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Clinical antifungal efficacy trials in invasive aspergillosis: Consensus standards for trial design and room for improvement

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Despite recent improvements in outcome of invasive aspergillosis there are still high failure and fatality rates. The trial comparing voriconazole to amphotericin B deoxycholate has become a reference for clinical trials in invasive aspergillosis due to the large number of patients included, the use of definition criteria close to the international consensus criteria, the inclusion of the halo sign on chest computed tomography for the definition of probable cases, the extensive review of the data by a panel of experts including radiologists, and most important, the successful efficacy results. Similar strict methodology for eligibility and assessment of outcome has been applied in the recently completed liposomal amphotericin B trial. This study compared a standard daily dose of liposomal amphotericin B (3 mg/kg) versus a high loading dose (10 mg/kg/d). The option to include possible cases in this trial and to give the investigators a few days to upgrade the diagnosis to a probable or definite level proved to be an effective strategy, saving four months in the duration of the recruitment period. Additional progress can be expected in future trials with the use of a standardized cutoff for the galactomannan detection test and a stratification at randomization on the most critical prognostic factor such as progressive underlying malignancy or allogeneic stem cell transplantation.

Keywords invasive aspergillosis, voriconazole, amphotericin B deoxycholate

Introduction

Invasive aspergillosis is a severe disease that occurs in severely ill patients. The response rate to first line therapy is only in the range of 50% with the most active anti-*Aspergillus* agents [1,2]. The three-month mortality rate is approximately 30% in the most recent clinical trials. The mortality continues to increase with the time of observation as many of these patients die from their underlying condition even if aspergillosis has been cured. It has been shown that the two-year overall mortality rate after onset of an invasive aspergillosis is as high as 74% in patients with a hematological malignancy [3].

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A consensus for clinical trial design? No, but a reference

Currently there is no consensus for the design of clinical trials in invasive aspergillosis. However there is a reference: the voriconazole comparative trial [1]. This trial compared voriconazole to amphotericin B deoxycholate as initial therapy in invasive aspergillosis. It has become a reference for several reasons:

- Three hundred and ninety-one patients have been recruited. This is the largest number of patients ever included in a first line therapy of aspergillosis trial. Of these 391 cases, 277 have been fully qualified for eligibility.
- Patients have been included in 95 centers located in four different continents demonstrating that two groups, the European Organization for Research and Treatment of Cancer and the Global Aspergillus

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Group on the American side of the Atlantic Ocean, can work together.

- The case definitions used for this trial have been very close to the international consensus definition criteria anticipating appropriately the criteria later developed and published by the EORTC/MSG [4].
- The halo sign, a progressive attenuation of the density around a nodule visible on CT-scan and very suggestive of invasive aspergillosis, has been accepted as a criterion to determine eligibility for a clinical trial in the voriconazole study [5].
- A data review committee of 12 members reviewed eligibility and response assessment of all cases. As another innovation, four radiologists were members of this data review committee and they played a major role in confirming or not the eligibility of the cases qualified on the basis of a halo sign.
- And mostly important, the successful achievements of the endpoints of this study with, for the first time in the antifungal therapy area, a benefit in survival: patients treated with voriconazole had a significantly improved response rate and 12-week survival compared to amphotericin B treated patients. It must be noted that the final results were very close to the estimated response rates for both amphotericin B deoxycholate (estimated response rate of 35%, observed response rate of 32%) and voriconazole (estimated response rate of 55% on the basis of an open label study [6] and observed response rate of 53%). The 32% response rate in the amphotericin B arm reflected the true response rate to this drug and was not underestimated by any flaw in the design of the study. One large multicenter series of cases treated in 1994–1995 demonstrated a complete plus partial response rate to amphotericin B deoxycholate of 32%, identical to the observed response rate in the amphotericin B arm of the global comparative voriconazole study [7].
- The comparative voriconazole trial has a major impact on the practice. Amphotericin B has been the gold standard for the primary therapy of invasive aspergillosis. Voriconazole has been approved in 2002 for primary therapy of invasive aspergillosis on the basis of the increased favorable response rate, the survival benefit and of the much better tolerance. All guidelines published since this approval recommend voriconazole as the best choice for first line therapy of invasive aspergillosis [8–12]. A similar recommendation has been formulated at the first European Conference on Infections in Leukemia held in September 2005 [R. Herbrecht *et al*., unpublished results].

