Latest developments in fungal lung infection in solid organ transplantation (SOT)

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The incidence of invasive mycoses following solid organ transplant (SOT) ranges from 5 to 42% depending on the organ transplanted. Despite the increasing impact of viral infections in SOT, fungal infections still have a main role in transplant recipients. In fact, they remain a common cause of morbidity and mortality in the early and late post-transplant periods. *Aspergillus* spp. and *Candida* spp. account for most IFI, but recent epidemiological and clinical studies suggest the emergence of mycelia fungi other than *Aspergillus* as well as resistant strains of *Candida* in these patients. Due to the difficulty in making a definitive diagnosis, the treatment is sometimes delayed or is not prescribed (post-mortem diagnosis). Serological and molecular detection of *Aspergillus* antigens or fungal DNA, in blood and/or BAL samples, may improve the diagnosis of pulmonary aspergillosis, but in SOT the sensitivity is variable and more studies are needed. Another pendent issue is antifungal prophylaxis in SOT recipients; it is unknown which is the best agent or the time duration, and in which receptors must be applied. Treatment combining AmB preparations, newer antifungal drugs, early surgical resection of infected tissue and discontinuation or modulation of immunosuppressive treatment can to be necessary in selected patients and in certain occasions, and all of them may improve prognosis of IFI. However, there are two main handicaps in the management of FI in transplant recipients: firstly, to establish an early diagnosis, secondly, delays in applying early treatment with antifungal drugs. Development of new early diagnostic tools more precise and well-designed multicenter evaluations of diagnostic methods and therapeutic regimens available at present are the important work in the next 3-5 years.
This review highlights changing spectrum of invasive fungal infections, risk factors, antifungal prophylaxis, and treatment following SOT.

Key words: **Fungal infections. Solid organ tranplantation. Opportunistic infections. Invasive aspergillosis. Prophylaxis.**

Últimos avances en las infecciones micóticas pulmonares ocurridas en el trasplante de órganos sólidos (TOS)

La incidencia de las micosis invasivas después del trasplante de órganos sólidos (TOS) oscila del 5 al 42%, según el órgano trasplantado. A pesar del creciente impacto de las infecciones víricas sobre el TOS, las infecciones micóticas desempeñan todavía un papel principal en los receptores de trasplantes. De hecho, siguen siendo una causa común de morbilidad y mortalidad en las fases precoz y tardía postrasplante. Aunque *Aspergillus* spp. y *Candida* spp. son responsables de la mayoría de infecciones fúngicas invasivas (IFI), recientes estudios epidemiológicos y clínicos sugieren la eclosión de micelios diferentes de *Aspergillus*, así como de cepas resistentes de *Candida*, en estos pacientes. Debido a la dificultad de realizar un diagnóstico definitivo, a veces se retrasa el tratamiento o no llega a prescribirse (diagnóstico post-mortem). La detección serológica y molecular de los antígenos de *Aspergillus* o del ADN fúngico, en muestras de sangre y/o lavado broncoalveolar (LBA), puede mejorar el diagnóstico de la aspergilosis pulmonar, pero en el TOS la sensibilidad es variable y es necesario realizar nuevos estudios al respecto. Otro tema pendiente es la profilaxis antifúngica en los receptores de TOS; se desconoce cuál es el agente más idóneo, así como la duración adecuada de la profilaxis y a qué receptores debe aplicarse. Los tratamientos combinados con preparados de anfotericina B, nuevos fármacos antifúngicos, resección quirúrgica precoz de los tejidos infectados e interrupción o modulación del tratamiento inmunosupresor, pueden ser necesarios en determinados pacientes y en ciertas ocasiones, y todos ellos pueden mejorar el pronóstico de las IFI. Sin embargo, existen dos obstáculos importantes para el tratamiento de las infecciones fúngicas en los receptores de trasplantes: en primer lugar, el establecimiento de un diagnóstico precoz;

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y en segundo lugar, los retrasos en el tratamiento precoz con fármacos antifúngicos. El desarrollo de nuevos métodos de diagnóstico precoz más precisos, y los estudios multicéntricos bien diseñados sobre los métodos diagnósticos y las pautas terapéuticas disponibles actualmente, son trabajos importantes a realizar durante los próximos 3-5 años. En la presente revisión se subrayan el espectro cambiante de las infecciones fúngicas invasivas, la profilaxis antifúngica y el tratamiento después del TOS.

Palabras clave: **Infecciones fúngicas. Trasplante de órganos sólidos. Infecciones oportunistas. Aspergilosis invasiva. Profilaxis.**

State of the art (Dr. A Solé)

This review highlights the changing spectrum of invasive fungal infections, risk factors, antifungal prophylaxis, and treatment following solid organ transplantation (SOT). Infection following SOT continues to be a severe and life-threatening complication, despite the fact that incidence has decreased with new prophylactic strategies and more refined immunosuppression[1]. The lung is the main target in heart and lung transplant, the second target in liver transplant (after intra-abdominal infection), and its involvement is less common in kidney transplant.

