laboratory diagnosis of invasive mycoses involves one or more of three mechanisms: isolation of the fungus by culture, serological detection of antibody, antigen, or metabolites from the fungus, or histopathological evidence of invasion. The challenge for innovative laboratory methods is to provide evidence of infection earlier than is possible with cultures, or to complement culture results by giving a positive result when the cultures are negative.

Correspondence: Dr Miguel Dictar, Servicio Infectología y Microbiología Clínica, Instituto Alexander Fleming, Cramer 1180, CP 1426, Capital Federal, Buenos Aires, Argentina. Tel: +54 11 45552900; fax: +54 11 45556053; e-mail: mdictar@yahoo.com

This discussion will focus on recent advances in laboratory technology for the diagnosis of candidiasis and aspergillosis as well as on the infectious complications that commonly occur in transplanted patients.

Updates in culture methods

Isolation procedures

Many of the traditional methods for the recovery of fungi from clinical specimens remain in use in clinical laboratories today. One area in which major advances have been made over the past 20 years is in the detection of fungemia. As the culture methodology has improved, more fungal pathogens are being detected.

Several early studies revealed that venting and aerobic incubation of conventional broth medium bottles improve the recovery of fungi, as does continuous agitation to increase aeration [1]. The addition of an agar slant to the bottles was the first major advancement in the technique. This biphasic system was found to improve the time of early detection of fungemia over traditional broth-based systems. An innovative break from broth-based culture systems was the introduction of a lysis-centrifugation method that incorporates the use of tubes containing an anticoagulant and a lysing agent, and into which blood cultures are drawn. This technology was found to be an excellent method for recovering pathogenic yeasts (*Candida* and *Cryptococcus* species) as well as thermally dimorphic fungi, particularly *Histoplasma capsulatum*. Several studies demonstrated the lysis-centrifugation technology to be superior to the biphasic systems in the overall rate of recovery as well as the time to recovery of yeast from the blood [1]. The major disadvantages to this technology are that it is labor-intensive and is associated with a high rate of culture contamination despite the use of laminar flow hoods [2].

In addition to the several manual approaches for performing blood cultures, four automated, continuous monitoring instrument systems (Bactec System [Becton-Dickinson, Sparkes, MD, USA], Difco ESP system [Difco, Detroit, MI, USA], BacT/Alert System [Organon Teknika, Turnhout, Belgium] and VITAL System [bioMerieux, Marcy l'Etoile, France]) are now available. Of these automated blood culture systems, the Bactec and BacT/Alert systems are superior in their capacities for the recovery of yeast from blood. Studies have documented that these systems match the performance of lysis-centrifugation methods for the detection of yeast including *Candida* species and *Cryptococcus neoformans* in blood [3,4]. Although the initial investments in these automated instruments are costly, these systems are advantageous in laboratories that process a high volume of blood cultures.

Identification methods

Biochemical assays

Rapid identification of fungi is of vital importance due to differing sensitivities to various antifungal agents. The mainstay of yeast identification to the species level is carbohydrate assimilation, which provides the basis for several commercially available systems designed for yeast identification. However, identification with these systems still takes several days. More rapid tests for the identification of yeasts commonly encountered in the clinical laboratory are now available. These newer tests are based on the presence of specific and unique preformed enzymes. For instance, *Candida albicans* produces the enzymes l-proline aminopeptidase (PRO) and 4-methylumbelliferyl-*N*-acetyl-β-galactosaminidase (MUGAL) whereas other species are positive for one or the other of these enzymes, but not both [5]. This test is available commercially and depending on the particular kit used takes 5–30 min to perform.

Specialized media

Media have also been formulated to provide the presumptive identification of yeasts based on colony morphology. These media are based on the direct detection of specific enzymatic activities by adding certain substrates or fluorochromes to the media. CHROMagar *Candida* (CHROMagar France, Paris, France) is one such medium that can be used for simultaneous isolation and presumptive identification of *C. albicans*, *C. krusei* and *C. tropicalis*. The benefits of using this media is that it shortens the time for presumptive identification of the organisms and it also allows for easier detection of multiple yeast species present in a specimen [6].

