Microsporidiosis: Epidemiology, clinical data and therapy

La microsporidiose : épidémiologie, manifestations cliniques et prise en charge thérapeutique

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Summary Microsporidiosis is an emerging and opportunistic infection in AIDS patients, organ transplant recipients, children, travelers, contact lens wearers and the elderly. It is associated with a wide range of clinical syndromes of microsporidiosis in humans. The disease is caused by microsporidia, obligate intracellular microorganisms that were recently reclassified from protozoa to fungi. The 14 species of microsporidia currently known to infect humans, Enterocytozoon bieneusi and Encephalitozoon intestinalis, are the most common causes of human infections and are associated with diarrhea and systemic disease. Species of microsporidia infecting humans have been identified in water sources as well as in wild, domestic and food-producing farm animals, raising concerns of water-borne, food-borne and zoonotic transmission. Various molecules have been tested for treating microsporidiosis in humans with variable success. Albendazole is effective against Encephalitozoon species such as Encephalitozoon intestinalis but not against Enterocytozoon bieneusi. This species has shown excellent clinical therapeutic response to direct action with fumagillin, but this drug is toxic when administered systemically to mammals. Its analog, TNP 470, could be promising alternative. Further work is necessary to identify other drugs, which are both effective and devoid of adverse effects.

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Résumé La microsporidiose est une infection opportuniste émergente chez les personnes atteintes du sida, les greffés d’organes, les enfants, les voyageurs, les porteurs de lentilles de contact et les personnes âgées. Elle est caractérisée par un spectre clinique varié chez l’homme. Elle est causée par des microsporidies, des microorganismes intracellulaires obligatoires, récemment classées parmi les champignons. Actuellement, 14 espèces sont incriminées en pathologie humaine dont Enterocytozoon bieneusi et Encephalitozoon intestinalis sont les espèces les plus fréquentes. Elles sont associées surtout à des manifestations intestinales ou disséminées. Les espèces de microsporidies infectant l’homme ont été identifiées aussi bien

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Introduction

Microsporidiosis is a well-known parasitic disease in animals and in the last two decades has become an increasingly common pathology in humans due to the growing number of persons with immunodepressive states [1]. Microsporidia are eukaryotic organisms currently classified as fungi [2]. There are 150 genera and more than 1300 species. Eight genera have been described in human hosts: Enterocytozoon, Encephalitozoon, Pleistophora, Trachipleistophora, Vittaforma, Brachiola, Nosema and Microsporidium. Fourteen species are implicated in human pathology, including Enterocytozoon bieneusi and Encephalitozoon intestinalis [3—5].

Considering the multiple and frequently severe manifestations of microsporidiosis, several groups have attempted to develop an effective treatment [5—8]. The purpose of this article is to examine the clinical manifestations of microsporidiosis and clarify the current status of drugs proposed for its treatment.

Pathophysiology

Little is known about the pathophysiology of microsporidiosis [3]. Microsporidia can infect any tissue, although some species exhibit adaptation to individual cell types [7,8]. Species with a tropism for the gastrointestinal tract, Enterocytozoon bieneusi and Encephalitozoon intestinalis, colonize the epithelium of the small bowel. Enterocytozoon bieneusi is found preferentially in the apical part of the villosities while Encephalitozoon intestinalis infects not only the villosities but also the cryptic cells, invading macrophages, fibroblasts and endothelial cells of the chorion [3,4,8]. An alteration of the intestinal mucosa can be seen as a flattened epithelium with accumulation of fat and significant desquamation, with the subsequent atrophy of the villosities and the brush border and the compensatory elongation and hyperplasia of the crypts reducing the absorption surface area up to 40%. Lymphoid exocytosis with edema, vesiculation and enterocyte necrosis can be observed [3—5,7,9—11]. Encephalitozoon intestinalis can induce severe ulceration of the small bowel associated with mucosal atrophy, acute and chronic inflammation and zones of submucosal macrophage infiltration [3,7]. Dissemination of Encephalitozoon intestinalis infection throughout the organism induces inflammatory reactions in infected organs such as the liver, the pancreas, the lungs and the kidneys [9].

The functional impairment results from decline in the enzymatic activities (alkaline phosphatase and the α-glucosidase found in the basal portion of the villosities) [4,12], malabsorption of lipids, deficiencies in potassium, magnesium, vitamin B12, and α-xylose and a fall in serum bicarbonates [9,12,13].

