Case Report

Multifocal osteomyelitis caused by *Candida dubliniensis*

Nele Wellinghausen,¹ Angelika Moericke,¹ Silke Bundschuh,²
Wilhelm Friedrich,² Ansgar S. Schulz² and Susanne A. Gatz²

Correspondence

Nele Wellinghausen
nele.wellinghausen@web.de

¹Institute of Medical Microbiology and Hygiene, University Hospital of Ulm, Ulm, Germany

²University Hospital for Children's and Adolescent Medicine, Ulm, Germany

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Candida dubliniensis is an emerging fungal pathogen, especially in immunodeficient patients. We report what is to the best of our knowledge the first case of multifocal osteomyelitis following disseminated infection in a patient after haematopoietic stem cell transplantation. PFGE for typing of *C. dubliniensis* was developed and the necessity of long-term antifungal therapy is discussed.

Case report

A 19-year-old white man with congenital haemolytic anaemia of undefined genetic origin, son of consanguineous parents, was referred for peripheral blood stem cell transplantation (PBSCT) to our institution. He suffered from the sequelae of lifelong transfusion therapy including haemosiderosis of the liver (histological grade IV), pancreas and endocrine system, and showed incomppliance to chelate therapy.

The conditioning regimen for PBSCT consisted of radio-immunotherapy with an yttrium-90-labelled CD66 antibody for myeloablation (calculated bone marrow dose 17 Gy), and chemotherapy with fludarabine (40 mg m⁻² for 4 days) and melphalan (140 mg m⁻² for 1 day). In addition, antithymocyte globulin [rabbit (Sangstad) 3.3 mg kg⁻¹ for 3 days] was given. On day 0 the patient received peripheral blood stem cells of a 10/10 human leukocyte antigen-matched unrelated female donor. The number of CD34⁺ cells in the graft was 4 × 10⁶ cells kg⁻¹, the CD3 cell count was adapted to 1 × 10⁷ cells (kg body weight)⁻¹. Graft versus host disease prophylaxis included cyclosporine and mycophenolate.

The patient developed fever on day -1 when ceftazidime was started (100 mg kg⁻¹ per day). On day +4 vancomycin (40 mg kg⁻¹ per day) was added, and on day +7 ceftazidime was changed to meropenem (80 mg kg⁻¹ per day). However, spiking temperatures continued and C-reactive protein (CrP) increased to a peak level of 298 mg l⁻¹ on day +10. No infectious focus was detected by computed tomography (CT) scan of the thorax. However, on day +8, he developed generalized papulopustulous skin efflorescences on his trunk and extremities suggestive of septic metastases.

Abbreviations: CrP, C-reactive protein; CSF, cerebrospinal fluid; CT, computed tomography; IHA, indirect haemagglutination; MRI, magnetic resonance imaging; PBSCT, peripheral blood stem cell transplantation; PET-CT, positron-emission-tomography computed tomography.

A skin biopsy was performed at day +9 and grew *Candida* spp. in pure culture after 24 h of incubation on Sabouraud dextrose agar. Blue colonies on Candida ID-2 agar (bioMérieux) (Eraso *et al.*, 2006), an abundance of chlamydospores on rice agar, and growth on Sabouraud dextrose agar at 42 °C and 45 °C, suggested *Candida albicans*; however, biochemical identification by API ID32 (bioMérieux) was ambiguous, suggesting *Candida dubliniensis* after 48 h (code 7142140015, 99.3 %, T0.67) and *C. albicans* after 72 h incubation (code 7347150015, 98.4 %, T0.53). In the Bichro-Dubli latex agglutination test (Fumouze) the isolate reacted positively. By sequencing a 550 bp fragment of the internal transcribed spacer regions 1 and 2 using the primers Fungi for (5'-TCCGTAGGTGAACCTGCGG-3') and Fungi rev (5'-TCCTCCGCTTATTGATATGC-3') identification of *C. dubliniensis* was confirmed. The strain showed 99.6 % homology to the *C. dubliniensis* reference strain DSM 13628 (GenBank accession number DQ105856) using the BLAST algorithm. Susceptibility testing by Etest on RPMI agar revealed low MICs for fluconazole (0.25 µg ml⁻¹), amphotericin B (0.023 µg ml⁻¹), flucytosine (0.016 µg ml⁻¹), itraconazole (0.023 µg ml⁻¹), voriconazole (0.008 µg ml⁻¹) and caspofungin (0.19 µg ml⁻¹).

