Acute isolated appendicitis due to *Aspergillus carneus* in a neutropenic child with acute myeloid leukemia

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SUMMARY

We describe a case of isolated acute appendicitis due to *Aspergillus carneus* in a neutropenic child with acute myeloid leukemia (AML) treated according to the AIEOP AML 2002/01 protocol. Despite prophylaxis with acyclovir, ciprofloxacin and fluconazole administered during the neutropenic phase, 16 days after the end of chemotherapy the child developed fever without identified infective foci, which prompted a therapy shift to meropenem and liposomial amphotericin B. After five days of persisting fever he developed ingravescent abdominal lower right quadrant pain. Abdominal ultrasound was consistent with acute appendicitis and he underwent appendectomy with prompt defervescence. PAS+ fungal elements were found at histopathology examination of the resected vermiform appendix, and galactomannan was low positive. *A. carneus*, a rare species of *Aspergillus* formerly placed in section *Flavipedes* and recently considered a member of section *Terrei*, was identified in the specimen. Treatment with voriconazole was promptly started with success. No other site of *Aspergillus* localization was detected. Appendicitis is rarely caused by fungal organisms and isolated intestinal aspergillosis without pulmonary infection is unusual. To our knowledge, this is the first report of infection due to *A. carneus* in a child and in a primary gastrointestinal infection.

KEY WORDS: Aspergillus carneus, Appendicitis, Galactomannan, Leukemia, Voriconazole.

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INTRODUCTION

Invasive fungal infections are an important cause of morbidity and mortality in immunocompromised neutropenic cancer and transplant patients (Pagano *et al.,* 2006; Pagano *et al.,* 2007; Pagano *et al*., 2010). In recent years, a shift in the most prevalent etiological agents has been observed from *Candida* spp. to filamentous fungi, which constitute, at present, the majority of invasive fungal infections in severely immunocompromised patients (Pagano *et al.,* 2006; Pagano *et al.,* 2007).

Aspergillus fumigatus, A. terreus and *A. flavus* are the *Aspergillu*s species most frequently involved in invasive infections in neutropenic patients, but new species are emerging (Kontoyiannis *et al.,* 2010; Steinbach *et al.,* 2012). In these cases, mortality rates range from 20 to 47% despite prophylaxis with new antifungal drugs active against molds (Lass-Flörl *et al*., 2005). Furthermore, the clinical diagnosis of these infections is challenging, often being misdiagnosed as bacterial infections, with fatal consequences (Auberger *et al.,* 2008; von Eiff *et al.,* 1995)

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Aspergillus infections usually involve the respiratory tract, with vascular invasion and subsequent dissemination. On the contrary, primary aspergillosis of the gut is a rare event (Eggiman *et al.,* 2006; Li *et al.,* 2014). We describe a case of primary aspergillar appendicitis in a child with acute myeloid leukemia (AML) diagnosed by histological examination and confirmed by cultural isolation and molecular identification.

CASE REPORT

A 6-year-old Romanian boy was diagnosed in the pediatric department of IRCCS San Matteo Hospital Foundation, Pavia, Italy, with AML, French-American-British classification M5. A first cycle of induction chemotherapy with ICE scheme (Ifosfamide, Carboplatin, Etopside) was started according to AIEOP (Italian Association for Pediatric Hematology/Oncology) AML 2002/01 protocol, and prophylaxis with acyclovir, ciprofloxacin and fluconazole was administered during the neutropenic phase. Sixteen days after the end of chemotherapy, the child developed fever without apparent infective foci (negative blood, stool, and urine cultures, chest X-ray, and galactomannan antigen test [Platelia™ Aspergillus EIA, BIO-RAD] 0.24 n.v. <0.5). Anti-infective therapy was shifted to meropenem and liposomial amphotericin B (3 mg/kg/day). After five days of treatment and persisting fever, he developed severe abdominal right lower quadrant pain. Abdominal ultrasound showed an aperistaltic, fluid-filled, non compressible, distended appendix with pericecal inflammatory changes and an appendicolith. These findings were consistent with acute appendicitis, and the patient underwent appendicectomy. The histopathology examination of the resected vermiform appendix showed gangrenous inflammation and secondary peri-appendiceal peritonitis. Periodic acid–Schiff (PAS) positive fungal elements with dichotomous branching hyphae were observed both in the appendix and in the vessels, demonstrating vascular invasion (Figure 1). A galactomannan antigen test on a serum sample obtained on the same day resulted weakly positive (0.69 n.v. <0.5). These findings prompted initiation of voriconazole therapy, initially intravenously for 10 days, with a loading dose of 14 mg/kg/day in two divided doses, then orally with a dose of 400 mg/day in two divided doses, subsequently adjusted through monitoring of circulating drug concentration, as recommended by the Fourth European Conference on Infections in Leukaemia (ECIL-4) pediatric recommendations (Groll *et al.,* 2012). Fever disappeared soon after surgery. Galactomannan levels turned negative after two weeks of treatment. No other site of *Aspergillus* localization was detected on total body CT scan performed one week after surgery and repeated before transplant. In the following months, the patient remained in complete hematological remission throughout the chemotherapy protocol, and underwent hematopoietic stem cell transplantation in first complete remission from a matched unrelated donor four months after appendicitis. Voriconazole secondary prophylaxis was maintained during neutropenia and until