PCR

The identification of *Candida* species in blood cultures by a rapid polymerase chain reaction (PCR) method has recently been described. This assay used fungus-specific universal primers for DNA amplification and species-specific probes to identify *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* amplicons in positive blood culture bottles. An enzyme immunosorbent assay based format was then used for amplicon detection. Testing required only 7 h and the test correctly identified all yeasts from 73 positive blood cultures with no false positive results obtained [7]. It is hoped that this technology will soon be available for the clinical laboratory.

In vitro antifungal susceptibility testing

The Antifungal Subcommittee of the National Committee for Clinical Laboratory Standards (NCCLS) has de-

veloped a standardized reference method for antifungal susceptibility testing of yeasts. This standard, the M27-A broth macrodilution method, provides a basis on which different laboratories may assess the minimum inhibitory concentration (MIC) of an antifungal agent and arrive at a value within a close level of agreement [8]. These methods represent a major advancement in diagnostic mycology as clinicians now have a guide to antifungal therapy as they are trying to decide between potentially toxic agents. Unfortunately, the method is labor intensive and requires 48–72 h incubation. This, together with expense, prohibits the routine testing of all clinical isolates. Commercial systems that decrease preparation time are now available (YeastOne; Trek Diagnostic Systems, Ltd., West Sussex, UK), but correlation with manual testing in the clinical setting is pending. Another *in vitro* antifungal susceptiblity testing method that may soon be available for use in the clinical laboratory is the E-test agar diffusion system. This system is also less labor intensive than the M27-A method and preliminary data suggest that its results correlate reasonably well with the reference method [9–12]. It should be noted that no standards for performing susceptibility tests on filamentous fungi are available yet although studies are in process. The NCCLS M27-A standards are only to be applied to *Candida* and *Cryptococcus* species.

Update in non-culture methods

Fungal isolation and identification remain the standard for diagnosing fungal infections. Unfortunately, studies have shown that patients with early invasive candidiasis are not likely to have positive blood cultures [13]. In addition, even when present in clinical specimens, fungi may take days to weeks to grow in the laboratory. Much attention has therefore been paid to non-culture methods for the diagnosis of mycotic infections. The ability of immunosuppressed patients to produce antibodies is limited and unpredictable and therein lies one of the difficulties of using antibody detection as a major diagnostic tool for invasive fungal infection in this population. The advantages of searching for fungal antigens rather than antibodies as markers for fungal infection is that the assay would not be dependent on host humoral responses. Several fungal antigens have been targeted for this purpose.

The detection of the galactomannan polysaccharide antigen in the serum and urine of patients with invasive aspergillosis was first reported in 1979 [14]. Development of various assays for the detection of galactomannan soon followed. Of these assays, the enzyme-linked immunosorbent assay (ELISA) antigen detection technique appears to be superior in its performance characteristics. Maertens *et al*. [15] demonstrated sensitivity and specificity with serial monitoring to be 92·6 and 95·4%, respectively (positive predictive value, 93%; negative predictive value, 95%). Unfortunately, a false positive rate of 8% was noted to occur in the first month post-bone marrow transplant, the explanation for which is still under investigation.

Tests to detect circulating antigens of *Candida* species in urine and serum as an indicator of invasive disease are in various stages of development for use in the clinical laboratory. To date, the list of detectable *Candida*-specific products/antigens in clinical specimens includes D-arabinitol, cell-wall mannoprotein (CWMP), enolase, aspartyl proteinase, and heat-labile antigen. Unfortunately, none of the commercially available tests for the detection of these antigens provide sufficient predictive value for the definitive diagnosis of invasive candidiasis to recommend their routine use in clinical mycology laboratories at this time. Although the antigen detection tests are useful for the diagnosis of candidemia, all assays have certain limitations, reflected by their sensitivity, specificity or both. A combination of two assays on the other hand, may increase the accuracy of diagnosis. Repeat serum sampling may also improve the reliability of antigen detection.