Invasive fungal infections (IFI) are the third cause of infection in SOT with an incidence of 5 to 42%[2-4] depending on the transplanted organ, although the global incidence has fallen over the last 10 years. This is due in part to better control of risk factors, improved surgical technique, a shorter post-operative period, and more appropriate immunosuppression. *Candida* (mainly non-albicans) and *Aspergillus* spp. cause most IFI. The incidence of invasive candidiasis has fallen, and the main challenge now lies with invasive aspergillosis (IA), which presents in 2 ways: early (in the 90-day period following transplantation) and late (beyond 3 months after transplantation). During recent years, approximately 50% of cases of IA have been late onset, and the lung is involved in 90% of all SOT[5-8]. Infections by *Aspergillus* fumigatus are the most common, with a progressive increase in infections by amphotericin B-resistant *A. terreus*. Other emerging fungi, such as *Scedosporium* and *Fusarium*, are still rare in SOT, although they are characterized by more severe infections, with mainly disseminated forms and a worse prognosis than infections caused by *Aspergillus* spp.[9,10].

In general, *Aspergillus* infections appear despite prophylaxis (breakthrough infection) and are accompanied by high mortality (40 to 80%), and almost 100% are disseminated forms, regardless of the treatment administered.

Risk factors in SOT

Risk factors are specific to each solid organ transplanted[6,11-16]. Nevertheless, some are common to all SOT recipients, generally those with poorer health, complicated post-operative period, and more aggressive immunosuppression. Thus, in the pre-transplantation period, early IFI are associated with corticosteroids, broad-spectrum antibiotics, colonization by fungi, and poorer clinical status. Furthermore, intra-operative factors such as complicated surgery or intravascular devices (ventricular assist devices, pacemaker) have also been related with early IFI. In the post-transplant period, the main factors associated with infection are reoperation, excessive use of antibiotics, CMV infection, high-level immunosuppression, chronic rejection, retransplantation, and renal insufficiency (dialysis). Finally, neutropenia induced by ganciclovir or valganciclovir can increase the risk of infection during the post-transplant period. In general, late IFI are associated with older age, renal failure, and chronic rejection[2,3,5-8].

Incidence

At present, *Candida* infections, mainly non-albicans infections, are clearly predominant in transplants involving the abdomen (liver, kidney, pancreas), mainly during the first 2 months (liver and pancreas). The Spanish multicenter study on IA in SOT (RESITRA Network) with a total of 4338 recipients had 113 cases of IA, that is, a global incidence of 2.6% (heart 4.4%, liver 2.8%, kidney 0.9%, and lung 17%). These figures are close to those published in other countries (heart 6%, liver 1 to 3%, kidney 0.7%, and lung 5 to 10%)[6,8].

Lung recipients are the most susceptible to infection by *Aspergillus* of all the SOT. The authors of a study on IA in lung transplant from 1 transplant center reported a 20% incidence of colonization, 6% incidence of tracheobronchitis, and 8% incidence of invasive forms, data that are similar to those reported in the international literature (26, 5, and 5%, respectively)[13-15].

Diagnosis

IFI in SOT are difficult to diagnose; in fact, in the early stages, more than 30% are asymptomatic, and both hemoptysis and chest pain are usually observed late. Furthermore, X-ray is not very specific, and the halo sign, which is very frequent in neutropenic patients, appears in around 30% of cases in SOT. The difficulties of clinical and radiological diagnosis are compounded by the low sensitivity of respiratory cultures, even in proven IFI. Thus, culture of sputum and bronchoalveolar lavage is positive in 8 to 34 and 45 to 63% of IFI, respectively. Furthermore, asymptomatic colonization is very common in 28 to 55% of cases (false positives mainly in lung transplant). Different biopsy techniques (transbronchial, videothoracoscopy, transparietal, and open chest) are often impossible due to the condition of the patient. Lastly, different culture techniques, which can detect fungal structures in plasma (1→3-β-D–glucan, galactomannan, and PCR), are not very sensitive in SOT. This is compounded by the sparse radiological experience in the diagnosis and follow-up of IFI in SOT, with different computed tomography modalities (high resolution CT, spiral CT, and positron emission tomography scanning)[17-21].