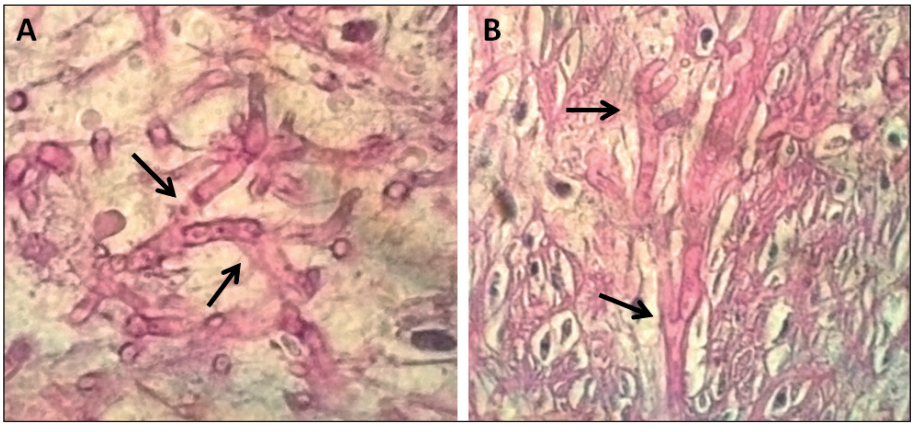

FIGURE 1 - *Histopathology examination of the resected vermiform appendix. Images from 2 fields of view (100X magnification) showing periodic acid–Schiff (PAS) positive fungal elements with dichotomous branching hyphae (arrows).*

neutrophil engraftment, and was switched to fluconazole 10 mg/kg/day for a further month until recovery of CD4+ counts. No aspergillosis relapse or other invasive fungal infections were observed.
Eight months after transplant, he persists in complete remission and in good clinical conditions.

MICROBIOLOGICAL IDENTIFICATION

Culture of the resected vermiform appendix on Sabouraud Dextrose Agar showed the growth of several light brown colonies of moulds after 3 days at 37°C, changing to pink with aging (Figure 2A). Conidial heads radiated to loosely columnar, 150-200x25-35 µm, were observed at microscopic examination using lactophenol cotton blue staining. Conidiophore stipes were variable in length, up to 1 mm, smooth-walled, hyaline to very light brown. Vesicles were hemispherical, 5.5-10.0 µm in diameter. Conidiogenous cells were biseriate. Metulae covered the upper third to half of the vesicle. Conidia, 2.5-3.5 µm in diameter, were spherical and smooth-walled (Figure 2B). These macroscopic and microscopic features were indicative of *Asperigillu*s genus. Molecular identification was then performed.
Genomic DNA was extracted using the Ultra Clean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) and amplified using universal fungal-specific primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) targeting internal transcribed spacer regions of fungal ribosomal DNA (White *et al.*, 1990). PCR was performed in a 2700 thermal cycler (Applera, Foster City, CA, USA) using the following conditions: denaturation 94°C for 5 min; 40 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, and final extension at 72°C for 5 min. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. The amplicon was sequenced using Big Dye terminators (Applera) in a 310 ABI PRISM® sequencer (Applera). Nucleotide sequences were analyzed using Finch TV software Version 1.4.0. and the GenBank BLAST database (White *et al.*, 1990). The strain was identified as *Aspergillus carneus* (Reference number of deposited sequence in Genbank: KP979648 100% homology for the first and second closest).
In vitro antifungal susceptibility testing to amphotericin B, itraconazole, posaconazole and voriconazole was performed with Sensititre Yeast One (TREK Diagnostic Systems, East Grinstead, West Sussex, UK) using the cut-off values of the Clinical and Laboratory Standards Institute (CLSI M38-A2, 2008), according to the manufacturer's instructions. Conidia and sporangiospores were induced in Potato Dextrose Agar cultures at 35°C and used to prepare the inoculum suspensions. Minimum inhibitory concentrations (MIC) were visually identified as the lowest concentrations retaining a blue color. Clinical breakpoints have not been established for mold testing yet, therefore epidemiological cutoff values were used (Pfaller *et al.*, 2009; Espinel-Ingroff *et al.*, 2011). These are available for *Aspergillus terreus,* section *Terrei*

