

Successful Treatment of Disseminated *Anncaliia algerae* Microsporidial Infection With Combination Fumagillin and Albendazole

Mélissa Boileau,¹ José Ferreira,² Imran Ahmad,¹ Christian Lavallée,³ Yvonne Qvarnstrom,⁴ and Simon F. Dufresne³

Departments of ¹Hematology-Oncology, ²Pathology, and ³Infectious Diseases and Medical Microbiology, Hôpital Maisonneuve-Rosemont, Université de Montréal, Canada; and ⁴Division of Parasitic Diseases and Malaria, Centers for Disease Control and Prevention, Atlanta, Georgia

Anncaliia algerae myositis is a life-threatening, emerging microsporidiosis among immunocompromised hosts. We report a case of disseminated *A algerae* infection in a man previously treated with alemtuzumab. Due to failure of albendazole-based therapy, fumagillin was added as a novel approach to management, with a good clinical response and patient survival.

Keywords. *Anncaliia algerae*; fumagillin; immunocompromised; microsporidiosis; myositis.

A 49-year-old-man was referred to our center for refractory chronic lymphocytic leukemia (CLL) with TP53 deletion. He was treated for concurrent diabetes mellitus. Over the past years, he had received several chemotherapy regimens including chlorambucil, fludarabine, cyclophosphamide, and rituximab. He was treated with alemtuzumab and high-dose dexamethasone for 2 months. This regimen also failed; hence, ibrutinib was initiated as a bridge to allogeneic hematopoietic stem cell transplantation. Trimethoprim-sulfamethoxazole (TMP-SMX), posaconazole, and valacyclovir were given as primary prophylaxis from the introduction of alemtuzumab onwards.

One month after ibrutinib initiation, the patient was admitted at our hospital for febrile neutropenia. No infection was identified upon initial investigation. However, the patient complained of upper and lower limb pain that had started 2 months earlier. Creatine kinase (CK) was elevated over 3 times the

upper limit of normal value (625 U/L). A lower extremity electromyogram was done and suggested diffuse acute myopathy. Magnetic resonance imaging (MRI) of the 4 limbs identified stigmata of polymyositis involving deltoids, triceps, and quadriceps. Myocardial involvement was also suspected because of elevated high-sensitivity cardiac troponin T (hs-cTnT). However, initial electrocardiogram and transthoracic echocardiogram were both normal. A biopsy of the vastus lateralis was performed, and light microscopy demonstrated a necrotizing myositis with numerous minute (2 micrometers), poorly stained intramuscular microorganisms. Immunohistochemistry with anti-*Toxoplasma gondii* antibodies was negative, as was *T gondii* polymerase chain reaction (PCR) on whole blood. Electron microscopy was consistent with microsporidial myositis [1, 2]. A fresh frozen muscle tissue sample was sent to the Centers for Disease Control Prevention ([CDC] Atlanta, GA), where species-level identification was performed by PCR and sequence analysis, which revealed *Anncaliia algerae*. Primers specific to that species (NALGF1-TCA CCA GAG CCT ATG TGC AGG; NALGR2- CTT CAT AAA AAC ATC CAT CTC) were used and amplified from 405-base pair segment of the small ribosomal subunit ribonucleic acid gene, which showed 100% identity with previous GenBank entries (accession numbers AY963290 and AF024656). Because toxoplasmosis was initially suspected, cerebral MRI was performed and revealed multiple parenchymal lesions with fine contrast enhancement and slight oedema. The *A algerae*-specific PCR was performed on the patient's cerebrospinal fluid (CSF) at the CDC and was positive. Urine and stools were negative for microsporidia by light microscopy (modified trichrome stain).

The patient was initially treated with a combination of albendazole (400 mg twice daily), pyrimethamine (50 mg once daily), and TMP-SMX (160–800 mg twice daily). Access to sulfadiazine was denied by Health Canada for this indication. After a few days without fever and diminished myalgia, the patient was discharged from the hospital with presumed effective therapy. The clinical and biological response to therapy is shown in Figure 1.

After 25 days of the initial regimen, the patient was readmitted because of recurring fever and myalgia, with peripheral oedema and progressive weakness involving all limbs (Medical Research Council Scale for Muscle Strength grade 4 proximally and grade 3 distally). Levels of CK (>1000 U/L) and hs-cTnT (>1500 ng/L) had risen significantly. Worsened signs of polymyositis were observed on control MRI while a second muscular biopsy showed a higher parasitic burden. A second cardiac MRI was still normal. Because fumagillin is known to be effective against certain microsporidia species [3], a 2-week trial

Received 27 April 2016; accepted 19 July 2016.