Prophylaxis

Prophylaxis of fungal infection in SOT remains unsolved[22-31]. In fact, there is wide variability between centers performing the same type of transplant[27] and this is representative of the little evidence in the literature on antifungal prophylaxis in SOT. Correct prophylaxis must take into account transplant-specific risk factors such as location of the organ in the abdomen or thorax (*Candida* or *Aspergillus*, respectively), complexity of the operation, retransplantation, prosthesis, transfusion requirements, renal failure, CMV infection, type of immunosuppression, lymphocyte depletion therapy, withdrawal or not of corticosteroids, incidence of chronic rejection, acute-chronic comorbidity (pulmonary, metabolic-diabetes, renal). In fact, the current trend in different transplant programs is "a la carte" prophylaxis, which is continued as long as there are risk factors. Traditionally, prophylaxis with fluconazole has reduced the incidence of invasive candidiasis in liver recipients, although it has not been proven to reduce mortality. In fact, in low-risk liver recipients, prophylaxis with azoles does not seem to offer any benefit. One important problem stemming from universal prophylaxis with fluconazole is the increasing incidence of non-albicans *Candida* and the development of cross-resistance to the new azoles[22]. Furthermore, fluconazole lacks activity against opportunistic molds (*Aspergillus*, *Zygomycetes*, *Scedosporium*, and *Fusarium* spp), which are responsible for the most severe IFI in SOT[10,22]; in fact, the development of strategies to prevent these invasive mycoses is crucial. Limited data are available as to the most effective antifungal agents for prophylaxis in SOT recipients. In the absence of clinical trials, there are considerable expectations regarding the efficacy of voriconazole or caspofungin for prophylaxis in high-risk SOT recipients. However, randomized controlled trials are needed to determine the most effective strategies for reducing the incidence of IFI in SOT patients.

This review analyzes the results of several studies on antifungal prophylaxis in 2 organs in which indications for this prophylaxis seems to be better classified.

Treatment

The currently available antifungal armamentarium has a wide range of drugs with different mechanisms of action[17,32-37]. Voriconazole is the drug of choice against invasive aspergillosis and has even proven to be more effective than amphotericin B as initial therapy in invasive aspergillosis, with greater survival[33]. However, voriconazole has no activity against Zygomycetes, with the result that amphotericin B continues to be the first-line drug until microbiological confirmation of infection by *Aspergillus* is available.

The role of echinocandins as a first-line drug remains to be determined; in fact, the initial approval by the FDA was for refractory aspergillosis. Caspofungin has not been tried alone as initial therapy in invasive aspergillosis, only as rescue therapy and combined with amphotericin B or azoles with activity against filamentous fungi[32,35,36]. Similarly, the role of posaconazole has yet to be determined–despite being indicated only for rescue treatment of aspergillosis, it has proven safe and efficacious in infections by filamentous fungi that were refractory to voriconazole[37,38].

Treatment should last between 10 and 12 weeks or at least 4 to 6 weeks after clinical/radiological resolution. Surgery is recommended in localized invasive pulmonary forms and at other sites (debridement of sinusitis, cutaneous mycetomas).

Despite the availability of potent antifungal drugs, treatment of SOT in IFI has a high percentage of failure. This may be due to a delay in the start of therapy, inappropriate therapy for fungal infection, inappropriate doses, deep neutropenia, high doses of corticosteroids, development of resistance, or even the fact that foreign bodies or central venous access devices can act as fungal reservoirs and must be withdrawn. The most suitable modality of antifungal therapy in SOT (monotherapy, sequential, or combined) has not been established either. Combination treatment offers promising results *in vitro*, in animal models, and in clinical practice. Nevertheless, prospective studies validating these results are necessary. In addition, there is no consensus with respect to which combinations are synergistic and which are antagonistic. Lastly, we must bear in mind that combinations can increase drug toxicity, pharmacological interactions, and cost.

Fluconazole (for *C. albicans*) or echinocandins would seem to be the agents of choice for candidal infections, whereas voriconazole or lipid formulations of amphotericin B are indicated in patients with invasive *Aspergillus* infection.

Future strategies must include improved conditions for isolating patients, control of risk factors, more systematic introduction of surgery, increasing concentrations of antifungals being used alone and in combination, and boosting host defenses against infection. The latter must include the use of cytokines such as recombinant interferon γ, growth factors for the activation of neutrophils (G-CSF and GM-CSF), vaccines, and monoclonal antibodies[17,39,40].

Comments

Below, a group of Spanish physicians with an interest in the field of fungal infections in SOT discusses the most remarkable papers produced in this area during the last year. The following are the publications selected for discussion.

Prophylaxis

Husain S, Paterson DL, Studerd S, Pilewskid J, Crespod M, Zaldonis D, et al. Voriconazole prophylaxis in lung transplant recipients. Am J Transplant. 2006;6:3008-16.