Another antigen under study for potential use in diagnostic testing is $(1\rightarrow3)\beta$-D-glucan, a characteristic, major fungal cell wall constituent. Its concentration in normal human plasma is low [16]. Research has revealed that deep mycoses including aspergillosis and candidiasis are associated with high plasma levels of $(1\rightarrow3)\beta$-D-glucan. Assays to measure $(1\rightarrow3)\beta$-D-glucan have thus been developed and look promising in clinical trials [16–18]. Commercial assays for $(1\rightarrow3)\beta$-D-glucan are not yet available.

PCR technology

The most immediate need for nucleic acid detection methods is in the immunocompromised patient group. Fungal infections usually have a sudden onset, progress rapidly, and are often fatal unless treatment is initiated early in the course of the infection. Much work has already been done in this area and the potential for use of this technology in the laboratory diagnosis of fungal diseases offer great promise. However, more work must be done to enhance the sensitivity and reduce the costs of these tests before they can be routinely used in the clinical mycology laboratory.

Table 1 IFI prevalence rates in solid organ transplant

Allograft	Recipients (*n*)	IFI prevalence rates *n* (%)
Kidney	1434	50 (3·5%)
Liver/liver–kidney	305	22 (7·2%)
Heart	78	4 (5·1%)
Lung/heart–lung	22	2 (9·0%)
Pancreas–kidney	22	2 (9·0%)
Total	1861	80 (4·3)

Invasive fungal infections in solid organ transplant recipients

Organ transplantation procedures were incorporated as a therapeutic option in many patients who lacked the normal organ functions in the heart, liver, kidney, lung, pancreas and small bowel. The development of new immunosuppressive agents and the improvement in surgical techniques led to increased survival of transplant recipients but infectious complications are major causes of morbidity and mortality in the post-transplant period. In an attempt to evaluate the impact of invasive fungal infections (IFI) in solid organ transplant recipients (SOTR) in Buenos Aires, a retrospective and descriptive study was performed.

Four SOTR centers were enrolled. Retrospective data on SOTR with diagnosis of deep fungal infections documented by culture, fungal antigen detection or histological diagnosis were included. Data recorded were analyzed in order to determine prevalence, time of onset, infection rate and associated mortality of IFI in SOTR. Statistical analyses were performed with the Statcalc Calculator EPI INFO, version 6.04c (Centers for Disease Control and Prevention, Atlanta, GA, USA and the World Health Organization, Geneva, Switzerland, October 1997).

From 1986 to 1999, 1861 solid organ transplants had been performed in adult recipients. Eighty cases (4·3%) of IFI were documented. As shown in Table 1 IFI were significantly more frequent in liver and liver–kidney recipients than in kidney recipients ($P < 0{\cdot}01$). *Candida* sp. was the most frequent pathogen (Table 2). Identification was made in eight cases: *C. albicans* ($n = 4$), *C. glabrata* ($n = 3$) and *C. tropicalis* ($n = 1$); the remaining isolates were not fully identified. Four *Aspergillus* spp. strains were identified as *Aspergillus fumigatus* ($n = 1$) and *A. flavus* ($n = 3$). Two patients had fungal coinfections; one of them had a locally invasive *Mucor* sp. and dematiaceous fungus infection and the other a *Pneumocystis carinii* and *Aspergillus* sp. pneumonia.

As seen in Table 3, *Candida* spp. were the most frequently isolated fungi in the early post-transplant period. *Aspergillus* spp. and *P. carinii* infections were related to

Table 2 Isolated fungi and organ involvement in SOTR

	C. neoformans $n = 12$ (15%)	*H. capsulatum* $n = 11$ (13·7%)	*Candida* spp. $n = 24$ (30%)	*Aspergillus* spp. $n = 15$ (18·7%)	*P. carinii* $n = 13$ (16·3%)	Others (6·2%)
CNS	6	1*	–	–	–	–
Lung						
Pneumonia	2†	3	–	3	13	–
Bronchitis	–	–	–	4	–	–
Liver	1	1	–	–	–	–
Esophagus	–	–	5	–	–	–
Upper UTI	–	–	7	–	–	–
Bone and joint	–	1	–	–	–	–
Andominal infection	–	–	3‡	–	–	–
Skin and soft tissues	1	–	–	2§	–	5¶
Eye	–	–	1	–	–	–
Bone marrow	1	–	–	–	–	–
Blood culture	–	–	8	–	–	–
Multiple organs	–	5	–	6	–	–
Not localized	1	–	–	–	–	–

