Microsporidiosis in patients with autoimmune diseases undergoing monoclonal antibody associated therapy

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ABSTRACT

We present *Enterocytozoon bieneusi* infection in four patients with autoimmune diseases undergoing prolonged monoclonal antibody therapies. Two patients suffered from inflammatory bowel disease and received anti-TNF therapies, whereas two other patients suffered from systemic lupus erythematosus with renal involvement and received anti-CD20 or anti-BLyS protein therapies. Three out of four patients consulted for diarrhea with abdominal pain without intestinal inflammation or bleeding at the time of sampling. The fourth patient did not declare intestinal troubles. Microsporidia genotype detected in this study were S9, C, Wildboard3 with one patient harboring 2 genotypes S6 and EBCMAP-038. Management of microsporidia infection included albendazole and reduction of immunosuppression treatment, but no specific treatment was implemented in two other patients. In conclusion, microsporidia infection occurs in patients with autoimmune diseases undergoing prolonged monoclonal antibody therapies. Diagnosis should be carefully assessed in this population and a thorough benefit-risk analysis is essential prior to initiating therapeutic interventions.

Short communication

Microsporidia are obligate intracellular fungi primarily transmitted through fecal-oral routes with sources of infection including contaminated water and food [1]. Three microsporidia species can induce intestinal diseases in humans, *Enterocytozoon bieneusi* and more rarely two species from the *Encephalitozoon* genus*, E. intestinalis* and *E. hellem*. The challenge of accurately identifying microsporidia through microscopy-based methods has resulted in many undetermined cases of microsporidia-associated diarrhea worldwide. The use of highly sensitivity molecular-based diagnostic methods has revealed that population susceptible to contracting microsporidiosis is highly varied [2]. Sporadic infections have been described in children, diabetic and elderly patients but highly immunocompromised individuals, HIV and solid organ transplant (SOT) patients, are the main at-risk population. To a lesser extent, microsporidiosis has been diagnosed in patients with hematologic malignancies and long terms treatment with corticoids [3, 4]. Patients suffering from auto-immune diseases (AID) and treated with monoclonal antibody (mAb) therapies such as tumor necrosis factor antagonist (anti-TNF) have been recently described as another category of at-risk patients [5, 6]. The proven benefits of mAb therapies have generalized their use in AID patients. However, in this emerging population, characterization of microsporidia genotypes to grasp the dynamics of microsporidia infections or clinical symptom management, remains unexplored.

To this aim, patients diagnosed with a microsporidia infection between 2019 and 2023 in the laboratory of Parasitology and Mycology of Saint Antoine Hospital, Paris, were retrospectively analyzed. Genomic DNA extraction from stool samples was performed with the QIAmp DNA Stool Mini Kit, according to the manufacturer's instruction. Microsporidia diagnosis was carried out using a molecular-based diagnostic method previously described [7]. Clinical data

and treatments were retrospectively collected from hospital data base. Genotype identification was performed by the French Microsporide Reference Center (https://cnrcryptosporidioses.chu-rouen.fr).

During a period of five years, a total of 20 cases of microsporidia were diagnosed in our institution, all due to *E. bieneusi*. Microsporidiosis was mainly detected in patients with a SOT (n=12; 60%). Human immunodeficiency virus (HIV) infected patients represented 20% (n=4) of the study population as well as patients suffering from an AID (n=4, 20%). In this retrospective study, we focused on AID patients. AID patients' characteristics are displayed in Table 1. They were two men and two women. The mean age was 36.5 years old (range: 24-49 years). Two patients were diagnosed with inflammatory bowel disease: one was diagnosed with Crohn's disease (CD) and the other was diagnosed with ulcerative colitis (UC). Two other patients suffered from systemic lupus erythematosus (SLE) with renal involvement. Three out of four patients consulted for diarrhea with abdominal pain without intestinal inflammation or bleeding at the time of sampling. The fourth patient did not declare intestinal troubles, but a stool analysis was done as part of the clinical assessment after being hospitalized due to a SLE flare**.** All enrolled patients had received one or several following drugs: corticotherapy (methylprednisolone or hydrocortisone), mycophenolate mofetil, azathioprine or 5 aminosalicylic acid combined with mAb therapies. Patients with inflammatory bowel diseases received anti-TNF therapies (Infliximab Adalimumab, Golilumab) whereas patients with SLE received anti-CD20 or anti-BLyS protein therapies (Rituximab or Belimumab) targeting B lymphocytes. Mean duration of immunosuppression treatment was 7.25 years (range: 3-10 years). At the time of the microsporidia diagnosis, lymphocytes count in patients ranged from 1200 to 2200 $CD4^+/mm^3$ with a mean of 1525 $CD4^+/mm^3$. Two patients had consumed raw