Lung transplant recipients have one of the highest rates of invasive aspergillosis (IA) in solid organ transplantation. Antifungal prophylaxis against aspergillosis is widely practiced in lung transplant recipients; however, the choice of antifungal drug and strategy employed vary considerably. Guidelines published by the American Society of Transplantation suggest several prophylactic regimens, including the use of azoles such as itraconazole, and in-

haled preparations of amphotericin at various dosages in high-risk lung transplant recipients.

This study examined the efficacy and toxicity of a strategy of universal de novo antifungal prophylaxis with voriconazole in 65 lung transplant recipients. End points were evaluated at 1 year and compared with those of 30 lung transplant recipients who were managed using a strategy of targeted antifungal prophylaxis (itraconazole ± inhaled amphotericin in patients at high risk of pre- or post-transplant *Aspergillus* colonization).

This study is the first to report the use of universal voriconazole prophylaxis in lung transplant recipients. The main finding is that the overall rate of IA at 1 year decreased to 1.5% with universal voriconazole prophylaxis compared with 23.5% in lung recipients managed with a targeted prophylaxis strategy (p = .001). Interestingly, the rate of *Candida* colonization, particularly non-*albicans* species, in the voriconazole group was significantly higher. In the voriconazole prophylaxis cohort, 27% of the lung recipients had normal liver enzymes throughout the course of the study. However, a 3-fold or higher increase in liver enzymes was noted in 37 to 60% of patients receiving voriconazole prophylaxis as compared with 15 to 41% of patients in the targeted prophylaxis cohort. In the voriconazole group, 14% of patients had to discontinue antifungal medications due to side effects compared with 8% in the targeted prophylaxis group.

Comments

The primary limitation of this study is the lack of randomization. Despite its limitations, the study has shown that universal voriconazole prophylaxis is an option for the prevention of IA in lung recipients. However, the use of voriconazole prophylaxis was associated with abnormal liver enzymes in a significant percentage of patients. Regular monitoring of liver enzymes and calcineurin inhibitors is required to avoid hepatotoxicity and nephrotoxicity. Other interesting findings were that universal voriconazole prophylaxis did not increase the rate of non-*aspergillus* fungal infections, there were no breakthrough infections with *Zygomycetes*, and voriconazole prophylaxis delayed the onset of *Aspergillus* colonization. This delay in the onset of *Aspergillus* colonization is advantageous, since by this time airway anastomoses are healed and patients are less immunosuppressed.

Corcoran TE, Venkataramanan R, Mihel KM, Marcinkowski AL, Ouc J, McCook BM, et al. Aerosol deposition of lipid complex amphotericin-B (Abelcet) in lung transplant recipients. American Journal of Transplantation. 2006;6:2765-73.

Lung transplant recipients have a higher incidence (3% to 16.6%) of invasive aspergillosis than other types of transplant recipients. In this type of transplant, inhaled medication is useful, both in prophylaxis and in treatment. This study analyzes the process of choosing and assessing the effectiveness of a nebulizer to administer amphotericin B lipid complex (ABLC) by aerosol.

Twelve nebulizers were studied, and the size of the particles produced and the quantity of antifungal drug released by each of the 12 devices was calculated. ABLC was marked with ^{99m}Tc and the relationship between the amount of antifungal and radioactivity was verified. A linear relationship was obtained.

The 12 patients (6 who underwent a unilateral transplant and 6 who underwent a bilateral transplant) received a total dose of ABLC of 35 mg. The patients underwent scintigraphy to monitor the distribution of the drug.

The most efficacious nebulizer was an AeroEclispe with a DeVilbiss 8650D compressor. In the 6 patients who underwent unilateral transplant, the mean total dose deposited was 8.3 ± 0.6 mg, which corresponds to 23.7 ± 1.7% of the total dose in the nebulizer. The native lung received a lower dose than the transplanted lung (36.4 vs. 63.6%). The drug was better distributed in the central compartment than in the peripheral compartment. In the 6 patients with a bilateral transplant, the mean total dose deposited was 9.9 ± 2.3 mg, which corresponds to 28.2 ± 6.5% of the total dose in the nebulizer. The right lung received a greater dose than the left lung (4 vs. 2.8 mg). The drug was better distributed in the central compartment than in the peripheral compartment. The patients had minimal symptoms after administration of the aerosol.

Comments

This analysis of the choice of nebulizer and compressor could be useful for later studies. The data from the clinical section are useful, although very preliminary. Other studies have examined prophylaxis with ABLC in lung transplant recipients. In general, a high-flow compressor with a nebulizer producing a nebulized particle < 5 µm should be chosen. The lung deposit depends on factors such as ventilatory pattern and underlying lung disease. No studies have determined the appropriate dose for prophylaxis with nebulized amphotericin and we do not know the most appropriate MIC_{90} of nebulized amphotericin B in the lung. In lung transplantation, when there are structural abnormalities (either due to pathologic chronic or native rejection in unilateral transplants), the deposit is deficient and early therapy with voriconazole would be recommended in these cases.