*, Brain abscess; †, solitary pulmonary nodule; ‡, cholangitis, abdominal abscess, peritonitis; §, one surgical wound infection and one venopuncture site infection; ¶, subcutaneous abscess. Fungi isolated are dematiaceous fungi ($n = 2$), *Paecilomyces lilacinus* ($n = 2$), *Trichophyton rubrum* ($n = 1$); *Mucor* sp. ($n = 1$).
UTI, Urinary tract infections.

Table 3 Time of fungal infections after transplantation

Fungus isolated	Post-transplant period		
	<30 days *n*	30–180 days *n*	>180 days *n*
C. neoformans	0	2	10
H. capsulatum	0	0	11
Candida spp.	14	7	3
Aspergillus spp.	2	7	6
P. carinii	0	13	0
Locally invasive infections*	0	0	5

* Include *Mucor* sp., dematiaceous fungi, *Trichophyton rubrum* and *Paecilomyces lilacinus*.

the maximal immunosuppression period. The remaining fungal infections were more frequent in the late post-transplant period.

The overall mortality of IFI in SOTR was 23·7%. Related mortality of IFI in different allografts was: kidney 23·9%; liver 23·8%; heart (3/4 infected patients) 75% (Table 4).

From the above data, the following conclusions can be made:

1. Prevalence of IFI in SOTR was 4·3%. Rates of fungal infections varied in different allograft recipients ranging from 3·5% in kidney transplantation to 9·0% in lung, heart–lung and pancreas–kidney transplants.
2. A significant difference ($P < 0{\cdot}01$) was observed in infection rates in kidney recipients (3·5%) and liver/liver–kidney recipients (7·2%).
3. *Candida* spp. was the most frequent pathogen isolated (30%). Rates of the other isolated fungi were similar (range 13·7–18·7%).
4. *Candida* infections appeared earlier in post transplant than the others fungal pathogens. *Cryptococcus* and *Histoplasma* infections were observed in the late post transplant period.
5. The highest mortality rate was observed in *Aspergillus* infections (60%) followed by *Candida* infections (29%). Mortality related to the other fungal agents ranged 7·6–9%.

Invasive mycoses in BMT recipients in Argentina

In order to describe the current situation of documented IFI in bone marrow and peripheral blood stem cell transplant patients in Argentina, a multicenter, retrospective and descriptive study was performed. To this effect, a questionnaire was sent to 12 centers asking for demographic data, antifungal prophylaxis, room characteristics, amphotericin B empirical treatment, frequency of microbiologically and/or histopahotologically documented IFI, description of each case of IFI. All statistical analyses were performed with the Statcalc Calculator EPI INFO version 6.04c.

The study period was from 12th September 1986 to 30th October 1999, and the results revealed the following points: 12 centers answered the questionnaire but three failed to give all the data requested. In the nine centers answering fully, the total number of transplanted patients was 2066. From these, 1451 (70%) received an autologous- and 615 (30%) an allogeneic transplant. Fourteen (three centers, 2%) were non-related. Most patients (1658, 80%) were adults but the pediatric population (≤ 15 years) was also considered (408, 20%). According to gender (11 centers, 1992 transplants), there were 1091 (55%) males and 901 (45%) females. As it concerns the characteristics of the patients' rooms, 61 had high efficiency particulate (HEPA) filters (median of 4·5 rooms per center, range 2–10), and 23 (38%) had laminar flow.