Despite empirical antifungal therapy with liposomal amphotericin B (3 mg kg⁻¹) and caspofungin (70 mg on day +9, 50 mg on days +10 to +17), the patient continued to deteriorate over the following days showing a clinical picture of sepsis, with temperatures above 40 °C, arterial hypotension and coagulation abnormalities. Decreased pulmonary and renal function required intensive care unit treatment (days +16 to +27) with tracheal intubation and ventilatory support. After final identification of the *Candida* species as *C. dubliniensis* (day +14), antifungal therapy was, therefore, changed to voriconazole (14 mg per day intravenously).

A significant rise in *Candida* antibody titres [indirect haemagglutination (IHA) test; Virion/Serion] was

observed, from 1:40 on day +9 to 1:2560 on day +20. Both *Candida*-specific IgM and IgG antibodies (Classic ELISA; Virion/Serion) were detectable (Fig. 1a). A total of eight blood cultures taken between days +5 and +22 remained negative even after manual subculturing following 7 days incubation in the BACTEC system. Cerebrospinal fluid (CSF) taken on day +16 was sterile. *Candida* spp. were not detectable by culture in urine or respiratory secretions. Fundoscopy of the eyes was unremarkable. Central vascular catheters were changed on days +13 and +36, and did not grow bacteria or fungi.

The patient showed prompt regeneration of granulocytes by day +11 with >2000 cells μl^{-1} by day +14. Donor cell chimerism was complete when tested on day +33. Granulocytes were even higher, >5000 cells μl^{-1} by days +14 to +45. Due to cytomegalovirus reactivation ganciclovir (10 mg kg^{-1}) was added on day +18. Fever, headaches and elevated CrP levels above 40 mg l^{-1} persisted but no infectious focus was detected by cranial CT scan on day +16 and whole-body positron-emission-tomography CT (PET-CT) scan on day +43. Thus, assuming sufficient antifungal therapy of the *C. dubliniensis* infection, voriconazole was discontinued on day +43.

Starting on day +95, the patient complained about bone pain in his legs, his right buttock and right lateral malleolus, as well as severe headaches. Magnetic resonance imaging (MRI) scans of the head (days +112 and +126) and the entire spinal cord (day +128) revealed no leptomeningeal enhancement or other signs of inflammation of the brain or spinal cord. CSF samples obtained on days +110 to +128 showed an abundance of granulocytes (170–1110 cells μl^{-1}) and markedly elevated protein levels (up to 5569 mg l^{-1}); however, cultures for bacteria and fungi were sterile. Mycobacterial culture (BACTEC MGIT and solid media incubated for 12 weeks) and *Mycobacterium tuberculosis* complex PCR (COBAS Amplicor; Roche) with CSF were negative on day +126 and +128. PCR with CSF testing for cytomegalovirus, Epstein–Barr virus, human herpes virus 6, herpes simplex virus 1 and 2, varicella-zoster virus, parvovirus B19 and enterovirus were negative on days +112, +126 and +128. A total of 11 blood cultures taken at days +102 to +134 remained negative even after manual subculturing following 7 days incubation in the BACTEC system.

Skeletal scintigraphy on day +133 showed enhancement in the right distal tibia and distal part of the right sacroiliac

Fig. 1. *Candida*-specific antibodies determined by IHA (●) and *Candida*-specific IgG (△) and IgM (■) (U ml^{-1}) determined by ELISA (a), and B cells (▲) and T cells (□) (b) in patient blood pre- and post-PBSCT. Relevant clinical findings (a) and periods of antifungal therapy (b) are marked. PBSCT was given on day 0.

joint. Corresponding MRI scans confirmed foci in the right distal tibia (day +135, Fig. 2a), foci in the right os sacrum and bilateral foci in the sacroiliac joints (day +142; Fig. 2b). Around day +150 the patient developed severe back pain in addition to his above-mentioned complaints. Further lesions in the thoracic and lumbar spine, including signs of inflammation of the caudal thoracic and lumbar meninges, were detected by MRI (day +162, Fig. 2c).