fish or seafood in the week leading up to the onset of intestinal symptoms. Microsporidia genotype detected were S9, C, Wildboard3 with one patient harboring 2 genotypes S6 et EBCMAP-038. Only in patient 3, a concomitant infection with a parasite, *Dibothriocephalus latus* was diagnosed, a parasite also associated with intestinal troubles. Albendazole treatment (400 mg twice per day for 4 days) was implemented in patient 1. In patient 2, resolution of intestinal symptoms was obtained after an adequate management of his UC flare, so specific anti-microsporidia treatment was not implemented. In patient 3, the treatment with mycophenolate mofetil was suspended for 6 weeks and renewed after verification that microsporidia infection was cleared. She also received praziquantel treatment to clear *D. latus* infection. In patient 4, microsporidia carriage was not considered for clinical symptom management of SLE and later analysis were negative. All patients had a favorable evolution.

The treatment options for AID such as SLE, UC and CD have substantially improved in recent years thanks to the introduction of mAb therapies. However, serious fungal infections in patients receiving anti-TNF treatments have been described requiring the implementation of antifungal treatment [8]. Microsporidia infection in patients treated with anti-TNF treatments has been poorly evaluated but a previous study reports a prevalence of 17% [6], whereas microsporidiosis in patients receiving therapies targeting B lymphocytes has not been described yet. In the present report, we illustrate 4 cases of *E. bieneusi* infection in patients with AID and undergoing mAb treatment for more than 3 years confirming that microsporidiosis can occurs after long-term treatment with these immunomodulators [6]. We have detected five genotypes with one patient bearing two different genotypes. All genotypes belonged to the phylogenetic group 1, which predominantly contains genotypes with low host

specificity and a high potential for interspecies transmission [1]. Genotype C and Wildboar3 are common in France and the genotype C has been mainly found in HIV-negative transplant recipients [9]. On the contrary S6, EBCMAP-038, which is closely related to S6, and S9 genotypes have been less frequently described. Although S9, detected in patient 1 suffering with CD, has been previously isolated in a patient with ulcerative colitis [10], our study reveals that our AID population is not preferentially infected by a specific genotype.

The impact of *E. bieneusi* infection on AID progression also remains poorly understood, largely due to the scarcity of suitable animal models for experimentation and the lack of epidemiological studies. Nonetheless, certain microsporidia species, such as *E. cuniculi*, have been associated to inflammatory pathways [11]. Should this extend to individuals with AID like inflammatory bowel diseases, it is conceivable that the clinical manifestations could exacerbate with the presence of *E. bieneusi* infection. Consequently, microsporidia diagnosis using molecular methods should be considered when AID flares occur in patients undergoing mAb therapies.

It is crucial to underscore that microsporidiosis treatment in this polytreated population carries significant implications. Fumagillin is the recommended treatment for *E. bieneusi* infection, but severe secondary effects have been described, requiring patient hospitalization for monitoring. In countries where fumagillin is not available reducing immunosuppression to enable recovery of the necessary immunity to eliminate the infection is one option to achieve medical cure in SOT patients [12]. However, reducing immunosuppression can potentially result in organ rejection in transplant recipients, as well as trigger flare-ups in AID patients. Our research indicates that this reducing immunosuppression approach could effectively clear the infection in patient 3, demonstrating its potential utility in treating AID patients.

