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Early molecular diagnosis of aspergillosis in a patient with acute myeloid leukaemia

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ABSTRACT

Diagnosis of invasive fungal infection remains challenging. Here we report a case of early diagnosis of invasive aspergillosis in a neutropenic patient affected by acute myeloid leukaemia, achieved through the detection of *Aspergillus fumigatus* species-specific ribonucleic acid sequences by a sensitive multiplex real-time polymerase chain reaction-based molecular assay. Thanks to the early diagnosis, targeted therapy was promptly established and the severe fungal infection controlled, allowing the patient to subsequently receive allogeneic hematopoietic stem cell transplantation from a haploidentical donor, her only curative option. Also in this instance, targeted secondary antifungal prophylaxis with voriconazole avoided any other fungal infection afterwards. This report suggests how the implementation of molecular assays in combination with routine diagnostic procedures, can improve microbiological diagnosis in sepsis, particularly in case of fungal infection, difficult to detect with standard microbiological culture methods.

Keywords: *aspergillus fumigatus, molecular diagnosis, sepsis, allogeneic transplant, voriconazole.*

INTRODUCTION

Febrile neutropenia and sepsis are frequent and life-threatening complications in patients with haematological malignancies (1). Severe sepsis with multiple organ failure remains the most frequent cause of morbidity and mortality after autologous or allogeneic hematopoietic stem cell transplantation (HSCT). Although the proportion of infectious deaths in haematological patients has decreased over the last two decades, much remains to be done to further reduce these events. More effective preventive and prophylactic strategies for high-risk patients are necessary (2, 3). Crucial issues for the management of sepsis are the early diagnosis of the underlying infection and the rapid administration of a targeted antimicrobial therapy, together with an efficient cardiorespiratory resuscitation (4). Blood cultures identify a pathogen in only 20 to 30 % of febrile episodes and the culturing and pathogen identification process is very long, postponing the start of a pathogen-targeted treatment. In particular, life-threatening invasive fungal infections (IFI)

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are very difficult to detect with standard methods and this may often delay the start of aggressive antifungal therapy. Thereby, a sensitive tool to promptly recognize pathogens causing sepsis is of high clinical relevance. The possible adjuvant role of molecular techniques in the diagnostic flow-chart for IFI has been repeatedly discussed (5, 6). LightCycler® Septi*Fast* test (Roche Molecular Systems) is a PCR-based multiplex assay that can be performed in uncultured peripheral blood. Septi*Fast* is reportedly capable of detecting 25 of the most common species isolated in blood cultures. The assay uses dual fluorescent resonance energy transfer (FRET) probes against the species-specific internal transcribed spacer (ITS) regions, a non-coding sequence interspaced among highly conserved bacterial and fungal RNA. Time from processing to result is remarkably short (less than 6 hours). In our Institution we performed two investigational trials with LightCycler® Septi*Fast*. The first study analysed blood samples from febrile neutropenic patients and demonstrates a significant correlation between the molecular test and the standard blood cultures (83 %), thus allowing the prompt start of a targeted therapy based on the molecular result. The molecular approach allowed the detection of *Aspergillus fumigatus* in two patients with diagnosis of IFI (7). The second study confirmed the high specificity and sensitivity of Septi*Fast* and took into account 869 blood samples from haematological patients with febrile neutropenia (8). Septi*Fast* was also tested by other groups, confirming its ability to rapidly diagnose bloodstream infections in immunocompromised patients (9) and its clinical utility in the management of sepsis (6, 10) and febrile neutropenia (11). In this case report Septi*Fast* was the only microbiological assay able to detect a fungal sepsis in a patient affected by high-risk acute myeloid leukaemia.

CASE REPORT

A 41-year-old Caucasian female received in November 2011 the diagnosis of Acute Myeloid Leukaemia (AML), M4 subtype according to French-American-British (FAB) classification, with intermediate cytogenetic risk. Initially treated without benefit with induction chemotherapy, the patient received salvage chemotherapy with high-dose cytarabine combined with idarubicin and cyclosporin A, obtaining complete disease remission, followed by consolidation chemotherapy with high-dose cytarabine. In June 2012 the patient underwent a first allogeneic HSCT from a 8/10 HLA-matched unrelated donor (MUD) upon busulfan/cyclophosphamide conditioning regimen and in vivo T cell depletion by thymoglobulin. Fluconazole antifungal prophylaxis was administered.