Epidemiology. Risk factors

Pappas G, Andes D, Schuster M, Hadley S, Rabkin J, Merion RM, et al. Invasive fungal infections in low-risk liver transplant recipients: a multi-center prospective observational study. Am J Transplant. 2006;6:386-91.

The incidence of invasive fungal infection (IFI) in orthotopic liver transplant (OLT) ranges from 5 to 42% with a mortality of 25 to 56%. In approximately 80% of cases, the causal agent is *Candida* spp. *Aspergillus* spp. is the second cause of IFI, with a mortality of 75 to 100%. There are no standard guidelines for selecting patients at risk of developing IFI and who might benefit from antifungal prophylaxis. In 2 retrospective studies, Collins and Karchmer observed that 40% of OLT recipients with IFI had 2 or more factors defined as being of high-risk, and that the presence of under 2 risk factors was associated with IFI in less than 5% of patients. In the study by Pappas involving 193 OLT recipients (no antifungal prophylaxis and

100 days' follow-up) in which definite low-risk criteria were established, only 7 (4%) patients developed IFI. Patients with 1 risk factor did not present more episodes of IFI than those who had none.

Comments

Taking into consideration the low frequency of IFI that could have been prevented using prophylaxis with fluconazole (0.5 to 1.5%), the absence of IFI-associated mortality, toxicity, the high cost of systemic antifungals, and the potential development of antifungal resistance, the authors believe that these criteria are useful for identifying patients with OHT for whom systemic antifungal prophylaxis would not be indicated.

This study is interesting as it is prospective and stratifies risk, although only 74 patients had only 1 risk factor. With respect to the risk criteria, retransplantation due to primary dysfunction of the graft is an independent risk factor for invasive aspergillosis. Similarly, renal insufficiency is another risk factor for IFI in general, including invasive aspergillosis. The need for a renal replacement technique is also an independent risk factor for the development of IFI and, therefore, for invasive aspergillosis. In both situations (primary graft dysfunction and need for renal replacement techniques) and in patients requiring laparotomy due to bleeding during the 5 days after transplant, other centers use prophylaxis with liposomal amphotericin B by considering them high-risk for IFI including invasive aspergillosis.

Singh N, Pruett TL, Houston S, Muñoz P, Cacciarelli TV, Wagener MM, et al. Invasive aspergillosis in the recipients of liver retransplantation. Liver Transpl. 2006;12:1205-9.

Retransplant is necessary in 10 to 15% of orthotopic liver transplant (OLT) recipients. This is early when it is performed during the first 30 days after the first transplant and late after 30 days. The incidence of invasive aspergillosis (IA) in OHT recipients is 1 to 8%. Liver retransplantation is an important risk factor for IA. This is the first in-depth analysis of IA after liver retransplantation. Twenty-five percent (17/67) of all cases of IA in OLT occurred in patients who had undergone retransplantation. The main indication for early retransplantation was primary graft dysfunction and in late retransplantation it was HCV infection or rejection in 78% of cases. At the start of IA, 88% of patients needed dialysis, 29% had CNS involvement and 47% disseminated IA. Retransplantation was performed during the first 30 days in 53% of cases and in the same percentage infection was late. It is important to point out the higher incidence of CNS involvement and the high mortality (100%) in late forms compared with early forms.

Comments

Several important observations can be taken from this study with respect to schedule and duration of antifungal prophylaxis, since half of all cases occur during the first 30 days after retransplant. The Spanish Transplant Infection Group (GESITRA) is carrying out a study with caspofungin for prevention of IFI in high-risk liver transplant recipients (21-28 days of prophylaxis in an attempt to cover the time for the maximum incidence of IFI). To date, and after a 100-day follow-up, only 1 surgical wound infection by Mucor has been observed. Studies on antifungal prophylaxis are necessary in patients with a high-risk of IFI (after criteria have been classified) with other antifungals that are active against *Aspergillus* spp. such as voriconazole or posaconazole, which can be administered orally without the need for intravenous administration or admission to hospital.

Knowledge of these epidemiological changes of IA in patients who had undergone retransplantation can help us evaluate these patients when they present an illness compatible with IA, so that in a late retransplantation with IA, we must initiate potent and aggressive treatment due to the risk of disseminated disease and its unfortunate prognosis.

Musk M, Chambers D, Chin W, Murray R, Gabbay E. Successful treatment of disseminated *Scedosporium* infection in 2 lung transplant recipients: review of the literature and recommendations for management. J Heart Lung Transplant. 2006;25: 1268-72.

The authors are from the Lung Transplant Unit at Perth Hospital in Australia and report 2 cases of disseminated scedosporiosis in lung transplant recipients (both unilateral) who responded satisfactorily to therapy with voriconazole. These are the first 2 cases reported in the literature of favorable outcome in this type of patient with medical treatment only.