There are however two limitations to the voriconazole comparative trial:

- First, the halo sign accepted as an entry criterion was not clearly defined in the protocol. Although this sign has been recognized and described nearly ten years earlier [13], it was not well known by radiologists, hematologists or infectious diseases physicians at the time this trial was conducted. As a consequence there was a high rejection rate of the investigators assessment of the halo sign by the data review committee. Overall 16% of the cases were disqualified by the data review committee for this reason.
- Second, the trial has been criticized as it compared voriconazole to amphotericin B deoxycholate and not to a lipid formulation. The reason of the choice of amphotericin B deoxycholate as comparator was due to the fact that none of the lipid formulations of amphotericin B was licensed for primary therapy of invasive aspergillosis. This is still the case. Due to regulatory and ethical constraints, the comparator had to be a drug licensed for the indication and therefore could only be amphotericin B deoxycholate. Investigators who designed the study were aware that median duration of therapy would certainly be much shorter in the amphotericin B deoxycholate arm than in the voriconazole arm for safety reasons and possibly for efficacy reasons. It was therefore planned to assess, as primary objective, the efficacy at week 12 including the course of initial randomized therapy and also of any other licensed antifungal agent given after failure or intolerance to the primary therapy. An additional analysis demonstrated that patients failing amphotericin B deoxycholate did not benefit much from the replacement of the conventional form of amphotericin B by a lipid formulation [14].
- Other critiques have included the possible bias owing to non-blinding of therapy, and the non-uniformity in use of amphotericin (premedication, fluids) between centers.

A second step in improvement of clinical trials

In December 2005, Cornely *et al*. presented the results of another large scale trial in first line therapy of invasive filamentous fungal infections [2]. This study, called the Ambiload study, compared two dosages of liposomal amphotericin B: a standard dose of 3 mg/kg per day and a high dose regimen of 10 mg/kg/d given for 14 days and followed by the standard dose. Response definitions and inclusion criteria were similar to the criteria used in the voriconazole trial

demonstrating that this voriconazole trial has been considered as a reference for clinical trials in first line therapy of invasive aspergillosis. The only significant change in eligibility criteria was the acceptance in the Ambiload study of a positive *Aspergillus* galactomannan antigen detection test as an entry criterion as opposed to the voriconazole trial designed before the test was routinely available. Among all the patients entered in the Ambiload study over an 18-month period, 201 were qualified by a data review committee.

The conclusions of the Ambiload study were that increasing the dose to 10 mg/kg per day did not improve the efficacy results but was associated with a higher rate of nephrotoxicity. The very good news was that favorable response rate and the 12-week survival rate in standard dose liposomal amphotericin B arm were similar to the rates obtained with voriconazole in the voriconazole versus amphotericin B deoxycholate comparative trial [1]. The recruitment was very successful in this Ambiload trial. One of the reasons was the possibility of enrolling patients with a possible aspergillosis according to the EORTC/MSG criteria. To reach definite eligibility, the diagnosis had to be upgraded to a probable or a definite aspergillosis within four working days. This delay gave enough time to obtain the results of the tests done before enrollment and nevertheless to treat efficaciously the patients in the study. This strategy allowed the inclusion of 20% more patients than restricting the inclusion to only probable or proven cases at randomization, saving approximately 4 months in the completion of the study.

Less than 10% of the cases of the Ambiload study were rejected for non confirmation of the halo sign by the data review committee, also including a radiologist. This low rejection rate can be explained by a better recognition of a halo sign by the investigators, an access to high resolution CT-scan in most centers and also by the transmission of digitalized CT-scan pictures on CD-rom rather than copies of the films. Copies often can result in a loss of quality while originally digitized pictures provide more information than the films. In addition, by using appropriate software, measurements of the lesions are much more precise on digital pictures than on films.

