Invasive sino-orbital mycosis in an aplastic anemia patient caused by *Neosartorya laciniosa*

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We report the first case of *Neosartorya laciniosa* invasive sinusitis involving the orbit in an immunocompromised male with aplastic anemia. Treatment included surgical debridement with enucleation of the eye, combination voriconazole and micafungin therapy followed by voriconazole alone. The fungus was identified using sequencing of partial *benA* and calmodulin genes.
A 66-year-old male was diagnosed with aplastic anemia in March 2011 and subsequently treated with bone marrow transplant (BMT) followed by immunosuppressive therapy with lymphocyte immune globulin anti-thymocyte globulin (Atgam, Pfizer Canada Inc., Kirkland, QC), steroids and cyclosporine. On May 19, he was referred from a community hospital to the University of Alberta Hospital with a history of pancytopenia (absolute neutrophil count (ANC) < 500 cells/µL) beginning in February and intermittent fevers since mid April. Additional findings included increased left-sided periorbital swelling and radiographic evidence of pneumonia, but no respiratory symptoms were evident. He had not received antifungal prophylaxis. On admission he was hemodynamically stable and febrile (temperature of 39.1°C). A complete blood count (CBC) revealed pancytopenia with a white blood count of 400 cells/µL (ANC 0 cells/µL), a creatinine of 112 µmol/L (Estimated Glomerular Filtration Rate 57 mL/min). Electrolytes and liver enzymes were normal. Urine and blood cultures were negative. The patient was treated empirically with vancomycin and ciprofloxacin for presumed periorbital cellulitis. A computed tomography (CT) of the head showed left-sided proptosis and progression of fluid and high density material causing opacification within all of visualized paranasal sinuses on the left side. This finding was interpreted to represent probable chronic paranasal sinus inflammation. A prior head CT done at the time of the BMT was normal. The patient deteriorated clinically with adverse changes including advancing opacification of frontal, ethmoidal and maxillary sinuses with erosions of bony septations of ethmoidal air cells on the left side seen on repeat CT on day 6 post admission. An abscess along the wall of the left medial orbit was documented on CT on day 13 and the patient was sent to the operating room the next day for drainage of a subperiosteal enhancing fluid collection. Intraoperative tissue samples were
sent for pathological analysis as well as for bacterial and fungal cultures. Bacterial culture yielded only coagulase negative *Staphylococcus* (CoNS). No fungal elements were seen on direct smear with calcofluor white (BD Diagnostic Systems, Sparks, MD) and fungal cultures were negative. However, the pathology report documented hemorrhagic necrotic sinus mucosal tissue with numerous branched, septate hyphae and focal vascular invasion consistent with invasive fungal infection (Fig. 1A). The patient was promptly started on liposomal amphotericin B (5 mg/kg/day). Tissue from a second debridement done on day 16 was again negative for hyphae by direct microscopy but culture yielded growth of a mold within three days that was suspected later to be a possible “white, non-sporulating *Aspergillus.*” An exoantigen immunodiffusion test, in which a filtrate from the culture was tested against commercially available antibody reagents (Pulse Scientific Inc., Burlington, ON), gave a positive result for *Aspergillus fumigatus.* The isolate was identified as a *Neosartorya* species when characteristic ascospores were produced two weeks later. A serum galactomannan enzyme immunoassay (Platelia™ *Aspergillus* EIA, Bio-Rad Laboratories (Canada) Ltd., Montreal, QC) performed on day 21 was also positive with an optical density (OD) index value of 2.8 (0.5 used as cut-off value) confirming the diagnosis of invasive aspergillosis. Antifungal therapy was switched to voriconazole 300 mg twice daily and the patient continued on vancomycin, ciprofloxacin in addition to cyclosporine and acyclovir for immunosuppression and antiviral prophylaxis respectively.

Despite the antifungal and broad antimicrobial coverage, the patient continued to be pancytopenic and was persistently febrile. A CT scan on day 26 showed suggestion of dehiscence of the sphenoid sinus and possible communication with the anterior cranial fossa could not be excluded. No intracranial venous thrombosis was noted on magnetic resonance imaging of the brain. A repeat CT on day 33 revealed evidence of gas within a large collection of
fluid infraorbitally measuring 5.6 cm in coronal diameter and again extensive mucoperiosteal thickening within frontal and ethmoidal regions as well as the right maxillary sinus. The patient was taken for surgical enucleation of the left eye on day 35 and further debridement was performed three days later. Fungal hyphae accompanied by tissue necrosis were again seen on histopathology of tissue and cortical bone samples and bacterial cultures were positive for Enterococcus species and CoNS. The patient was continued on vancomycin for a total of 6 weeks of therapy. Unfortunately, fungal culture was not requested at surgery but a repeat serum galactomannan on day 40 remained positive with OD index value of 2.075.