Antifungal prophylaxis with fluconazole was used by eight centers: for patients colonized by non-*C. krusei Candida* (two centers); in one center for all allogeneic and in only autologous patients with leukemia, as well as in allogeneic patients with gammaglobulin antilymphocitic (GAL) and/or corticosteroid treatment and/or expected

Table 4 Etiological agent and related mortality in SOTR

	Kidney	Liver	Heart	Lung/heart–lung	Mortality %
C. neoformans	1/10	0/2	–	–	8·3
H. capsulatum	1/10	0/1	–	–	9·0
Candida spp.	2/9	4/14	1/1	–	29·2
Aspergillus spp	6/9	1/1	2/3	0/2	60·0
P. carinii	1/10	0/3	–	–	7·6
Locally invasive	0/4	0/1	–	–	0

protracted neutropenia (two centers), and universally during the neutropenic period (three centers). Amphotericin B at low doses was given in one center because of a high rate of aspergillosis secondary to building work. No treatment was given in three centers. Amphotericin B was used empirically (10 centers) during the neutropenic period in 553/1768 transplants (31%, range 15–53%). Eight centers indicated use of empirical treatment with amphotericin B in autologous (373/1138; 33%, range 14–51%) and allogeneic transplants (93/265; 35%, range 17–64%) (P = not significant).

Documented IFI

Microbiologically and/or histopathologically documented IFI were found in 101/2066 transplants (4·8%, range 0–24%); from these 54/1451 transplants (3·7%, range 0–10%) were autologous and 47/615 (7·6%, range 0–24%) allogeneic ($P < 0·01$). Eighty-three IFI were confirmed in patients with underlying diseases, as follows: leukemias 34 (41%; acute 24–29%, chronic 10–12%), lymphomas 27 (32·5%), solid tumors 11 (13%), aplastic anemia 6 (7%), multiple myeloma 4 (5%), Fanconi's disease 1 (1%).

Twelve centers (101 IFIs) reported on the fungi isolated, the majority of which corresponded to yeasts (73, 72%), most non speciated *Candida* spp. (69, 68%). Non-*C. albicans Candida* was isolated in 41 (40·5%) cases, *C. tropicalis* in 13, *C. krusei* in 10 (10%), *C. parapsilosis* in nine, *Candida* sp. in six, *C. glabrata* in three and *C. albicans* in 28 (28%). Other yeasts (*Rhodotorula* sp., *Hansenula anomala*, *Trichosporon pullulans*, *P. carinii*) were also isolated in 4% of the population.

There were 28 (28%) cases in which filamentous fungi were isolated. Causative agents were: *Aspergillus* spp. ($n = 15$, 15%), including *Aspergillus* sp. ($n = 7$), *A. fumigatus* ($n = 4$), *A. flavus* ($n = 3$), *A. niger* ($n = 1$); *Fusarium* sp. ($n = 6$, 6%), *Mucor* sp. ($n = 1$, 1%). Other fungi were also identified in six patients: *Pseudallescheria boydii*, *Penicillium* sp., *Alternaria* sp., *Exophiala jeanselmei*, *Acremonium* sp. and unidentified moulds.

No differences were found in type of fungi (filamentous fungi vs. yeasts) isolated in autologous and allogeneic transplants.

Time of IFI diagnosis

These data were from 11 centers with 83 cases of IFI. During the neutropenic and post-neutropenic periods, there were 60 (72%) and 17 (20·5%) cases, respectively. Three (3·5%) cases were diagnosed before transplantation and three (3·5%) lacked information. Yeasts were diagnosed during neutropenia more frequently than filamentous fungi: 48 (80%) vs. 12 (20%) transplants ($P = 0·01$), respectively. Filamentous fungi were diagnosed in the post-neutropenic period more frequently than yeasts: nine (53%) vs. eight (47%) ($P = 0·01$), respectively.

Site of IFI

In 83 IFIs, diagnosed fungal involvement was confirmed in 110 sites corresponding to: blood ($n = 36$, 34%), lungs ($n = 17$, 15%), skin ($n = 11$, 10%), catheter tip ($n = 10$, 9%), gastrointestinal tract ($n = 9$, 8%), genitourinary tract ($n = 8$, 7%), paranasal sinus ($n = 5$, 4·5%), disseminated ($n = 3$, 3%), heart ($n = 2$, 2%), external otitis ($n = 2$, 2%), upper respiratory infections ($n = 2$, 2%), liver ($n = 2$, 2%), bile ducts ($n = 1$, 1%), spleen ($n = 1$, 1%) and brain ($n = 1$, 1%).