The next steps to improving the clinical trials?

Standardization of the cut-off for Aspergillus galactomannan detection

Antigen testing was not available for the voriconazole study in most of the participating centers and thus was not included in the entry criteria. However, sera have been collected prospectively during the study in the European centers and run in a central laboratory after the end of the study. Results were rather disappointing with a low rate of positive samples even in cases well documented by cultures [15]. However this translational investigation led to a conclusion that persistence of positive galactomannan beyond day 5 is associated with a worse outcome.

Nowadays the galactomannan detection test is an important test to identify the cases despite its insufficient sensitivity. The test has been licensed in Europe for many years with a recommended index cutoff value of 1.5. Many European investigators felt this index cutoff was too high and used a lower one for their daily practice. Index cutoff values of 1.0, 0.8 and 0.7 have been proposed [16–18]. Later Marr *et al*. assessed the test and suggested the lowering of the index cutoff value to 0.5 with a substantial increase in sensitivity and a reduced loss in specificity [19]. The index cut-off value of 0.5 has since been approved by the Food and Drug Administration. Most of the recent trials have set the index cutoff value to 1.0 [2,20]. For the next trials we need a standardization of the index cut-off value and it seems that 0.5 could be the best compromise.

Stratification at randomization

Stratification at randomization is critical in a clinical trial when one or more factors can significantly affect the outcome. Imbalance for these factors of poor or good prognosis between the two arms may lead to inappropriate conclusions.

Prognosis factors are not well known in invasive aspergillosis except for the allogeneic hematopoietic stem cell transplant recipients [21–23]. In a recent study by Cordonnier *et al*., seven factors of poor prognosis have been identified in 51 hematopoietic stem cell transplant patients (41 allogeneic and 10 autologous transplantation): aged 12–35 years, dissemination of invasive aspergillosis, presence of a pleural effusion, monocyte count of less than 120 cells/μl, prolonged administration of steroids within the previous 2 months, receipt of a steroid dose equivalent to 2 mg/kg prednisone or more at the time of diagnosis, and uncontrolled graft versus host disease [22].

In this study the 4-months survival was especially low in allogeneic stem cell transplant patients (32% in 41 patients) not significantly different from survival of autologous transplanted patients (40% in 10 patients). In general, allogeneic hematopoietic stem cell transplant patients experience a worse outcome than non-allogeneic transplanted patients. Both the voriconazole comparative trial and the Ambiload trial have

Table 1 Voriconazole comparative trial: twelve-week response rates according to the underlying condition in voriconazole and amphotericin B deoxycholate arms. Adapted from Herbrecht *et al*. [1].

Patient's condition	Voriconazole arm	Amphotericin B arm
Allogeneic stem cell transplantation	32%	13%
Neutropenic hematological condition	63%	38%
Other immunosuppressed condition	50%	32%

demonstrated the lower response rate (Table 1) or survival (Fig. 1) of allogeneic stem cell transplant recipients [1,24].

Another factor of poor prognosis identified in the Ambiload trial is the absence of complete response of the underlying malignancy. Although precise data are lacking, we can speculate that a part of the deaths occurring within three months after the start of the antifungal therapy in patients with an invasive aspergillosis are more likely due the progressive underlying malignancy than to the fungal infection. Information on the exact cause of death is critical in a clinical trial although we recognize that determining the causality of death is often difficult in these patients. Precise recommendations should be formulated in a clinical trial to help the investigator to classify the cause of death assuming that in the absence of evident other cause of death or in the absence of documentation of a favorable clinical response prior to the death, deaths must be attributed to the invasive aspergillosis to be as conservative as possible.

Recognition of prognosis factors is not easy in phase III clinical trials as some of the exclusion criteria apply to the patients with the worst prognosis. Usually patients with a severe renal or hepatic impairment, with a concomitant infection or a non-infectious pulmonary disease, patients who are intubated or have a short life expectancy for any other reason are excluded from these trials as these factors may interfere with the response and tolerance assessments.