Correspondence: S. F. Dufresne, Department of Infectious Diseases and Medical Microbiology, Hôpital Maisonneuve-Rosemont, 5415 Boul. de l'Assomption Montréal, QC H1T 2M4, Canada (sf.dufresne@umontreal.ca).

Open Forum Infectious Diseases®

© The Author 2016. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com. DOI: 10.1093/ofid/ofw158

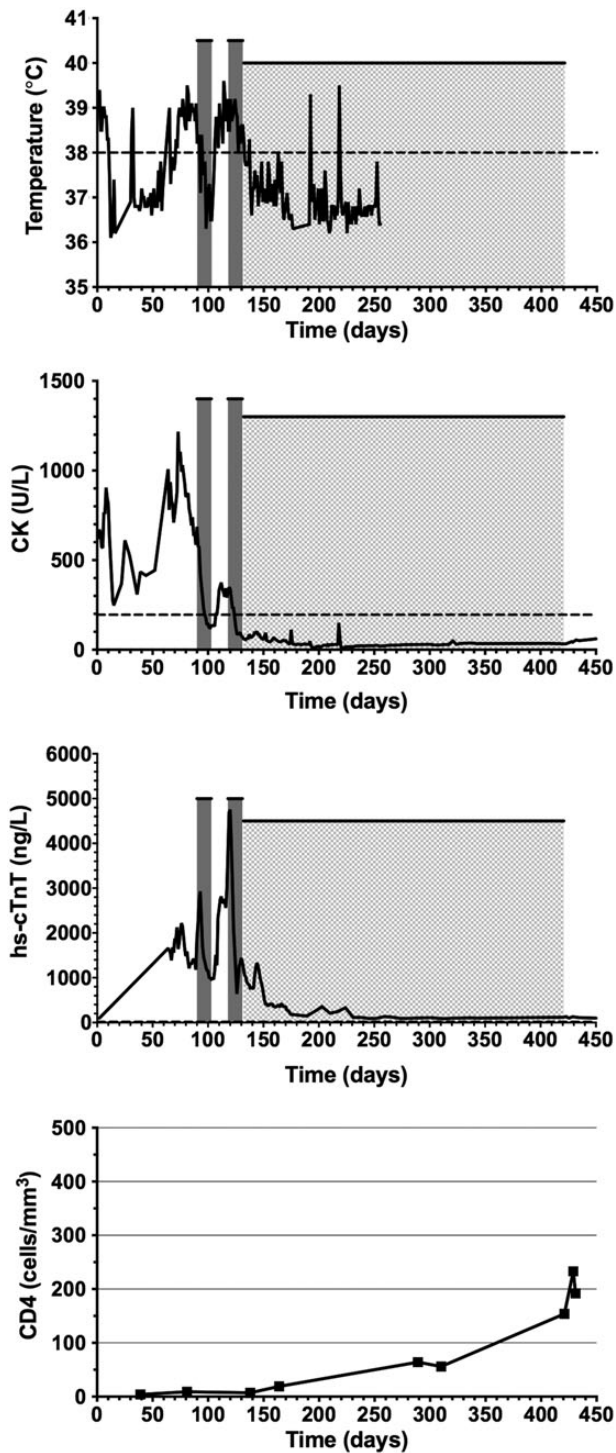


Figure 1. Clinical and biological response to therapy and CD4 cell count. Creatine kinase (CK), high-sensitivity cardiac troponin T (hs-cTnT), body temperature (highest measure of each day, any body site) and CD4 cell count are shown according to time after diagnosis. Dashed lines represent the upper normal limit value for each parameter. Fumagillin treatment (20 mg 3 times daily) and maintenance (20 mg once daily) therapy periods are shown in full dark gray and checkered light gray, respectively. Albendazole was given concomitantly with fumagillin.

was attempted at full therapeutic dose (20 mg 3 times daily). Albendazole (400 mg twice daily) was maintained whereas

pyrimethamine was discontinued. Prophylactic TMP-SMX was continued (80–400 mg once daily). Upon fumagillin initiation, significant improvement occurred: fever ceased, and the patient’s muscular endurance and balance improved. Prompt CK normalization and a 60% reduction in hs-cTnT were also observed.