Based on our patient’s tenuous clinical picture, we elected to continue him on oral voriconazole 300mg twice daily and to add intravenous micafungin 150mg daily based on reported uniform susceptibility to this drug among Neosartorya and related species classified within Aspergillus section Fumigati (1, 2). A final CT done on day 40 documented mucosal thickening within the remainder of paranasal sinuses and no fluid collections. He remained transfusion dependant for his pancytopenias, but defervesced and had gradual clinical improvement resulting in his transfer to a community hospital 13 days later. The patient completed a total of six months of antifungal therapy (7 weeks of combination voriconazole and micafungin and four months of monotherapy with voriconazole). He was discharged from hospital seven months after his initial presentation. He continued on cyclosporine for his aplastic anemia and his blood counts stabilized. He was scheduled to have reconstructive surgery to his left orbital area. There was no evidence of recurrence of his fungal infection two months after stopping voriconazole therapy.

**Mycology.** As per review of the literature which indicated variable susceptibility to voriconazole depending on species of Neosartorya, the isolate was submitted for further
identification to the University of Alberta Microfungus Collection and Herbarium (UAMH) where it was accessioned as UAMH 11627. Subcultures on potato dextrose agar (PDA) (BD) and oatmeal salts agar (OAT; prepared in-house) were incubated at 30ºC and 35ºC. Growth was faster at 35ºC with the colony on PDA reaching a diameter of 6 cm after 3 days of incubation. The submitting laboratory reported growth at 45ºC but no growth at 50ºC. Colonies remained yellowish white with no blue-green surface coloration after 14 days. Conidial heads were sparse. The vesicle was about 15 µm wide and bore few phialides on the upper surface. Characteristic yellowish-white, thin-walled ascomata were produced on both media within 7 days, but were more profuse on OAT (Fig. 1B). Ascospores were broadly lenticular with two prominent equatorial crests and measured 4.5 to 5 µm long (Fig 1C). Sequences of the beta-tubulin and calmodulin gene regions were obtained for species identification. DNA was extracted using the E.Z.N.A. SP Fungal DNA kit (United Bioinformatica Inc., Saskatoon, SK). The partial \textit{benA} and calmodulin genes were sequenced using primers previously described (3, 4). Sequences were edited using Sequencher ver. 5.0 (Gene Codes Corp., Ann Arbor, MI) and compared with available sequences in the Genbank nucleotide database using a Blast search. Results were similar for both searches with the case isolate showing 99-100% \textit{benA} similarity and 98-100% calmodulin similarity with several sequences of \textit{Neosartorya spinosa} and \textit{N. laciniosa}, including the ex-type strain KACC 41657. To further assess the genetic relationship between the case isolate and these \textit{Neosartorya} species, a dataset of combined \textit{benA} and calmodulin sequences was obtained and a parsimony analysis was performed using PAUP* v.4.0b10 (http://paup.csit.fsu.edu/) and with \textit{N. fischeri} as outgroup. The robustness of the trees obtained was evaluated by 1,000 bootstrap replications. In the resultant tree, the case isolate is shown
grouping with isolates of *N. laciniosa* with 100% bootstrap support and the group is sister to *N. spinosa* (Fig 2).

Susceptibility testing performed retrospectively on isolate UAMH 11627 yielded MICs (mg/L) of amphotericin B 0.5, 5-fluorouracil 8, fluconazole >64, itraconazole 1, posaconazole 0.25 mg, voriconazole 1, caspofungin 0.5.

Invasive aspergillosis (IA) is the most common filamentous fungal infection among immunocompromised patients with very high mortality rates. Although the majority of infections are attributed to *Aspergillus fumigatus*, there is increasing recognition of the role of related species in causing invasive disease especially since the advent of molecular methods allowing for more precise identification of the etiologic agent involved. There are currently nine *Aspergillus* species and 27 *Neosartorya* teleomorphs included within the *Aspergillus* section *Fumigati* (2, Mycobank http://www.mycobank.org/). *Neosartorya* species are generally recognized by their rapidly growing, poorly sporulating white to pale green colonies in primary culture, by their thermotolerance (all growing at 37ºC and a few up to 50ºC), and by formation of ascomata containing ascospores on sporulation media. Currently, *N. hiratsukae, N. pseudofischeri, N. udagawae* are confirmed as opportunistic pathogens, with the latter species gaining recent attention (5-13). Although *N. fischeri* and *N. spinosa* have been identified in some reports, their roles in causing infection have not been reliably confirmed. Isolates from some older cases have been re-identified as *N. pseudofischeri* and isolates from more recent cases have not been identified using sequencing of genes appropriate for *Neosartorya* identification (14-17).