Comments

These are the first 2 cases of disseminated *S. apiospermum* infection in lung transplant recipients who survived with antifungal treatment (voriconazole). One is the first case ever reported of disseminated scedosporiosis with renal involvement. The increase in immunosuppression and prophylaxis with nebulized amphotericin B may have favored dissemination of the fungal infection. *Scedosporium* spp. is as ubiquitous and vasculotropic as *Aspergillus* spp., although it is more difficult to treat due to the frequent resistance presented (*S. apiospermum* to amphotericin B and *S. prolificans* to all available antifungals). In the case of an IFI caused by hyalohyphomycetes in a lung transplant recipient, voriconazole should be considered the first-line therapy until the causal agent is identified and its antifungal sensitivity is verified. Disseminated scedosporiosis in an SOT recipient can be treated successfully, although we must bear in mind that voriconazole controls it efficaciously but may not be able to eradicate the fungus and that antifungal therapy may therefore be indefinite. Both patients were unilateral lung transplant recipients: this was also the case in other series where this type of transplant is associated more frequently with fungal infections, since the other lung acts as a "natural" reservoir for these pathogens. A mycological culture of bronchoalveolar lavage in these patients does not rule out a fungal infection of the lung. Good-quality microscopy with chemoluminescent agents (such as calcofluor white) allows immediate presumptive diagnosis (in real time) of a

mycosis, and it is recommendable in any clinical microbiology laboratory thanks to its ease of use, speed, and low cost, making it much better than molecular biology techniques, which have not yet been standardized for these purposes. Terbinafine can be used in these patients in combination with azoles.

Singh N, Limaye A, Forrest G, Safdar N, Muñoz P, Pursell K, et al. Late-onset invasive aspergillosis in organ transplant recipients in the current era. Medical Mycology. 2006;44:445-9.

This study aims to determine, in the era of new immunosuppressive agents, the risk factors and clinical characteristics of late-onset invasive aspergillosis (IA) (≥ 90 days) in solid organ transplant (SOT) recipients. They therefore carried out a prospective multicenter study including 40 patients (aged 19 to 68 years) who underwent the following transplants: lung (13), heart (9), liver (8), kidney (4), small intestine (1), multiorgan (5): 13% of patients had to undergo retransplantation.

The diagnosis of IA was made by positive culture in 87.5% of cases: 50% of cases were late-onset (≥ 90 days post-transplant) and the risk factors significantly related to IA were sirolimus combined with tacrolimus (5/5 vs. 0/20; $P = .047$) and previous CMV infection ($P = .09$). The other variables studied and administration of monoclonal antibodies had no effect on the late onset of IA.

Comments

At present, half of all cases of post-SOT IA are late-onset (≥ 90 days). The delayed onset of IA in SOT patients has important implications for prophylaxis and recommendations for therapy in patients with pulmonary infiltrates. The association between late-onset IA and sirolimus combined with tacrolimus may be skewed because this drug was commonly used for immunosuppression in chronic rejection and kidney transplant (20% of cases). In a recent publication by RESITRA (Transplantation. 2006;82:1457-62), the risk factors associated with late-onset IA in SOT include (unlike the present study) renal failure, chronic rejection, 2 or more early acute rejections, recurrent bacterial infections, and the need for dialysis during the first month after transplantation. Unlike this study, other authors did find a direct relationship between the use of immunomodulators, including alemtuzumab, and late-onset IA in SOT when they are used to treat rejection (Clin Infect Dis. 2007;44:204-12) or lymphoproliferative disorders (Clin Infect Dis. 2006;43:16-24.)

Diagnosis

Champion L, Stern M, Israël-Biet D, Mamzer-Bruneel MF, Peraldi MN, Kreis H, et al. Sirolimus-associated pneumonitis: 24 cases in renal transplant recipients. Ann Intern Med. 2006;144:505-6.

Sirolimus (rapamycin) is a potent immunosuppressor used as an alternative to calcineurin inhibitors in solid organ transplantation. Sirolimus-associated pneumonitis has been reported sporadically in kidney, heart, liver, and lung transplant recipients. Nevertheless, this complication is still relatively unknown in part due to the absence of specific diagnostic criteria. Champion et al have evaluated the clinical and laboratory characteristics of sirolimus-associated pneumonitis in 24 (11%) of 217 kidney transplant recipients treated with this drug. The mean duration of previous treatment with sirolimus was 5.5 months. Trough levels of sirolimus before pneumonitis varied from 12 to 30 ng/mL (median, 20 ng/mL). The most frequent symptoms included cough (96%), asthenia (87%), fever (70%), dyspnea (33%), hypoxemia (42%). All the patients had pulmonary infiltrates in computed tomography of the chest that were indicative of bronchiolitis obliterans organizing pneumonia in 79% of cases. Bronchoalveolar lavage–carried out in all cases–mainly revealed lymphocytic alveolitis (79%). Although the manifestations of pneumonitis improved transitorily in 2 patients after reducing the dose of sirolimus, the drug had to be suspended in all cases, with clinical and radiological resolution. Survival at 6 months was 100%.

