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Achievements and goals of the EORTC Invasive Fungal Infections Group

B.E. de Pauw[a], R. Herbrecht[a,*], F. Meunier[b] on behalf of the EORTC Invasive Fungal Infections Group

[a]*Department of Hematology, University Medical Centre Nijmegen, PO Box 9101—Geert Groteplein 10, NL-6500 HB Nijmegen, The Netherlands*
[b]*EORTC Central Office, Avenue E. Mounier 83 bte 11, B-1200 Brussels, Belgium*

Abstract

Invasive fungal infections are an increasing complication for patients with cancer. These infections still are difficult to diagnose and to treat and thus still have a high fatality rate. New strategies should include evaluation of new diagnosis tools and large-scale assessment of these new methods will need multidisciplinary collaboration. High-quality clinical trials dedicated to establish 'state-of-the-art' prevention and treatment are also directly needed. Created in 1991, the EORTC Invasive Fungal Infection Group has faced several of these challenges and significantly improved the knowledge and management of these infections in Europe.

Keywords: Fungal infections; Empirical therapy; Prevention; Treatment; Antifungal agents

1. Introduction

Invasive fungal infections are the principal cause of death of both remitting and unremitting patients who required intensive, bone-marrow ablative chemotherapy. Familiar opportunistic fungi, as well as hitherto obscure specimens, can be encountered as culprits in a kaleidoscopic pattern of clinical manifestations that may carry a high mortality, even in patients with a remitting underlying disease. Facing this challenge, the EORTC Invasive Fungal Infections Group (IFIG) was created in 1991 on the initiative of Françoise Meunier, who became the first Chairperson. The aims of the group were defined as:

a. To conduct, develop, coordinate and stimulate clinical research on epidemiology, diagnosis, prevention and treatment of invasive fungal infections.
b. To increase the awareness of physicians, nurses and all other healthcare workers with regard to the morbidity and mortality associated with invasive fungal infections.
c. To provide insight in the economical burden originating from fungal infections to the society and healthcare systems.

To achieve these purposes, international cooperation remains mandatory to guarantee a high quality of clinical research in this difficult field of assessing optimal strategies. Moreover, a multicentre setting helps to preserve the capacity for medical excellence throughout Europe. In fact, in the early 1990s, it was inevitable to conclude that, due to the lack of reliable data, virtually all actual antifungal strategies were based on assumptions rather than on solid evidence.

As of 31 December 2000, the Group comprised 45 active, 33 probationary and 18 corresponding centres distributed over the European Union countries, as well as Croatia, Czech Republic, Hungary, Israel, Kuwait, Norway, Poland, Saudi Arabia, Slovakia, Switzerland and Turkey. Many members of the Invasive Fungal Infections Group are simultaneously members of other EORTC Groups such as the Leukemia Group, Lymphoma Group and International Antimicrobial Therapy Group. Due to her appointment as Director-General, Françoise Meunier chose to resign from the Chair of the Invasive Fungal Infections Group. In September 1995, she was replaced by Ben de Pauw who, after having served two terms, was succeeded by Raoul Herbrecht in October 2001. During the years 1992–2001, in total

* Corresponding author. Based at: Hopital Universitaire Hautepierre, Dept. Onco/Hematologie, Rue Moliere, F-67098 Strasbourg, France. Tel.: +33-3881-27688; fax: +33-388-127681.
E-mail address: raoul.herbrecht@chru-stasbourg.fr (R. Herbrecht).

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more than 1300 patients were entered in clinical investigational projects.

2. Activities

2.1. Epidemiological work

Several surveys have helped to identify the principal clinical problems to be solved by designing new clinical trials.

Four epidemiological surveys were conducted, partly in collaboration with the European Bone Marrow Transplantation Register (EBMT) and with the EORTC Leukemia Group:

- the incidence and outcome of fungaemia (Coordinator: C. Viscoli),
- invasive aspergillosis (Coordinator: D. Denning),
- the occurrence of aspergillosis during therapy for multiple myeloma (Coordinator: O. Lortholary),
- the outcome of patients with invasive fungal infections undergoing a bone marrow transplant procedure (Coordinator: F. Offner).

These surveys were published in leading, peer-reviewed journals [1–4].