Unfortunately, after a week of fumagillin discontinuation, fever recurred, muscular strength declined, and CK and hs-cTnT bounced back. In addition, the patient developed symptomatic congestive heart failure, and a cardiac MRI confirmed moderate biventricular dysfunction with a reduced left ventricular ejection fraction (LVEF) of 30%. A second 2-week trial of full dose fumagillin was initiated and again led to significant clinical and biological improvement. At that time, CD4⁺ T lymphocyte count was still extremely low ($19 \times 10^9/L$); therefore, we decided to provide reduced-intensity fumagillin as maintenance therapy (20 mg twice daily for 4 days, then 20 mg once daily) until immune recovery. Hospitalization was complicated by central venous catheter infection (*Pseudomonas aeruginosa*) and nosocomial pneumonia that accounted for 2 additional episodes of fever (Figure 1).

Fumagillin and albendazole maintenance therapy was stopped after 290 days, because the patient’s condition had improved significantly and the CD4⁺ T lymphocyte count had reached 200. Before fumagillin introduction, the patient was bedridden because of severe generalized myopathy; by the end of therapy, he was in a rehabilitation center and able to walk over 30 meters. A lower limb MRI confirmed an almost complete resolution of polymyositis stigmata. Creatine kinase normalized and hs-cTnT improved for approximately 8 months and stabilized approximately 90 ng/L. The LVEF remained at approximately 30%. Follow-up cerebral MRIs showed reduction in parenchymal lesions, with minimal residual abnormalities consistent with scars. To this date, 6 months after therapy was ended, the patient has shown no sign of relapse.

DISCUSSION

The microsporidia *A algerae*, formerly called *Nosema* and *Brachiola*, was long known as an insect parasite, but it is now recognized as an emerging human pathogen. *Anncaliia algerae* has been reported to cause severe myositis in 5 patients under immunosuppressive treatment for rheumatoid arthritis and solid-organ transplantation [2, 4, 5]. Acquisition mode is not known, but the current lead hypothesis is that the infection could be transmitted through contact with spore-contaminated water inhabited by infected mosquitoes [6]. The optimal therapy of this infection is yet to be determined, and *A algerae* myositis was fatal in all but 1 case [2].

We described a case in a man heavily immunosuppressed due to treatment for CLL. The patient was from a rural area of Québec, Canada, and reported living in an area where bodies of

water and mosquitoes are abundant. Of note, this is the first case of *A. algerae* infection where central nervous system (CNS) and cardiac involvement could be substantiated along with skeletal muscles, although it was not proven by histopathology. Central nervous system infection was supported by positive PCR on CSF and cerebral MRI lesions, whereas cardiac infection was suggested by progressive congestive heart failure, cardiac MRI abnormalities, and high hs-cTnT, all of which subsided with antiparasitic therapy. It is interesting to note that both cardiac and CNS disorders had been suspected to be associated with *A. algerae* infection in previous cases [2]. Immunosuppression of our patient was multifactorial, but it was mainly driven by a profound alemtuzumab-induced CD4⁺ T-cell lymphopenia. Unfortunately, this effect is not readily reversible, and yet reducing immunosuppression was thought to be a key factor in the management of the only surviving patient with *A. algerae* microsporidial myositis [2]. Therefore, it was paramount to optimize antimicrobial therapy.

Albendazole is an antitubulin polymerization drug whose efficacy against *A. algerae* is supported by genomic, in vitro, and clinical data. *Anncaliia algerae* β -tubulin gene sequence exhibits conserved regions associated with sensitivity to this drug among other microsporidia [7]. In cell culture, albendazole can attenuate *A. algerae* infection and inhibit new spore production [7]. Moreover, the survival of the case described by Watts et al [2] may have been attributable to this drug. However, in other cases and this one, albendazole appeared to slow disease progression, but it seemed insufficient to control the infection.

Fumagillin, an antibiotic derived from *Aspergillus fumigatus*, has traditionally been used to treat microsporidiosis in honeybees [8]. In vitro and animal studies suggest that fumagillin and its derivative (TNP-470, ovalicin) are active against several microsporidia [9–11], probably through their irreversible inhibition of methionine aminopeptidase type 2 (MetAP2) [12]. In a randomized placebo-control trial, fumagillin was identified as an effective treatment for *Enterocytozoon bieneusi* in immunocompromised patients [3]. Direct data supporting fumagillin activity against *A. algerae* is lacking, although this species was shown to harbor a MetAP2 gene homologous to that of various other microsporidia [13]. Based on these previous reports and the failure of initial therapy in our patient, we decided to attempt fumagillin, which was obtained from Sanofi (Paris, France) through Health Canada Special Access Program. Manifest improvement was observed, albeit a 14-day course was not long enough to control the disease. A second intensive phase followed by maintenance therapy until immune recovery and disease resolution was successful in this case. It is unlikely that immune reconstitution contributed significantly to control of infection during intensive fumagillin courses, because CD4 cell count was still extremely low at that point.