Comments

Approximately 1 of every 10 kidney transplant recipients treated with sirolimus developed pneumonitis. Sirolimus-associated pneumonitis must be taken into consideration in the differential diagnosis of pulmonary infiltrates in transplant recipients treated with this immunosuppressor, especially if a loading dose and/or doses greater than 5 mg/day or levels greater than 15 ng/mL are used. The withdrawal of sirolimus usually leads to resolution of clinical-radiological manifestations, although fatal cases have been reported.

Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. Clin Infect Dis. 2006;42:1417-27.

One of the central problems of medical mycology is the low yield of tests to diagnose invasive fungal infection. Culture is limited due to the frequent impossibility of taking samples from the site of infection, the lack of sensitivity, and slow growth of the fungus. Therefore, attempts have been made to develop other methods based on the detection of metabolites or fungal antigens as well as DNA, the most notable being detection of galactomannan, a cell wall polysaccharide that is secreted in the surrounding medium as the fungus develops. Although this polysaccharide is most abundant in all species of *Aspergillus*, in other genera, such as *Penicillium*, it is also present. The company BIORAD has developed a method for detecting galactomannan using a monoclonal antibody, EBA2. The authors of the study carried out a search of the literature to identify those articles related to the diagnosis of invasive fungal infection using detection of galactomannan. Once the search was made, the following articles were included in the meta-analysis: (i) those in which the diagnostic criteria are based on those published by the EORTC/MSG or similar; (ii) those in which the sample analyzed was serum; and (iii) those that offer sufficient data to calculate sensitivity and specificity. Twenty-nine articles were included, 3 of which involve solid organ transplant (SOT) recipients. The limits for considering a test

as positive were 0.5 in 5 studies, 1 in 13 studies, and 1.5 in 11. The global sensitivity of the technique for tested invasive aspergillosis was 0.71 (95% CI, 0.68-0.74) and the specificity was 0.89 (95% CI, 0.88-0.9). Sensitivity is higher in liver recipients (0.82; 95% CI, 0.7-0.9) and the specificity, although lower, continues to have acceptable values (0.86; 95% CI, 0.83-0.88). On the contrary, sensitivity is very low in SOT recipients (0.22; 95% CI, 0.03-0.6), but the specificity is acceptable (0.84; 95% CI, 0.78-0.88). The analysis of sensitivity and specificity taking into account the value considered as positive indicates that the greater the value, the greater the accuracy of the test. Thus, the sensitivity for a cutoff point of 0.5 was 0.27 (95% CI, 0.06-0.61) and for a cutoff of 1 it was 0.79 (95% CI, 0.71-0.87). Lastly, sensitivity and specificity do not vary substantially when probable cases are included in the analysis.

Comments

The technique is moderately useful for the diagnosis of invasive aspergillosis in patients with hematological conditions. It has little value in the diagnosis of invasive aspergillosis in SOT. Specificity is high in all studies and, therefore, the test is more useful for ruling out invasive aspergillosis. The positive predictive value has a direct relationship with prevalence, whereas the negative predictive value is maintained independently of this. Therefore, this technique should be used in populations with a high risk of invasive aspergillosis. The study analyzes the usefulness of this marker in monitoring for invasive aspergillosis, and not as a diagnostic test in patients with signs and symptoms compatible with invasive aspergillosis.

With the available data, detection of galactomannan is more useful for ruling out the existence of invasive aspergillosis than for diagnosing it. Perhaps the importance of this study lies in stressing what we do not know and should try to find out.

Copp DH, Godwin JD, Kirby KA, Limaye AP. Clinical and radiological factors associated with pulmonary nodule etiology in organ transplant recipients. Am J Transplant. 2006;6:2759-64.

Several respiratory complications have been reported in solid organ transplant (SOT) recipients. Nodular pulmonary images or pulmonary nodules are observed relatively frequently on the chest X-ray and differential diagnosis has become a considerable clinical challenge with different etiological possibilities. The severity of many of these entities means that diagnosis is often aggressive and empiric therapy is usually started until a definitive diagnosis is obtained.