In September 2012 the patient relapsed and received a fludarabine-based salvage therapy without benefit. Since the diagnosis of AML, the patient had experienced several infectious episodes: a catheter-related sepsis from *Staphylococcus hominis* and an episode of diarrhoea caused by *Clostridium difficile* after induction therapy, and, after the first allogeneic HSCT, a sepsis by *Escherichia coli*. No history of fungal infections was reported. In November 2012 the patient was referred to our Institute for a second allogeneic transplant.

The patient presented acute onset of fever and dry cough with sepsis at the hospital admission in November 2012. Blood analyses demonstrated pancytopenia, including grade IV neutropenia (secondary to the disease relapse), and high level of serum c-reactive protein. According to our institutional guidelines for febrile neutropenia, the patient was tested for standard blood, urine and sputum culture and galactomannan antigen test on blood, which all resulted negative. Chest X-ray was normal. A wide-

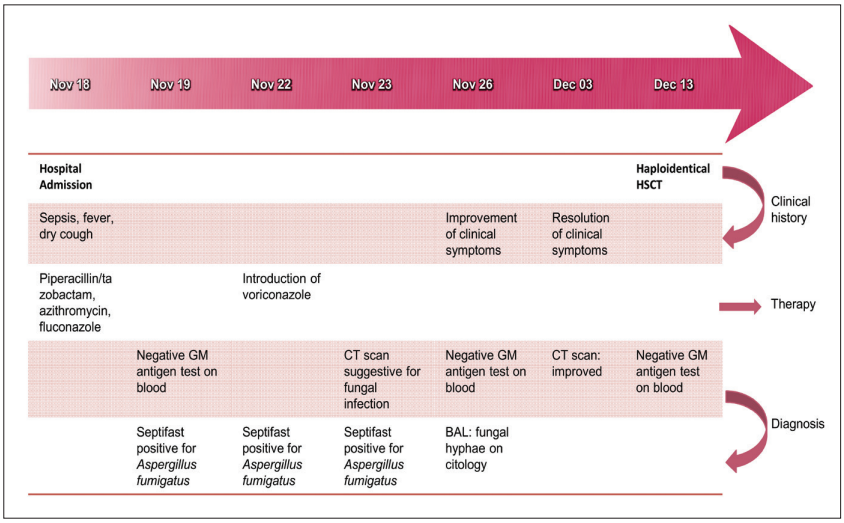

Figure 1 - *Temporal sequence of patient's sepsis, from hospital admission to haploidentical HSCT, with detailed clinical and laboratory steps.*
HSCT = hematopoietic stem cell transplantation; CT = computerized tomography; BAL = bronchoalveolar lavage; GM = galactomannan.

spectrum empirical antibiotic therapy with piperacillin-tazobactam and azithromycin was therefore initiated.

The initial management of the infection requires formulating a probable diagnosis, obtaining cultures, performing imaging studies to confirm the source of infection, and finally initiating appropriate and timely empirical antimicrobial therapy (4). High-risk patients require early hospitalization to receive broad-spectrum antibiotic therapy; in this setting the use of an antipseudomonal β-lactam agent, such as piperacillin-tazobactam, is recommended (12). Concomitantly to the other microbiologic analyses, Septi*Fast* was performed and resulted positive for *Aspergillus fumigatus* (*Figure 1*); the positive result was available after only 4 hours from patient's admission, and importantly was confirmed in three consecutive samples of patient's peripheral blood. Fluconazole antifungal therapy was promptly changed on the basis of this evidence, and the patient received voriconazole, a triazole antifungal agent that has fungicidal activity against *Aspergillus*.

Large studies have demonstrated the superiority of voriconazole to improve response rate and survival in highly immunosuppressed patients developing invasive aspergillosis (IA) after allogeneic HSCT (13, 14). The following day a computerized tomography (CT) scan of the chest (*Figure 2*) was performed, evidencing an extensive lobar pneumonia, highly suggestive for fungal infection. A bronchoalveolar lavage (BAL) was also performed and cytology resulted positive for septate hyphae suggestive for *Aspergillus* (*Figure 3*) classifying a diagnosis of probable IFI, while all other microbiological tests performed on BAL were negative. Importantly serial galactomannan

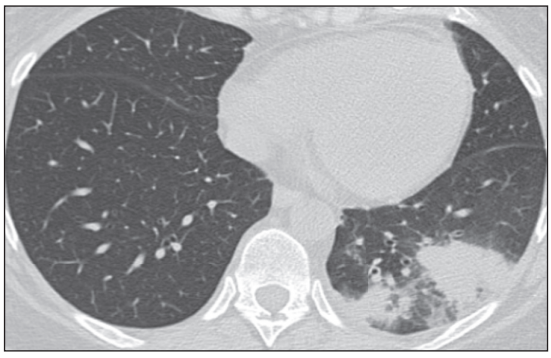

Figure 2 - *Chest CT scan of the patient shows a large consolidation area and peripheral ground-glass opacities in left lower lobe.*
CT = computerized tomography.