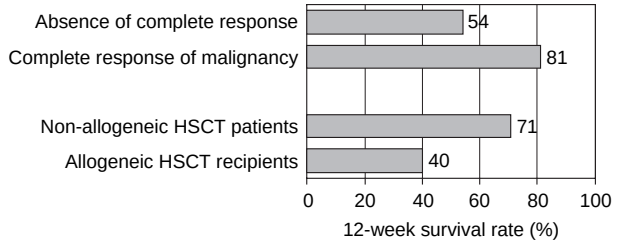

Fig. 1 Ambiload trial: 12-week survival rates of allogeneic hematopoietic stem cell transplant (HSCT) recipients versus non-allogeneic HSCT patients (bottom of the panel) and of patients in complete response of the underlying malignancy versus patients not in complete response (top of the panel). Adapted from Cornely *et al*. [24].

A large single center study (or pooled data from several centers, without exclusions as in clinical trials) would be more appropriate to identify the prognosis factors that have a major impact on the response or on the survival independently of the underlying condition and of factors that are usually exclusion criteria in clinical trials. These factors could then be used for stratification in clinical trials especially when combination antifungal therapy, intended for the most severe cases, is considered.

Inclusion of possible cases in clinical trials

The EORTC/MSG consensus definition criteria for invasive fungal infection divided the infections into three categories [4]. Applied to invasive aspergillosis these three groups are:

- The definite cases defined by a histopathological demonstration of tissue invasion by hyaline septate hyphae or on a positive identification of an *Aspergillus* spp. by culture from a usually sterile site.
- The probable cases defined by host factors, typical clinical or radiological signs and also by a positive mycological data, either microscopy, culture or *Aspergillus* galactomannan antigen.
- The possible cases defined by host factors and either radiological, clinical or mycological signs.

Definite cases are very stringently defined and there is no discussion about them. Probable cases are only rarely a matter of discussion. In rare instances diagnosis is inappropriate: e.g., in cases of respiratory tract colonization by *Aspergillus* spp. or false positive results of *Aspergillus* galactomannan test in the setting of a pulmonary infection due to another pathogen. Conversely, possible cases are loosely defined and may correspond to simple colonization of the respiratory tract, false positive galactomannan tests, to non fungal pulmonary infection or even to non infectious pulmonary lesion in the setting of a febrile neutropenia.

The loose definition of the possible cases was the main reason for the EORTC/MSG to propose new definitions criteria. These have been presented preliminarily at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, December 2005 [Donnelly JP *et al*., unpublished]. These new criteria should solve most of the conflicts that existed with the previous one. Therefore possible cases will now

be much closer to confirmed aspergillosis and there is no longer a reason to exclude this important group of patients who anyway need an antifungal therapy to be excluded from clinical trial. However stratification on degree of certainty of the infection is mandatory.

Cause of death

Attributing appropriately the cause of death to the aspergillosis or to the underlying malignancy would need an autopsy of all patients dying during the study period in a clinical trial. If autopsy is not possible, a careful review of all the existing data for each single patient must be performed. Although a narrative may be collected in the case report form for all serious adverse events, information is often insufficient for the data review committee to conclude formally. In the absence of precise data, it can be assumed that most of the early deaths, occurring less than 3 months after the onset of the aspergillosis, are more or less attributable to the infection and that deaths occurring later are mostly due to the worsening of the underlying condition or to another concomitant event.

Conclusions

The most recent clinical trials performed in invasive aspergillosis as well as in invasive candidiasis, in prophylaxis of fungal infections, or in empiric therapy of persistent febrile neutropenia have reached high levels of quality allowing very solid conclusions [1,2,25–29]. New standards for antifungal efficacy trials have thus been established. Some further improvements can be proposed especially with respect to a standardization of the cutoff of the *Aspergillus* galactomannan test, inclusion of other diagnostic tests such as the glucan test or PCR, and appropriate stratification at randomization according to the most relevant baseline prognosis factors.

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