Histopathology

Excepting ocular lesions, detailed descriptions of histological lesions due to microsporidiosis have only been reported in immunodepressed subjects. In this context, the infected tissue generally exhibits a minimal inflammatory tissue reaction, but with variable expression from normal tissue architecture to severe degenerative lesions of the epithelium [3,4]. Bile duct infections can be associated with papillary stenosis (secondary to the inflammatory reaction), bile duct dilatation, alithiasic cholecystitis and sclerosing cholangitis [3,4]. Kidney lesions are seen as a tubulointerstitial granulomatous nephritis with an inflammatory infiltration composed of macrophages, lymphocytes, plasma cells and langhans type multinucleated giant cells [3]. Infection of the ureters can lead to a lymphoplasmocyte and granulomatous inflammatory reaction [3]. In the bladder, the lesions produce an ulcerated cystitis with lymphohistiocyte infiltration [3]. Hepatic infections with Encephalitozoon spp. can cause a granulomatous necrosis with the presence of microsporidia carried in vacuoles disseminated within the hepatic parenchyma or a non-granulomatous inflammatory reaction [3,4]. Muscle infections with Pleistophora spp. lead to muscle atrophy and diffuse degenerative lesions with numerous microsporidia spores infiltrating the muscle fibers [3,4]. Ocular infections can cause deep inflammatory keratitis with polymorphous multinuclear cell, histiocyte and neutrophil polymorphonuclear cell infiltration and zones of necrosis associated with thickening of the cornea [17,18]. This reaction is however generally moderate or even absent in the event of superficial keratoconjunctivitis where the inflammatory infiltration is made up of neutrophil polymorphonuclears and mononuclears [3].
The immune response

Cell-mediated immunity dominates the defense against microsporida, associated with a humoral immune response [19].

The importance of cellular immunity has been demonstrated in particular in AIDS patients with a low CD4 T-cell count and with animal models of lethal infections in CD4 and CD8 T-cell depleted animals [1]. Among the mediators of cellular response, CD8 T-cells play an essential role in the organism’s defense against microsporida [20]. CD8 T-cells participate in the pro-inflammatory response via the production of cytokines such as gamma interferon (INFγ) and via their direct cytotoxic effect [21]. They also contribute to the regulation of the immune response by secreting interleukin-10 (IL10) [3, 5, 22]. A rapid rise in the level of circulating CD8 lymphocytes has been described after oral administration of Enterocytozoon cuniculi spores in the mouse [5].

Recent studies have demonstrated the importance of pro-inflammatory cytokines such as INFγ, tumoral necrosis factor α (TNFα) and interleukin-12 (IL12) in resistance against Enterocytozoon infections [21—23]. In addition, knock-out mice for genes coding for INFγ or its receptor lose their resistance against microsporidia infections and become infected with Enterocytozoon intestinalis [22].

The importance of humoral immunity in the organism’s defense against microsporidia has been demonstrated in animal models. The role of antibodies was elucidated by using anti-exospore monoclonal antibodies in immunodepressed mice infected with Enterocytozoon cuniculi. These antibodies contributed to prolonged survival [19]. Cases of deficient humoral immunity reported in the literature [24] have provided further evidence in favor of a role for humoral immunity in the organism’s defense against microsporidia.

The immune response to microsporidiosis caused by Enterocytozoon bieneusi has not yet been studied because of the failure of long-term tissue cultures and the lack of a small animal model for this species. Spontaneous natural infections caused by Enterocytozoon bieneusi have been reported in two strains of monkeys, which are at the present time the only animal models capable of simulating microsporidiosis infections in both immunocompetent and immunodepressed individuals [5].

Epidemiological data

Prevalence and geographic distribution

Microsporidiosis is a cosmopolite disease. Data on prevalence have varied greatly (Table 1) depending on the geographical region, the population studied and the diagnostic methods used [4, 5, 25—28].

Early in the 1980s, before the AIDS epidemic, microsporidiosis was rarely described in humans [1, 4, 25, 29]. The prevalence was determined on the basis of serology data for anti-Enterocytozoon cuniculi antibodies, the only species isolated in mammals. Worldwide, seroprevalence varies from 0 to 42%. The highest seroprevalences were reported from Sweden among homosexuals and in a population presenting other parasitic diseases, explaining the frequency of cross-reactions with microsporidia [1, 4, 25, 30].

The expansion of the AIDS pandemic led to the identification of several new microsporidia species. Most cases of clinical microsporidiosis are caused by two species, Enterocytozoon bieneusi and Enterocytozoon intestinalis.

Since 1990, the prevalence of microsporidia infections in AIDS patients has been estimated to vary from 1.5% to 50%, depending on the geographic region concerned and the diagnostic methods applied [1, 5, 25] (Table 1). Regarding the evaluations reported by these different studies, Bryan and Shwartz (1999) estimated the overall prevalence of microsporidiosis in AIDS patients to be about 15% [1, 25, 31].

HIV-infected patients account for the majority of cases of microsporidiosis, especially in Southeast Asia (India, Thailand), the Middle-East (Turkey), Europe, Africa (Tunisia, Mali, Ouganda, Senegal, Zimbabwe) and Latin America (Brazil, Peru) [4, 5, 9, 32—49].