The chief undesirable effect of fumagillin is hematotoxicity, namely thrombocytopenia and granulocytopenia. To the best of our knowledge, this is the first patient treated with fumagillin for longer than 14 days. Our patient has received a cumulative dose of 7560 mg (378 tablets) over an 11-month period. While receiving a therapeutic dose (20 mg 3 times daily) and then a maintenance dose (20 mg one daily), the patient's lowest platelet count was $14 \times 10^9/L$ and $50 \times 10^9/L$, respectively. However, because the patient was treated with low molecular weight heparin for a recent pulmonary embolism, he received platelet transfusions to keep his platelet count above $50 \times 10^9/L$. No clinically significant bleeding occurred, and, more importantly, after the first therapeutic attempt, the patient's platelet count normalized spontaneously. Transient neutropenia was observed concomitantly to *P. aeruginosa* bacteremia. Aspartate transaminase and alanine transaminase increased up to twice the upper limit of normal value during treatment. Hematotoxicity remains an issue and requires careful follow up. For our patient, the benefit of fumagillin outweighed the risk, although we cannot accurately evaluate long-term adverse effects.

CONCLUSIONS

In immunocompromised hosts, *A. algerae* has emerged as a new human pathogen. The hallmark of systemic infection is diffuse myositis, which can be accompanied by myocardial and CNS involvement. This infection is characterized by a high fatality rate. This case indicates that fumagillin may be an effective addition to albendazole, particularly where there is significant host immunosuppression. A prolonged course may be necessary with careful surveillance of adverse events and meticulous weighing of risks and benefits.

Acknowledgments

We thank Jean-Michel Molina and Matthew R. Watts for their valuable advice on the clinical management of this case. We are also grateful to Dr. Watts for his critical review of this manuscript.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

References

1. Ramanan P, Pritt BS. Extraintestinal microsporidiosis. *J Clin Microbiol* **2014**; 52:3839–44.
2. Watts MR, Chan RC, Cheong EY, et al. *Anncaliia algerae* microsporidial myositis. *Emerg Infect Dis* **2014**; 20:185–91.
3. Molina JM, Tourneur M, Sarfati C, et al. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med* **2002**; 346:1963–9.
4. Coyle CM, Weiss LM, Rhodes LV III, et al. Fatal myositis due to the microsporidian *Brachiola algerae*, a mosquito pathogen. *N Engl J Med* **2004**; 351:42–7.
5. Field AS, Paik JY, Stark D, et al. Myositis due to the microsporidian *Anncaliia (Brachiola) algerae* in a lung transplant recipient. *Transpl Infect Dis* **2012**; 14:169–76.
6. Cali A, Weiss LM, Takvorian PM. A review of the development of two types of human skeletal muscle infections from microsporidia associated with pathology in invertebrates and cold-blooded vertebrates. *Folia Parasitol (Praha)* **2005**; 52:51–61.
7. Santiana M, Pau C, Takvorian PM, Cali A. Analysis of the beta-tubulin gene and morphological changes of the microsporidium *Anncaliia*

- algerae* both suggest albendazole sensitivity. J Eukaryot Microbiol **2015**; 62:60–8.
8. Katznelson H, Jamieson CA. Control of nosema disease of honeybees with fumagillin. Science **1952**; 115:70–1.
 9. Didier PJ, Phillips JN, Kuebler DJ, et al. Antimicrosporidial activities of fumagillin, TNP-470, ovalicin, and ovalicin derivatives in vitro and in vivo. Antimicrob Agents Chemother **2006**; 50:2146–55.
 10. Coyle C, Kent M, Tanowitz HB, et al. TNP-470 is an effective antimicrosporidial agent. J Infect Dis **1998**; 177:515–8.
 11. Beauvais B, Sarfati C, Challier S, Derouin F. In vitro model to assess effect of antimicrobial agents on *Encephalitozoon cuniculi*. Antimicrob Agents Chemother **1994**; 38:2440–8.
 12. Alvarado JJ, Nemkal A, Sauder JM, et al. Structure of a microsporidian methionine aminopeptidase type 2 complexed with fumagillin and TNP-470. Mol Biochem Parasitol **2009**; 168:158–67.
 13. Zhang H, Huang H, Cali A, et al. Investigations into microsporidian methionine aminopeptidase type 2: a therapeutic target for microsporidiosis. Folia Parasitol (Praha) **2005**; 52:182–92.