In order to determine the pathogenesis of the foci in the bones, a biopsy of the distal tibia was performed on day +152. The biopsy grew sporadic *Candida* spp. and very sporadic *Staphylococcus epidermidis*. Simultaneous skin cultures from the biopsy area were negative for *Candida* spp. Biochemical and molecular methods (as above) identified the isolate as *C. dubliniensis* and susceptibility testing revealed MICs similar to the first isolate. In order to confirm the assumed clonal identity of both isolates, PFGE with RFLP analysis for typing of *C. dubliniensis* was developed. PFGE–RFLP was carried out with CHEF DRIII equipment (Bio-Rad) in 1% agarose at 14 °C, a voltage of 200 V with pulse rates of 3–70 s, and run times of 22–24 h, using various restriction enzymes like *PauI*, *EcoRI*, *SgsI*, *SfiI*, *SmaI* and *PdI* (Fermentas). A pulse rate of 5–60 s using enzyme *SgsI* showed the clearest banding patterns and the highest discriminatory power using a *C. dubliniensis* reference strain [external quality control strain 01-2004 from INSTAND (a Collaborating Center for Quality Assurance and Standardization in Laboratory Medicine of the World Health Organization)]. PFGE–RFLP revealed the very close genetic relationship of the skin and the bone biopsy isolates (Fig. 3).

After identification of the *Candida* isolate from the bone biopsy, antifungal therapy was restarted with fluconazole (400 mg twice a day for 3 days, then 200 mg twice a day),

casprofungin (50 mg per day), and flucytosine (2 g twice a day). On day +169 fluconazole was substituted by amphotericin B (0.5 mg kg⁻¹ per day up to day +233, followed by 1 mg kg⁻¹ three times a week in an outpatient setting). Under this therapy the bone pains and headaches, as well as elevated temperatures, were regressive. On day +241 casprofungin was discontinued and on day +254 amphotericin B was changed to voriconazole (200 mg twice a day). Therapy with flucytosine was continued until day +345 and oral voriconazole was continued as monotherapy. The foci in the spinal column had already decreased in size on an MRI scan on day +268. The sacroiliac changes were no longer visible on an MRI scan on day +310 and the foci in the os sacrum were markedly improved. Therefore, assuming sufficient therapy of the disseminated osteomyelitis, voriconazole was discontinued on day +400. The lesions in the lumbar spine further regressed and those in the os sacrum were no longer detectable by the time of the follow up MRI on day +470. Furthermore, skeletal scintigraphy (day +463) did not reveal any abnormalities. At the same time the patient recovered completely clinically. He was gaining weight and had no pain or fever. However, his CrP levels continued to be slightly elevated to approximately two to four times the upper normal range (i.e. 20–40 mg l⁻¹).

Four months later, weight loss and anorexia were noted. A follow-up PET-CT scan on day +716 revealed new lesions in the patient's liver and right femur without any sign of reactivation at the sites of the former lesions. In order to determine the cause of the new lesions an open biopsy of the right femur was performed after the patient had been treated again with voriconazole (4 g mg kg⁻¹ per os twice a day) for 14 days. Although bacterial, mycobacterial and fungal cultures remained sterile, budding fungal structures were observed in the direct Calcofluor white stain

Fig. 2. Representative images of the MRI scans at initial diagnosis of osteomyelitis for the main areas involved (displayed in T1): lesion in the right tibia (a), in the os sacrum and the sacroiliac joints (b), and in the lumbar spine (c). The corresponding days post-PBSCT are given below each image. Osteomyelitic lesions are indicated by arrows.

Fig. 3. PFGE-RFLP typing of *C. dubliniensis* isolates using *SgsI* digestion. Lanes 1 and 6, 48.5 to 970 kb molecular mass marker (λ clB57Sam7; Bio-Rad); lane 2, clinical isolate from skin biopsy; lane 3, clinical isolate from bone biopsy; lane 4, clinical isolate from bronchial aspirate from a different patient; lane 5, control strain 01-2004 from INSTAND.

suggesting infection with *Candida* spp. Therefore, anti-fungal therapy with voriconazole (7 mg kg⁻¹ twice a day) was restarted and caspofungin (50 mg per day) was added. Therapy was initiated intravenously for 14 days and voriconazole was still continued orally.

Discussion

C. dubliniensis, first recognized as a germ tube-positive yeast distinct from *C. albicans* in 1995 in Ireland (Sullivan *et al.*, 1995), is an emerging fungal pathogen, especially in immunodeficient patients. It has been primarily identified as a cause of oral candidiasis in human immunodeficiency virus-infected persons (Sullivan *et al.*, 1995) but systemic infections are increasingly reported (Brandt *et al.*, 2000; Jabra-Rizk *et al.*, 2005; Meis *et al.*, 1999; Sebt *et al.*, 2001). The importance of *C. dubliniensis* as a pathogen is highlighted by its ability to develop *in vitro* resistance against fluconazole after exposure to the drug (Moran *et al.*, 1997; Sullivan, *et al.*, 2004) and by the presence of a range of virulence genes similar to those of *C. albicans* (Sullivan *et al.*, 2004). *C. dubliniensis* is distributed worldwide and, recently, an environmental source has been described (Nunn *et al.*, 2007).