2.2. Harmonisation and 'state-of-the-art' treatments

Several members of the IFIG participated in a consensus committee with litigants from the USA and Japan to formulate guidelines for the treatment of yeast infections [5]. Indeed there were huge interinstitutional differences, not only in the incidence and approach of invasive mycoses, but also with regard to definition and their classification [6]. Terms like 'presumed', 'probable', 'possible', 'proven', 'histologically' or 'culturally documented', 'clinically documented' led to misinterpretation because there was no consistent agreement on the criteria required to place the patient in a given category. Moreover, the availability of new diagnostic techniques complicated the matter even further. By consequence, it became difficult to compare the results of different studies, even when they had been conducted by the same group of investigators. The IFIG took the initiative to constitute a task force to define the criteria for invasive fungal infection in conjunction with the Mycosis Study Group and other opinion leaders in the USA [7]. This project was coordinated by Dr Sibel Ascioglu, who worked for a year as a research fellow at the EORTC Data Center in Brussels. She also helped to complete the analysis of the aforementioned epidemiological surveys on fungi occurring in myeloma patients and bone marrow transplant recipients. This fellowship had been made possible by a fee from the Turkish government followed by an unrestricted grant from Pfizer International, New York, USA. As soon as consensus was achieved, the definitions proved to be widely used for both new clinical studies and reviews.

2.3. Clinical trials

Many ideas for clinical trials were generated, but their actual execution was impeded by factors such as financial restrictions and, above all, difficulties in establishing a firm diagnosis of infections. Even if all patients from all EORTC centres with invasive fungal infection as a complication of a malignant disease were entered in clinical trials, there still would hardly be a sufficient number of subjects available to conduct good quality studies within a reasonable time-span. It was therefore decided in 1997 that, after stratification, hosts with an invasive fungal infection who were immunocompromised due to causes not related to a malignancy could be entered into the Group trials in an attempt to enlarge the capacity to conduct statistically reliable clinical studies. Moreover, an official agreement was reached with the Mycoses Study Group from the USA to synchronise the study protocols as much as possible and to exchange strategic plans and scientific data.

Several trials were conducted successfully by the EORTC Invasive Fungal Infections Group:

- 19911: fluconazole versus itraconazole for the treatment of oropharyngeal candiasis in non-neutropenic patients; Trial Coordinator: R. DeBock [8]
- 19923: a study comparing Ambisome® 1 mg/kg/day with 4 mg/kg/day in proven and probable invasive fungal infections in neutropenic patients; trial coordinators: M. Ellis and D. Spence [9]
- 19962: a phase II study designed to explore the feasibility of liposomal nystatin in treatment of refractory invasive fungal infections; trial coordinators: F. Offner and G. Samonis [10]
- 19961: comparison of the safety and efficacy of amphotericin B with the new azole voriconazole for patients with invasive aspergillosis; Trial Coordinators: D. Denning and R. Herbrecht. This study had an identical sister protocol for patients to be enrolled in North America [11]

The presently running trial 19951 is designed to investigate the optimal time to initiate empirical antifungal therapy. In this study, therapy with 3 mg/kg/day of Ambisome® is started at day 3 or at day 6 of persistent fever in neutropenic patients who have no further objectively verifiable evidence of an infection; Study Coordinators: C. Viscoli and P. Ljungman.

3. Future developments

In the years to come, many key questions need to be answered; the main items for clinical studies are summarised in Table 1. It has to be recognised that such investigations will be difficult to realise for reasons apparently inherent to invasive fungal infections; these are listed in Table 2.

3.1. Diagnostic problems

Fungal infections account for 20–30% of the fatal events in patients with acute leukaemia. The incidence of documented invasive fungal infection is reported to constitute around 7% of all febrile episodes in neutropenic patients. Many specialists have the feeling that the incidence of invasive fungal infections is increasing, but it is far from clear whether this a real increase or a reflection of a greater awareness, better diagnostic tools, improved antibacterial therapy preventing death due to bacterial infections (including Gram-negative sepsis), a better cancer cure rate, etc.

At least as worrisome are reports that disclose an increasing incidence of unusual fungal pathogens in cancer patients. Furthermore, data on the interruption of treatment of the underlying malignancy are only anecdotal. More exact figures concerning these issues are required to estimate the size of the problem and to determine whether it warrants extensive material and personnel investments by both the scientific community and the pharmaceutical industry.

Estimations on the incidence of invasive fungal infections so far have also been inaccurate due to poor diagnostic tools. Most of the infections are, indeed, not diagnosed or treated ante-mortem. This is partly related to absence of the classic inflammatory response. Atypical pulmonary infiltrates, as well as unexplained disturbances of liver and kidney function and echographical lesions in these organs, may be suggestive of the diagnosis. Adequate culturing is frustrated by the low specificity of specimens such as sputum, whereas invasive diagnostic interventions are often precluded because of concomitant thrombocytopenia or hypoxaemia.