Albendazole was also proposed in the study to control microsporidial infection in patient 1. Albendazole is known to alleviate diarrhea in *E. bieneusi* infected patients but without clearing the infection. This implies that managing CD flare-ups in this patient could probably play a role in clearing the infection. This hypothesis is reinforced by the observation that microsporidiosis was also successfully eliminated in two other patients (patients 2 and 4) without undergoing any targeted anti-microsporidia treatment. The use of highly sensitive molecular methods has the advantage of detecting very low levels of parasites. However, a disadvantage is the inability of this methods to differentiate between infection-associated-symptoms and colonization. Therefore, this retrospective analysis highlights the possibility of resolving microsporidiosis infection during the management of AID flare-ups, emphasizing the need to prudently weigh the benefits and risks when considering specific treatments for AID patients and to follow-up of infection evolution using molecular methods.

The immunological mechanisms implicated in *E. bieneusi* infection control are unknown due to the lack of experimental animal models. However, the prevalence of this fungus in HIV patients with low $CD4^+$ T lymphocyte count strongly indicates the significant role played by this arm of the immune system in preventing the infection. Microsporidiosis in patients with CD has also been associated with δγT lymphocytes and IL-7 deficiencies, suggesting their potential etiological role in the microsporidia control [13]. Finally, the diagnosis of microsporidiosis in individuals undergoing long-term anti-TNF therapies confirmed in this study or in patients with B cell depletion, supports to the hypothesis that low levels of TNF, cytokine well known to be implicated in the trigger of a TH1 response, or reduction B lymphocytes may also play a role in this condition.

In conclusion, this study confirms microsporidia infection in AID patients with associated mAb therapies and illustrates the genotypes implicated. We also provide valuable guidance to clinicians in the diagnosis and management of microsporidiosis. Because clinical symptoms may be mild or due to the underlying disease and because of potential side effects of treatments, the initiation of a specific treatment should include a benefit-risk analysis. Studies involving a significant number of patients are necessary to ascertain whether this fungal infection can contribute to the etiology of an AID flare, and consequently, to suggest appropriate management.

NOTES

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Contribution: RK, CB, KL, LP, MM, DM, YS, JB and AMS analyzed clinical isolates and the associated medical records or participated to the clinical management of patients. CN, PP, analyzed microsporidia genotypes. RK, CH and AMS participated to the conceptualization, data curation, formal analysis, investigation, and methodology. RK, CH and AMS reviewed and edited the first version of the manuscript. Authors approved the final version before submission.

Table 1. *E. bieneusi* infected AID patients' characteristics and clinical management

Patient Nb	Age (years)	Gender	AID	IS treatment*	$CD4^+/mm^3$	Duration treatment (years)	Clinical symptom (time to diagnosis)	Transmission route	Genotype	Other infections	Microsporidia treatment
1	49	Male	CD	Infliximab Azathioprine	2200	10	Subacute diarrhea with abdominal pain without digestive inflammation (ND)	NA	S9	Non	Albendazole
2	39	Male	UC	Golilumab 5-SAS	1400	9	Watery diarrhea. No rectal bleeding (3 days)	Raw fish	C	Non	No treatment
3	34	Female	SLE	Prednisone Belimumab Mycophenolate mofetil	1200	3	Non-bloody non-inflammatory diarrhea (2 weeks)	seafood	Wildboard 3	*D. latus*	IS treatment reduction
4	21	Female	SLE	Corticotherapy Azathioprine Rituximab	1300	7	Non intestinal symptoms (ND)	NA	S6 and EBCMAP-038	Non	No treatment

AID: Auto-immune disease; CD: Crohn's Disease; UC: Ulcerative colitis; SLE: Systemic lupus erythematosus with renal involvement; IS: Immune suppressive treatment at the time of microsporidia diagnosis; 5-SAS: 5 aminosalicylic acid. *D. latus: Dibothriocephalus latus*; ND: no data.

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