We report what is to the best of our knowledge the first case of multifocal osteomyelitis caused by *C. dubliniensis*, preceded by a systemic infection that became manifest in disseminated skin lesions. PFGE-RFLP, which was used for typing *C. dubliniensis* for what is believed to be the first time in this study, revealed the very close genetic relationship of the skin and bone biopsy isolates. Since it

has been shown that *C. dubliniensis* undergoes genetic variation more frequently than other species (Gee *et al.*, 2002; Joly *et al.*, 2002), both isolates may represent variants of the same strain.

Systemic infection with *C. dubliniensis* evoked a *Candida*-specific antibody response (Fig. 1). Since the IHA test measures antibodies against *Candida* polysaccharide cell wall antigens, mainly of the IgM class, a T-cell-independent antibody response may be postulated. It is difficult to evaluate whether the early serological response was caused by residual host B cells or by donor B cells. The former is suggested by the finding that the first contact of the host B cells to the *Candida* antigen may have taken place already before conditioning of the patient, since colonization with *C. dubliniensis* often precedes invasive infection (Meis *et al.*, 1999). During the later phase of disease, when osteomyelitis developed, *Candida*-specific antibodies gradually decreased to negative titres although the patient had high numbers of donor B cells, accompanied by only low T-cell numbers in his peripheral blood. Importantly, the patient also showed a delayed T-cell reconstitution judged by a lack of sufficient numbers of CD4 T cells (<400 μ l⁻¹), especially of naive CD4 T cells, until 6 months after the PBSCT, and by poor T-cell proliferation in response to mitogens even 1 year after the PBSCT (data not shown). Therefore, a deficiency in both the B- and T-cell system may have contributed to the inability to clear the infection.

Optimal antifungal therapy for *C. dubliniensis* remains unclear. After initial therapy with liposomal amphotericin B, caspofungin and voriconazole (Salgado-Parreno *et al.*, 2006), the patient clinically improved. Since no infectious foci were detected in a whole-body PET-CT scan on day +43, antifungal therapy was discontinued after 5 weeks of therapy. The clinical course, however, suggested that the duration of the initial antifungal therapy was too short. Most probably, dissemination of *Candida* cells to the bones had already occurred during the initial phase of the infection. Interestingly, despite treatment with voriconazole the isolate remained susceptible to fluconazole and voriconazole. Evidence-based data regarding antifungal therapy of *Candida* osteomyelitis are scarce, but current guidelines recommend surgical debridement and an initial therapy with amphotericin B followed by fluconazole for a total duration of 6–12 months (Pappas *et al.*, 2004). Since surgical debridement was impossible in our case, a combination antifungal therapy was given for several months (Ashley *et al.*, 2006). Although the bone lesions were regressive and the patient clinically improved, the disseminated infection was apparently not cured since approximately 11 months later new lesions were detected in the femur and liver.

While *C. dubliniensis* was initially regarded as a *Candida* species with only low pathogenic potential, fatal courses of systemic infections have been reported recently (Carr *et al.*, 2005; Chan-Tack, 2005). Whether specific virulence factors of *C. dubliniensis* like phenotypic switching (Sullivan *et al.*,

2005) or the underlying condition of the patient are the primary determinants of the clinical course remains unclear. This case of disseminated *C. dubliniensis* infection highlights the pathogenic potential of *C. dubliniensis* in severely immunocompromised patients. Failure to detect *C. dubliniensis* in blood cultures as well as clinical improvement of the patient does not preclude dissemination of the pathogen. The appearance of new osteomyelitic lesions even after prolonged antifungal combination therapy stresses the necessity of long-term antifungal treatment of suspected disseminated *C. dubliniensis* infection, especially in immunocompromised patients.