Besides, even if tissue specimens are obtained, they rarely yield any growth, yet culture is a critical prerequisite to the correct identification of the offending fungus. In the near future, the rapidly evolving and promising techniques in molecular biology, i.e. the polymerase chain reaction, may provide a solution to detect very small amounts of diagnostic materials. Metabolites of the fungus, such as mannose, enolase, mannitol and arabinotol, are found in varying amounts and are not specific for invasive disease. Therefore, the employment of a panel of diagnostic tests and markers could be considered instead of relying on a single assay. Unfortunately, the potential value of these techniques have not yet been tested in studies that are sufficiently large to permit firm conclusions. Moreover, the majority of procedures available so far only allow semi-quantitative determination which renders them less suitable for therapeutic monitoring. In spite of all these limitations, the results of laboratory investigations and imaging techniques may be very helpful if communication between the clinician and microbiologist or radiologist is optimal.

3.2. Prevention and prophylaxis

There is increasing evidence for person-to-person transmission of yeast infections, as well as for the association between construction works and local outbreaks of *Aspergillus* infections, coinciding with a high air concentration of spores. Next to building activities, pigeon droppings, dry flowers, pepper and medication carton have been identified more or less anecdotally as sources of *Aspergillus* spores. However, the appropriate hygienic measures to prevent these events have not been studied consistently. It is generally assumed that yeast transmission can be prevented by proper hand washing, but the role of local disinfectants and protective

Table 1
Key questions in clinical fungal research

•How to avoid treatment interruption because of a disseminated fungal infection in patients with controllable underlying malignant disease?
•How reliable and reproducible are the presently available diagnostic procedures and what can be expected of the new techniques?
•Is it possible to prevent the occurrence of an invasive fungal infection by prophylactic use of antifungal drugs?
•Is personal and environmental hygiene important in the prevention of invasive fungal infections?
•What is the place of empirical antifungal therapy in the persistently febrile neutropenic patient?
•Which antifungal drugs should be preferred?
•Are combinations feasible and/or necessary?
•Are the lipid formulations of amphotericin B cost-effective?
•What is the optimal daily dose of systemically active antifungal agents?
•What can be expected from biological response modifiers such as interferon and haematopoietic growth factors?

Table 2
Problems in studying invasive fungal infections

•Uncommon diseases
•Difficult diagnosis
•Slow accrual
•Little interest among nurses
•Not limited to a particular population
•Major influence of indirect factors
•Recovery of immune status is a crucial factor
•Occurrence often in end-stage disease
•Protracted infections
•Long follow-up required

clothing is still obscure. Laminar flow systems and HEPA filters are believed to lower the concentrations of spores in the air, but prospective studies on the impact of expensive portable HEPA filter cabinets are not available.

The temptation to use one of the available antifungal drugs in a prophylactic setting is apparently irresistible. Unfortunately, the evidence to support such a strategy in the majority of patients at risk is rather thin [12]. Older studies on the prophylactic use of polyenes and older azoles are outdated because of major changes in cancer chemotherapy regimens during the last decennium. Moreover, there is little evidence that oral amphotericin B has any impact on the number of invasive fungal infections, and this agent is known for its limited compliance due to adverse events. Most studies on prophylaxis comprise too few patients which hampers the assessment of efficacy due to a large type II error.

In addition, the criteria used to describe the outcome of prophylaxis trials deserve attention; the number of proven infections and the number of deaths attributable to a fungal infection are crucial, but data on the latter parameter are seldom available. In contrast, surrogate parameters like the perceived need for systemic antifungals and overall survival are frequently reported. Many studies have a dual goal, namely the prevention from both moulds and yeasts, whereas these infections have a completely different epidemiology and portal of entry and, consequently, require separate analysis. The newer triazoles are better tolerated and appear to be more efficacious. Prophylaxis with fluconazole at a dose of 400 mg once daily was effective in preventing superficial and disseminated candidiasis in combination with a lower overall mortality in a population of bone marrow transplant recipients. However, the picture is far less clear for other patient groups. In several randomised studies, itraconazole was effective in diminishing colonisation of the gastrointestinal tract by *Candida* strains, but neither the ultimate incidence of documented and presumed *Aspergillus* infections, nor the perceived need for intravenous amphotericin B was influenced.