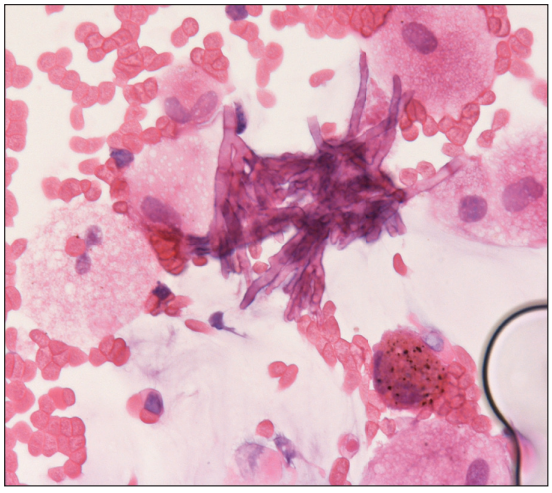

Figure 3 - *Cytology on bronchoalveolar lavage: septate hyphae, suggestive for Aspergillus, and foamy macrophages.*

antigen tests on blood resulted all negative. Clinical symptoms rapidly improved upon the start of voriconazole therapy, with resolution of sepsis and rapid response of the fever. A CT scan (*Figure 4*) performed 10 days after starting voriconazole showed a marked reduction of the lobar pneumonia and ground-glass opacities.
Septi*Fast*-guided early initiation of antifungal therapy prompted rapid resolution of the sepsis, enabling the patient to sustain a second allogeneic HSCT from a haploidentical donor in December 2012. The conditioning regimen consisted of treosulfan, fludarabine and melphalan followed by T cell-replete peripheral blood stem cells. Post-grafting immunosuppression consisted of high-dose post-transplant cyclophosphamide followed by oral administration of mycophenolate mofetil and sirolimus.
Of notice, the evidence of fungal infection was fundamental to guide the choice of the transplant protocol towards a conditioning devoid of anti-thymocyte globulins to favour rapid immune reconstitution and reduce the risk of fungal infection reoccurrence. In the aplastic phase and during the post transplant course the patient maintained a secondary prophylaxis with voriconazole; therapeutic drug monitoring allows a safe and effective administration of voriconazole along with sirolimus. Episodes of sepsis from *Pseudomonas aeruginosa* were reported after transplant, but all solved with targeted antibiotic therapy.
The targeted secondary antifungal prophylaxis and the rapid immune reconstitution (*Figure 5*) avoided any other fungal infection, despite the occurrence of cutaneous acute graft versus host disease (GvHD) initially, and a severe overlap chronic GvHD (involving skin, liver, mouth, eyes and lung) which required profound immunosuppression afterwards.
At the last follow up (1 year after transplant) the patient is still in complete remission of the haematological disease, without any sign of fungal infection.

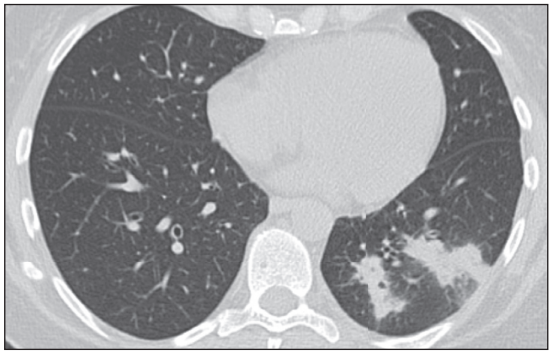

Figure 4 - *Chest CT scan of the patient, performed 10 days after starting of the targeted antifungal therapy, showed a marked reduction of the lobar pneumonia and particularly of the ground-glass opacities. CT = computerized tomography.*

DISCUSSION

Aspergillus spp. is the most serious fungal infection after allogeneic HSCT, and is the main cause of infectious death. Recipients of allogeneic transplantation show a high multifactorial risk of invasive fungal infec-