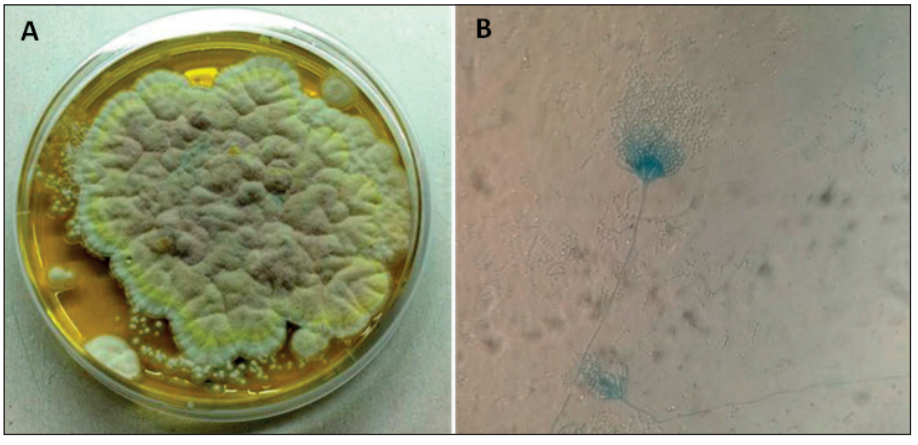

FIGURE 2 - *Panel A. Culture of Aspergillus carneus from resected vermicular appendix on Sabouraud Dextrose Agar. Panel B. Microscopic morphology of A. carneus conidial heads (40X magnification).*

TABLE 1 - *Minimum inhibitory concentration (MIC) of the antifungal agents tested using Yeast One Sensititre (TREK Diagnostic Systems).*

Antifungal Drug	*MIC (mcg/ml)*
Posaconazole	0.25
Voriconazole	0.5
Itraconazole	0.5
Fluconazole	256
Amphotericin B	2

(Samson *et al.,* 2011), which includes *A. carneus*. The fungus was sensitive to amphotericin B, itraconazole, posaconazole and voriconazole (Table 1). A quality control strain (*A. flavus* ATCC 204304) was also included in the test, whose sensitivity results were within the ranges indicated by CLSI.

DISCUSSION

Isolated intestinal aspergillosis without pulmonary involvement is unusual and only a few cases have been described up to date, mainly in patients with hematological malignancies (Catalano *et al.,* 1997; Eggiman *et al.,* 2006; Park *et al.,* 2010; Kazan *et al.,* 2011; Imola *et al.,* 2012). In such cases, *Aspergillus* penetrates through the intestinal mucosa damaged by chemotherapy. In this context, primary fungal appendicitis is extremely rare. The definitive diagnosis is largely based on histopathology evidence of fungi in resected specimens (Larbcharoensub *et al.,* 2013).

A. carneus, described by Blochwitz in 1933 (Blochwitz, 1933), is a rare species of soil *Aspergillus*, originally placed in the *Flavipedes* section before its current positioning into section *Terrei* (Samson *et al.,* 2011). This also includes the species *A. terreus sensu stricto*, *A. niveus*, *A. alabamensis* and *A. terreus* var. *aureus*, that are morphologically indistinguishable and can only be identified with molecular techniques. *A. terreus* is a widespread soil saprophyte and has been described as an emerging cause of infection in some hospital outbreak reports with a dissemination rate of 63% (Lass-Flörl *et al.,* 2005). *A. carneus* has rarely been reported as a pathogen. It was shown to induce lateral and truncal ataxia in mice inoculated intravenously, and it was reported as cause of lung lesion in a man (Morquer, 1957; Pore, 1968). Data from *in vitro* and *in vivo* experiments indicate that almost all *A. terreus* isolates are intrinsically resistant to amphotericin B (Blum *et al.,* 2013). However, the *A. carneus* isolate from our case was susceptible both to voriconazole and amphotericin B, based on the breakpoints proposed by CLSI for *A. terreus* isolates (Espinel-Ingroff *et al.,* 2011; Pfaller *et al.,* 2009). The lack of infection dissemination and the only weakly positive galactomannan serum levels observed in our patient could be explained by the prompt initiation of antifungal therapy with amphotericin B, then switched to voriconazole, characterized by a higher tissue penetration (Felton, *et al.,* 2014).

To our knowledge, this is the first report of infection due to *A. carneus* in a child and in a primary gastrointestinal infection. Our case suggests that this species shares the same *in vitro* antifungal susceptibility to azoles and amphotericin B as other species of *A. terreus* (Tortorano *et al.,* 2008), indicating voriconazole as the first treatment choice. In our patient, positive galactomannan levels guided the decisions to change antifungal therapy regime. This approach proved successful, confirming the importance of this test in guiding medical decisions, as well as in monitoring the response to treatment. Surgery was crucial for the clearance of infection and for tissue sampling, which allowed pathogen isolation, identification, and antifungal susceptibility testing. In our case, *in vitro* MIC values of antifungal drugs reflected *in vivo* efficacy. In conclusion, a timely identification of the fungal pathogen, with subsequent specific therapy, is of vital importance to limit systemic dissemination.

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