3.3. Empirical antifungal therapy

Most trials commonly include patients in whom the diagnosis of fungal infection is only presumptive. It is generally contended that early antifungal therapy and clinical awareness of the possibility of an invasive fungal infection are crucial prerequisites to avoid a confrontation with a stage of infection that is beyond a reasonable chance of cure. By consequence, treatment is often commenced on an empirical basis, i.e. for fever not responding to adequate antibacterial therapy. This strategy is based on two studies that comprised a very low number of patients and the statistical power of both trials, especially in the subgroup analysis, is too low to allow any definite conclusions.

Nevertheless, the empirical use of intravenous amphotericin B in persistently febrile granulocytopenic cancer patients has become a very popular strategy. Starting at day 3 of persisting fever, two-thirds of the neutropenic patients with acute leukaemia and bone marrow transplant recipients in North America nowadays will receive empirical amphotericin B whilst most centres in Europe will commence intravenous antifungals after 5–7 days. Others are even more selective and prefer guidance by clinical, radiological and microbiological parameters that might be indicative of an invasive fungal infection. Indeed, some patients at high risk might benefit from a very early institution of systemic antifungals. Therefore, the question of the optimal time-point to start antifungal treatment remains and is currently investigated by the EORTC Invasive Fungal Infections Group.

3.4. Antifungal agents

Therapeutic options for invasive fungal infections are qualitatively and quantitatively limited. Amphotericin B is potentially toxic to the kidney and causes hypokalemia, among many other side-effects such as immediate allergic reaction. A reduced, better tolerated dose may be insufficient to arrest or control a proven mycosis. The antifungal activity of amphotericin B results primarily from its interaction with sterols in the cell membranes of eukaryotic cells leading to a leakage of cations and the subsequent loss of macromolecules such as nucleoproteins. Binding of amphotericin B to ergosterol, the cell membrane sterol characteristic of fungi, accounts for antifungal activity. Binding to cholesterol, the membrane-stabilising sterol characteristic of human cells accounts for toxicity. The addition of corticosteroids may blunt the antifungal activity since they impair the cellular immunity almost instantly and because they are sterol molecules themselves they may bind amphotericin B, rendering the drug unavailable to interact with ergosterol in fungi. Nephrotoxicity can be reduced by making certain that the patient is eunatremic. In the less critically ill patients administration of itraconazole may be an acceptable alternative to amphotericin B in patients with aspergillosis who require prolonged treatment. A high price and the lack of data on efficacy in comparative, randomised trials are the drawbacks of lipid formulations of amphotericin B introduced to circumvent the amphotericin B-related toxicity. The options and limitations of these formulations in comparison to traditional amphotericin B have been assessed at several institutes worldwide, but the number of adequate, randomised, prospective studies in proven or probable cases remains disappointingly limited. Formal

phase II trials assessed the activity of liposomal nystatin and caspofungin, the new intravenous candin with a different mechanism of action from the traditional antifungals, in the treatment of invasive aspergillosis; the results justify further evaluation in comparative studies [10]. Moreover, in view of the results from the recent EORTC study that showed the superiority of the voriconazole over amphotericin B for first-line treatment of invasive aspergillosis, a reappraisal of all other antifungals for this indication is mandatory [11]. Where and when they should be used, as well as what are the potential benefits of a combination of different classes of antifungals appear to be appropriate questions.

5-Flucytosine, an analogue of cytosine, is effective against *Candida* and *Cryptococcus* infections, but the fungi may become rapidly resistant if the drug is given alone. There are data to prove that amphotericin B and 5-flucytosine act synergistically. However, only a small series suggests that combination therapy may be of benefit in some cases. Patients with a high likelihood of invasive candidiasis may benefit from high-dose fluconazole. However, failures of fluconazole have occurred, particularly in infections caused by *Candida glabrata* and *Candida krusei*. Combining the drug with amphotericin B *ab initio* may address this problem, but there are no data to support such a strategy. Itraconazole might offer an alternative to fluconazole for treating disseminated candidiasis. Furthermore, the high expectations on the activity of the new candins against resistant *Candida* species require corroboration.