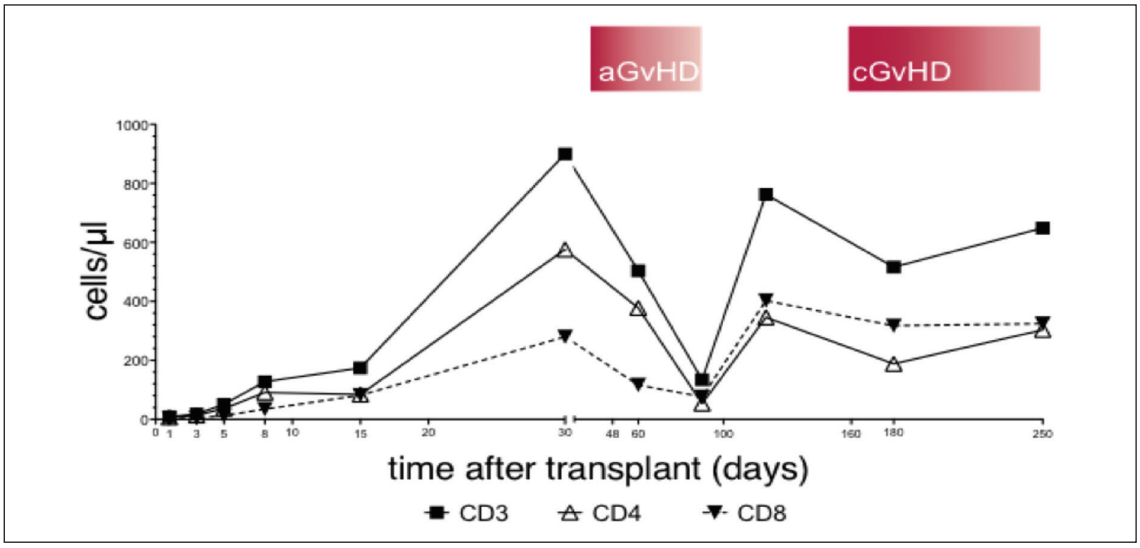

Figure 5 - *Immune reconstitution after allogeneic HSCT: absolute counts of lymphocyte subpopulations of CD3+, CD4+ and CD8+ T cells, assessed by whole blood immunophenotypic staining with specific monoclonal antibodies (all from Biolegend) and Flow-Count Fluorospheres (Beckman Coulter), acquired using a Gallios cytometer and analyzed with Kaluza software (all from Beckman Coulter). HSCT = hematopoietic stem cell transplantation; GvHD = graft versus host disease.*

tion that can be ascribed to: host characteristics, IFI before transplant, neutropenia after the conditioning regimen (particularly in leukemic patients who may have been colonised during the multiple chemotherapy cycles), cord-blood or haploidentical family donor, severe acute or chronic GvHD and cytomegalovirus infections (15). Despite some major improvements, the mortality of *Aspergillus* in allogeneic HSCT patients remains over 50% in recent series and the diagnosis of infection in immunocompromised patients is still challenging (13). Life-threatening infections could significantly delay or even impede the prosecution of malignancy-specific treatment of haematological patients, therefore only the timely diagnosis guiding a targeted therapy may give the opportunity to perform aggressive but potentially curative treatments such as intensified chemotherapy or allogeneic HSCT. The EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study Group) Consensus Group provided the standard definitions for IFI classification. "Proven" IFI requires the demonstration of fungal elements in diseased tissue (biopsy, culture). The presence of a host factor plus clinical features (i.e. CT scan) and mycological criteria (i.e. cytology, indirect tests) identifies a "probable" IFI. The category of "possible" IFI includes all those cases with clinical evidence of IFI but for which there is no mycological support (16). In recent years advanced diagnostic tests, such as high resolution CT scan and antigen-based tests, have considerably improved the identification of IFI (2, 17). Nevertheless available tools, like histopathology, culture and radiology, are often unable to timely detect the fungal pathogen and the presence of IFI (18). The clinical impact of more recent diagnostic approaches (galactomannan, β-glucan and PCR assays) is under investigation (2, 17). The contribution of molecular techniques could be potentially

relevant in a multidisciplinary approach for IFI diagnosis (5, 6). In this case report only the molecular assay was able to detect *Aspergillus* in different consecutive samples, whereas all the other microbiological assays resulted negative, and this allowed a prompt start of targeted antifungal therapy and a rapid resolution of patient's sepsis. Achieving control of this severe infectious complication made haploidentical HSCT suitable for this patient, who had in this treatment her only possibility for a definitive cure.

This case further evidences that a well-designed molecular test in combination with traditional assays can play an important role in the diagnosis of IFI and can potentially improve the standard algorithm, according to the EORTC/MSG Consensus Group, therefore leading to a rapid diagnosis and an earlier targeted antimicrobial therapy.

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