Type of IFI

Eighty-three documented IFIs were classified as:

1. localized (one organ with or without fungemia) in 44 (53%) patients, nine (20%) with fungemia; fungi isolated were *C. albicans* ($n = 15$, 34%), non-*C. albicans Candida* ($n = 11$, 25%), *Aspergillus* spp. ($n = 9$, 20%) and others ($n = 9$, 20%). The most frequent sites of infection were lung ($n = 12$), skin ($n = 9$), genitourinary tract ($n = 8$) and paranasal sinus ($n = 5$);
2. disseminated acute or chronic (two or more non contiguous organs) in 11 (13%) patients, two (18%) with fungemia; fungi isolated were *Aspergillus* spp. ($n = 5$, 45%), *C. albicans* ($n = 2$, 18%), non-*C. albicans Candida* ($n = 2$, 18%), others ($n = 2$, 18%);
3. fungemia without clinical site of infection in 18 (22%) cases; fungi isolated were non-*C. albicans Candida* ($n = 14$, 78%), *C. albicans* ($n = 3$, 17%) and others ($n = 1$, 5%);
4. catheter related infection in 10 (12%) patients, seven (70%) with fungemia; fungi isolated were non-*C. albicans Candida* ($n = 5$, 50%), *C. albicans* ($n = 3$, 30%) and others ($n = 2$, 20%).

Treatment of IFI

In 83 cases of IFI, 70 (84%) transplant patients were treated with amphotericin B (56 standard, 13 liposomal, one colloidal), six (7%) with fluconazole (three during neutropenia, two post-neutropenia, one unknown), five (6%) received no treatment (four deaths), one (1%) trimethoprim-sulphametoxazole and one (1%) unknown.

Outcome of IFI

In 83 cases of IFI, 53 (64%) patients were cured, four (7·5%) of which required surgical treatment. The IFI mortality was 22% (18 cases). Other causes of death were registered for 11 (13%) patients, with one unknown cause (1%). Mortality directly related to filamentous fungi was 10/26 (38·4%) versus yeast 8/57 (14%) ($P = 0·02$). The fungi isolated were *Aspergillus* sp. ($n = 6$), non-*C. albicans Candida* ($n = 4$), *C. albicans* ($n = 3$), *Fusarium* sp. ($n = 2$) and others ($n = 3$).

Conclusions

The above results draw attention to the following points: (i) nine centers (75%) used antifungal prophylaxis while three did not; (ii) one-third of transplant patients required empirical amphotericin B treatment; (iii) IFI frequency was 4·8% with a wide range (0–24%) probably due to different populations and/or techniques for diagnosis; (iv) IFIs were significantly more frequent in allogeneic (7·6%) than in autologous (3·7%) transplant patients ($P < 0·01$); (v) IFI underlying diseases were most frequently acute and/or chronic leukemia ($n = 34$, 41%) followed by lymphomas ($n = 27$, 32·5%); (vi) most frequent yeasts were non *C. albicans Candida* in 40·5%, followed by *C. albicans* in 28%; *C. krusei* was isolated in 10%; (vii) most frequent filamentous fungus was *Aspergillus*; (viii) IFI were diagnosed mostly during neutropenia; yeasts were diagnosed mostly during neutropenia and filamentous fungi during the post-neutropenic period ($P = 0·01$); (ix) most common sites of IFI were blood, lungs, skin, catheter tip, gastrointestinal tract and genitourinary tract; (x) localized IFI was the most common clinical presentation; (xi) fungemia was detected in 18–22% of cases with localized, disseminated or no focused site of infection; (xii) localized IFI were caused by *C. albicans*; disseminated cases by *Aspergillus* sp. and fungemia, without clinical site of infection or catheter-related, by non *C. albicans Candida*; (xiii) standard amphotericin B therapy was the most frequent treatment used; (xiv) mortality directly related to IFI was 18 deaths (22%) with a significantly higher mortality related to filamentous fungi ($P = 0·02$).

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Contributors

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