The objective of this study was to identify clinical and radiological factors to determine the etiology of pulmonary nodules in the SOT recipient in order to guide early empiric treatment. A bibliography search was made on PubMed as was a retrospective study of all SOT recipients with pulmonary nodules between 1990 and 2005 at the University of Washington Medical Center, Seattle, USA. The definitive diagnosis of the etiology of pulmonary nodules was based on clinical, radiological, histological, and microbiological criteria. The authors determined the association between different clinical/radiological characteristics and specific etiologies of pulmonary nodules. Three specific etiologies were chosen for analysis: infectious vs. noninfectious, *Aspergillus* vs. non-*Aspergillus*, and post-transplant lymphoproliferative syndrome vs. absence of post-transplant lymphoproliferative syndrome. Logistic regression models were used to calculate the odds ratios and confidence intervals. The significant risk factors in univariate models were included in multivariate model, which were used to study 55 of the 94 SOT recipients with pulmonary nodules on the X-ray. Lung biopsy confirmed 82% of the diagnoses. Infection caused 56% of the pulmonary nodules observed (mycosis 33%, bacterial infections 22%). Within the category of mycoses, aspergillosis (20%) and cryptococcus (7%) were the most frequent. Nocardiosis (11%) were the most common bacterial cause of pulmonary nodule followed by mycobacteria (6%) and *Legionella* (4%).

The most common noninfectious causes were post-transplant lymphoproliferative syndrome (26%) and carcinoma (16%). Seronegativity for the Epstein-Barr virus was a significant predictive factor for the post-transplant lymphoproliferative syndrome (PTLD), although the number of patients studied using these conditions was low. Radiological evidence of pulmonary consolidation was significantly associated with an infectious etiology. Infection by *Aspergillus* was associated with diagnosis of pulmonary nodules within the first 90 days after transplantation with a sensitivity of 0.64 and a specificity of 0.89. There are no specific radiological characteristics for distinguishing aspergillosis from other etiologies of pulmonary nodule.

Comments

Some radiological images combined with other clinical data could contribute to an early diagnosis of pulmonary infections and guide antimicrobial therapy in patients with SOT until a definitive diagnosis is established. For example, the presence of consolidation is significantly related to an infectious etiology, an EBV-seronegative recipient to PTLD, and nodules during the first 90 days post-transplant most probably to *Aspergillus*. However, these findings must be confirmed by multicenter prospective studies.

Therapy

Singh N, Limaye AP, Forrest G, et al. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: a prospective, multicenter, observational study. Transplantation. 2006;81:320.

This is the first published study on the clinical efficacy of combination therapy for invasive aspergillosis in organ transplant recipients. Combination therapy provides the most benefit when used as initial therapy, as in this study, because salvage antifungal regimens for refractory fungal infections have generally proven less effective than when the same drug was used as primary therapy.

This prospective, multicenter, observational study analyzed the efficacy and tolerability of voriconazole and caspofungin when used as primary therapy for invasive

aspergillosis in 40 organ transplant recipients compared with a control group of 47 transplant recipients who received a lipid formulation of amphotericin B as single-agent primary therapy.

In addition, it correlated *in vitro* interactions between *Aspergillus* isolates and the combination of voriconazole plus caspofungin with clinical outcome. Survival at 90 days in patients who received voriconazole plus caspofungin was 67.5% (27/40) in the cases and 51% (24/47) in the control group (ns $P = .117$). However, when 90-day mortality was analyzed in subgroups of patients, combination therapy was independently associated with reduced mortality in those suffering from renal failure ($P = .02$) and in those with *A. fumigatus* infection ($P = .019$), even when adjusted for other factors predictive of mortality in the study population. These features are very interesting because in organ transplant recipients with invasive aspergillosis, renal failure is by far the most significant factor that portends a higher risk for mortality. The other principal observation from this study is that combination therapy for invasive aspergillosis was independently associated with better survival in patients with *A. fumigatus* infection. This observation could not be explained by known differences in the clinical characteristics of the patients. Whether *A. fumigatus* is inherently more susceptible to this antifungal combination *in vivo* is not known. This study is one of the first attempts to correlate *in vitro* antifungal combinations with the outcome of invasive aspergillosis. Study data show a lack of correlation between *in vitro* testing and outcomes suggest that current laboratory methodology may not reliably predict clinically meaningful interactions *in vivo*. It is also plausible that factors that affect outcome are predominantly host-related. Further study is necessary to determine whether this lack of correlation is limited to specific combinations of antifungal agents or host groups with invasive aspergillosis.

Comments

Combination therapy is expensive, increases the likelihood of adverse effects, and potential for drug interactions. Caution should be exercised in recommending its use until robust data are available. Therefore, although not as rigorous as a randomized trial, if these conclusions are confirmed by other prospective studies, then combination therapy with a triazole and an echinocandin might be considered preferable for subsets of organ transplant recipients with invasive aspergillosis, such as those with renal failure or invasive aspergillosis due to *A. fumigatus*.

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