3.5. Dosing of antifungals for therapeutic purposes

Intravenous amphotericin B, at a dose of 0.5–1.0 mg/kg/day, is considered the standard therapy for granulocytopenic patients suspected to have a pulmonary or disseminated mycosis. The full therapeutic dose level should be achieved within the first 24 h and an administration time of 4 h is most common. However, these recommendations are rather based on assumptions and limitations set by inherent toxicity than by results obtained in randomised studies. There is increasing evidence from open studies that lipid formulations of amphotericin B are offering an improved therapeutic index. However, it remains questionable whether the higher doses of 3–5 mg/kg/day will produce enhanced efficacy as no direct comparisons with a standard dose of conventional amphotericin B or with lower doses of these lipid formulations have been made in adequate numbers of neutropenic patients. Indeed, preliminary results from an EORTC Invasive Fungal Infections Group study indicate that 1 mg/kg/day of Ambisome® may be as effective as the recommended dose of 4 mg/kg/day [9].

The optimal duration of treatment has not been established; in cases where parenteral amphotericin B was given for empirical purposes, it is usually given until neutropenia and fever have resolved. In other cases, therapy should be continued until all signs and symptoms have disappeared or, in case of a documented *Aspergillus* infection, objectively verifiable improvement has been registered. From a theoretical point of view, alternate-day therapy appears pharmacokinetically appropriate since the half life of amphotericin B is approximately 24 h. Such a strategy may be better tolerated and allows for improved nutrition and better hydration. For acute *Candida* infections, there is little objective evidence to support any firm recommendation. In chronic disseminated disease, signs and symptoms of chronic disseminated candidiasis can persist for more than 6 months, despite adequate antifungal therapy. For cryptococcosis, a minimum duration of treatment of 10 weeks is mentioned again, whilst for the other, more rare fungi, no useful recommendations on the duration of treatment can be found. Virtually all conclusions on the duration of therapy are based on assumptions and ought to be confirmed in a clinical setting.

3.6. Biological response modifiers

The mortality rate of invasive fungal infections in patients with cancer varies in major series between 40 and 75% with an average of about 60%. These studies, however, comprise very heterogenous patient populations. Even under optimal circumstances, the overall successful outcome of a documented invasive fungal infection may not exceed 20%. Retrospective data and one prospective study indicate that no significant differences in the rate of survival can observed between patients with disseminated mycoses who received intravenous amphotericin B and those who did not. With an increasing number of granulocytes, a response rate of approximately 85% can be anticipated and patients with a remitting underlying disease will recover in around 60% of cases. The failure to recover granulocytes is a poor prognostic factor. Hence, a possible mode to improve the outcome of invasive fungal infections in neutropenic patients is to enhance the patient's natural defence mechanisms. Granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) have direct effects on the number of circulating cells, mainly neutrophils and macrophages and influence their function resulting in an increased fungal killing activity. Presently, there is an intriguing discrepancy between the positive effect of growth factors on granulocyte recovery and objective clinical benefits. This may be partly due the heterogenous study populations. Likewise, interferon holds promise on the basis of studies in animal models, but data on its use in patients with invasive fungal infections are lacking.

Table 3
Arguments to adhere to large clinical trial groups

•To assess strategies or drugs that are commercially problematic
•Only way to do large strategic trials in a timely manner
•To cooperate with pharmaceutical companies which should consider clinical trialists as equal partners
•Continuous exchange of thoughts
•Personal biases/preferences are neutralised
•Minimise personal profits and keep scientific research as a priority

3.7. Improving the quality of clinical trials

Randomised clinical trials remain the single best tool known for minimising bias and generating high quality data. The areas of highest need are clearly invasive candidiasis and aspergillosis. Multiple new drugs, including entirely new classes of agents, are now entering development. The recent EORTC-MSG set of definitions for invasive mycoses are a step towards creating a global consensus about the populations to study, but there is also a need for an improved, generally accepted scoring system consisting of standardised reference points for the assessment of outcome in clinical trials [6].

4. Concluding remarks

Progress in the treatment of invasive fungal infections has been made during the last years and the IFI Group of the EORTC made a considerable contribution to this. There will be no further progress without high quality clinical research to define 'state-of-the-art' treatments. As indicated in Table 3, such duties should be fulfilled by a multidisciplinary effort and large, global networks of clinical scientists because only well-designed clinical trials with adequate numbers of patients will provide unequivocal answers on the basis of unbiased data. Particularly in the field of invasive fungal infections, clinical research relies on teams of experts, including doctors, statisticians, data-managers, ethicists, economists, etc. Careful design of clinical trials should include appropriate definitions of the various clinical entities to be investigated and of the parameters to be measured. Today, only less than 5% of available patients with cancer are enrolled into clinical trials. Only if this is improved can we face the challenge provided by fungal infections that often complicate the treatment of malignant diseases. Collaboration at an international level is mandatory to combat this threat which could become one of the major impediments to achieving a cure of the basic underlying malignancy.

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