*Neosartorya* infections in humans may include invasive infections of eye, ear or lung, endocarditis, peritonitis, multifocal brain abscesses and osteomyelitis (5-16). We report the first
case of *N. laciniosa* invasive sino-orbital aspergillosis (SOA) in a patient with aplastic anemia. How our patient acquired his infection is unknown. He did not live on a farm or acreage and had not worked for some time due to illness so no specific exposures could be identified. *Neosartorya laciniosa* was described in 2006 for 12 isolates mainly from agricultural soils with a single isolation from strawberry fruit pulp (4). The species has a broad geographic distribution in Korea, Dominican Republic, Kenya, Pakistan, Netherlands, Surinam and the USA. *N. laciniosa* is genetically closest to *N. spinosa* and these species could not be reliably distinguished based on results of blast search. However, the differentiation between the species is clearly supported in the phylogenetic tree based on the combined dataset (Fig 2).

*Neosartorya* species are uncommonly isolated in our hospital, so there was concern 4 months after this patient’s presentation when a pediatric patient with acute lymphocytic leukemia presented with a necrotic nasal lesion from which a *Neosartorya* species was again cultured. However, sequencing of the isolate from the second patient determined its identity as *N. pseudofischeri*.

SOA is a relatively uncommon form of aspergillosis, occurring in both immunocompetent and immunosuppressed humans, as well as in canines and felines. Most cases have been described based on clinical and histopathological features and the etiologic agent has been infrequently identified to the species level (e.g. 18). A recent study of the etiology of feline SOA in Australia determined that *Neosartorya* species were the cause of all 17 cases documented (19). Although sequencing of the internal transcribed spacer regions was insufficient to identify the isolates to the species level, the high % similarity, the production of ascospores in some mating studies, and the frequent treatment failure suggests that *N. udagawae* was the main
species involved. *N. udagawae* differs from *N. lacinosa* in being heterothallic, in failing to grow above 42°C and in being refractory to antifungal therapy (10, 12, 13, 19).

Our patient was successfully treated with voriconazole and micafungin together with surgical debridement. Antifungal susceptibility testing done retrospectively on the case isolate showed that *N. laciniosa* is similar to *N. hiratsukae* in showing low MICs to amphotericin, itraconazole, voriconazole and caspofungin (1). In contrast, both *N. udagawae* and *N. pseudofischeri* show high MICs to voriconazole and itraconazole (1, 10). It is therefore clinically important to not only differentiate *Neosartorya* isolates from the related *Aspergillus* within the section *Fumigati*, but also to identify *Neosartorya* isolates to the species level.

This case confirms that *Neosartorya* species should be suspected when a white thermotolerant *Aspergillus* with sparse conidiation is isolated and that identification to the species level can be obtained by sequencing of partial *benA* and calmodulin genes. Accurate identification is necessary to elucidate clinical or therapeutic differences between *Neosartorya* species.

**Nucleotide sequence accession numbers.** Sequences for *N. laciniosa* UAMH 11627 were deposited in GenBank under accession no. JX845619 for the partial calmodulin gene and JX845620 for the partial *benA* gene.

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**References**


Legend to figures

FIG 1. Panel A, Gomorri methanamine silver stained section of sinus mucosal tissue showing numerous branched, septate hyphae (Bar = 20 µm); Panel B, colony on OAT showing ascomata after 7 days growth at 35°C; Panel C, ascospores in face and side view showing two crests (Bar = 5 µm).

FIG 2. One of 19 equally parsimonious trees (CI 0.891, RI 0.867, HI 0.109) inferred from maximum parsimony analysis of combined partial beta-tubulin and calmodulin gene sequences showing the placement of the case isolate. Bootstrap values above 50% are shown. For each isolate, GenBank accession numbers and culture collection number are listed. Culture collection acronyms are CBS – Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; IBT – Culture Collection of Fungi, Technical University of Denmark, Lyngby, Denmark; KACC – Korean Agricultural Culture Collection, Suwon, South Korea; NRRL – USDA Agricultural Research Service Culture Collection, Peoria, IL; SUM – Medical Mycology Research Center, Chiba University, Chiba, Japan; UAMH – University of Alberta Microfungus Collection and Herbarium, Edmonton, Canada.