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References

- Dodds Ashley, E. S., Lewis, R., Lewis, J. S., Martin, C. & Andes, C. (2006). Pharmacology of systemic antifungal agents. *Clin Infect Dis* 43, S28–S39.
- Brandt, M. E., Harrison, L. H., Pass, M., Sofair, A. N., Huie, S., Li, R. K., Morrison, C. J., Warnock, D. W. & Hajjeh, R. A. (2000). *Candida dubliniensis* fungemia: the first four cases in North America. *Emerg Infect Dis* 6, 46–49.
- Carr, M. J., Clarke, S., O'Connell, F., Sullivan, D. J., Coleman, D. C. & O'Connell, B. (2005). First reported case of endocarditis caused by *Candida dubliniensis*. *J Clin Microbiol* 43, 3023–3026.
- Chan-Tack, K. M. (2005). Fatal *Candida dubliniensis* septicemia in a patient with AIDS. *Clin Infect Dis* 40, 1209–1210.
- Eraso, E., Sahand, I. H., Villar-Vidal, M., Marcos, C., Dolores, M. M., Madariaga, L., Ponton, J. & Quindos, G. (2006). Usefulness of *Candida* ID2 agar for the presumptive identification of *Candida dubliniensis*. *Med Mycol* 44, 611–615.
- Gee, S. F., Joly, S., Soll, D. R., Meis, J. F., Verweij, P. E., Polacheck, I., Sullivan, D. J. & Coleman, D. C. (2002). Identification of four distinct genotypes of *Candida dubliniensis* and detection of microevolution in vitro and in vivo. *J Clin Microbiol* 40, 556–574.
- Jabra-Rizk, M. A., Johnson, J. K., Forrest, G., Mankes, K., Meiller, T. F. & Venezia, R. A. (2005). Prevalence of *Candida dubliniensis* fungemia at a large teaching hospital. *Clin Infect Dis* 41, 1064–1067.
- Joly, S., Pujol, C. & Soll, D. R. (2002). Microevolutionary changes and chromosomal translocations are more frequent at RPS loci in *Candida dubliniensis* than in *Candida albicans*. *Infect Genet Evol* 2, 19–37.
- Meis, J. F., Ruhnke, M., De Pauw, B. E., Odds, F. C., Siegert, W. & Verweij, P. E. (1999). *Candida dubliniensis* candidemia in patients with chemotherapy-induced neutropenia and bone marrow transplantation. *Emerg Infect Dis* 5, 150–153.
- Moran, G. P., Sullivan, D. J., Henman, M. C., McCreary, C. E., Harrington, B. J., Shanley, D. B. & Coleman, D. C. (1997). Antifungal drug susceptibilities of oral *Candida dubliniensis* isolates from human immunodeficiency virus (HIV)-infected and non-HIV-infected subjects and generation of stable fluconazole-resistant derivatives in vitro. *Antimicrob Agents Chemother* 41, 617–623.
- Nunn, M. A., Schaefer, S. M., Petrou, M. A. & Brown, J. R. (2007). Environmental source of *Candida dubliniensis*. *Emerg Infect Dis* 13, 747–750.
- Pappas, P. G., Rex, J. H., Sobel, J. D., Filler, S. G., Dismukes, W. E., Walsh, T. J. & Edwards, J. E. (2004). Guidelines for treatment of candidiasis. *Clin Infect Dis* 38, 161–189.
- Salgado-Parreno, F. J., Alcoba-Florez, J., Arias, A., Moragues, M. D., Quindos, G., Ponton, J. & Arevalo, M. P. (2006). In vitro activities of voriconazole and five licensed antifungal agents against *Candida dubliniensis*: comparison of CLSI M27–A2, Sensititre YeastOne, disk diffusion, and Etest methods. *Microb Drug Resist* 12, 246–251.
- Sebti, A., Kiehn, T. E., Perlin, D., Chaturvedi, V., Wong, M., Doney, A., Park, S. & Sepkowitz, K. A. (2001). *Candida dubliniensis* at a cancer center. *Clin Infect Dis* 32, 1034–1038.
- Sullivan, D. J., Westerneng, T. J., Haynes, K. A., Bennett, D. E. & Coleman, D. C. (1995). *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology* 141, 1507–1521.
- Sullivan, D. J., Moran, G. P., Pinjon, E., Al Mosaid, A., Stokes, C., Vaughan, C. & Coleman, D. C. (2004). Comparison of the epidemiology, drug resistance mechanisms, and virulence of *Candida dubliniensis* and *Candida albicans*. *FEMS Yeast Res* 4, 369–376.
- Sullivan, D. J., Moran, G. P. & Coleman, D. C. (2005). *Candida dubliniensis*: ten years on. *FEMS Microbiol Lett* 253, 9–17.