Since the administration of antiretroviral therapy has enabled HIV-infected patients to restore their immune status, the number of cases of microsporidia infections has dropped off dramatically in developed countries [9, 29, 32—34]. In sub-Sahara Africa, however, where two-thirds of people with HIV infection reside, tritherapy is not widely available and by consequence microsporidiosis continues to be a cause of high morbidity and mortality [32—34].

In subjects free of HIV infection, e.g. blood donors, pregnant women, slaughter house employees, or subjects with diarrhea of undetermined cause, the seroprevalence of microsporidia is estimated at 1.3 to 22% [1, 25]. Similar prevalences in HIV-negative subjects have been reported using microscope studies and polymerase chain reaction (PCR) techniques in travelers to endemic zones (3.3 to 10%), children with diarrhea or not (1.7% to 17.4%) and elderly persons (17.2%) [1, 24, 25, 36].

Risk factors

Immunodepression

Depression of cell-mediated immunity, as occurs in HIV infection, is the main risk factor of human microsporidiosis [50, 51]. Microsporidiosis occurs at an advanced stage of immunodepression for lymphocyte CD4 counts <100 cells/mm³ [29, 38, 43, 45, 48, 50, 51].

Treatment-induced immunodepression in subjects with malignant homeopathies, organ transplant recipients and bone marrow graft recipients is another risk factor for microsporidiosis [15, 29, 52—55].

Other factors

Microsporidiosis occurs increasingly in non-HIV-infected populations, including immunocompetent travelers sojourning in a tropical zone, persons wearing contact lenses, children, elderly subjects due to increased frailty and undernourished people [1, 5, 9, 25, 36, 56—58].

Modes of transmission and reservoirs

Sporos are the infesting form of microsporidia. The resistance of these spores results from their protective walls formed of proteins and chitin. Microsporidia spores have a very particular content (Fig. 1); a fine polar filament is coiled up in the cytoplasm and fixed to the anterior end of the...
<table>
<thead>
<tr>
<th>Authors (year)</th>
<th>Country</th>
<th>Number of cases</th>
<th>Population studied</th>
<th>Prevalence</th>
<th>Diagnostic method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michiels et al. (1992)</td>
<td>France</td>
<td>207</td>
<td>HIV (+)</td>
<td>3.38%</td>
<td>Electron microscopy</td>
</tr>
<tr>
<td>Aoun et al. (1997)</td>
<td>Tunisia</td>
<td>12</td>
<td>HIV (−)(^a)</td>
<td>16.7%</td>
<td>Uvitex 2B + Weber’s trichrome</td>
</tr>
<tr>
<td>Liguory O. et al. (1996)</td>
<td>France</td>
<td>100</td>
<td>HIV (+)</td>
<td>39%</td>
<td>PCR</td>
</tr>
<tr>
<td>Billaud et al. (1997)</td>
<td>France</td>
<td>682</td>
<td>HIV (+)</td>
<td>2.9%</td>
<td>Uvitex 2B + Weber’s trichrome</td>
</tr>
<tr>
<td>Lebbad et al. (2001)</td>
<td>Guinea</td>
<td>52</td>
<td>HIV (+) and HIV (−)(^b)</td>
<td>HIV (+):11% HIV (−): 0%</td>
<td>Weber’s trichrome</td>
</tr>
<tr>
<td>Ferreira et al. (2001)</td>
<td>Portugal</td>
<td>215</td>
<td>HIV (+)</td>
<td>42.8%</td>
<td>Weber’s trichrome+ Calcofluor White M2R</td>
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<tr>
<td>Lores et al. (2002)</td>
<td>Spain</td>
<td>60</td>
<td>HIV (−)(^c)</td>
<td>17.02%</td>
<td>Weber’s trichrome</td>
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<tr>
<td>Bern et al. (2004)</td>
<td>Perou</td>
<td>2652</td>
<td>HIV (+)</td>
<td>3%</td>
<td>Weber’s trichrome</td>
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<tr>
<td>Konate et al. (2005)</td>
<td>Mali</td>
<td>70</td>
<td>HIV (+)</td>
<td>11.5%</td>
<td>PCR</td>
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<td>Lejeune et al. (2005)</td>
<td>Vietnam</td>
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<td>HIV (+)</td>
<td>1.38%</td>
<td>PCR</td>
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<tr>
<td>Endeshaw et al. (2006)</td>
<td>Ethiopia</td>
<td>243</td>
<td>HIV (+) and HIV (−)(^b)</td>
<td>HIV (+): 18.2% HIV (−): 0%</td>
<td>PCR</td>
</tr>
<tr>
<td>Chacin-Bonilla et al. (2006)</td>
<td>Venezuela</td>
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<td>HIV (+)</td>
<td>13.6%</td>
<td>Weber’s trichrome</td>
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<tr>
<td>Sarfati et al. (2006)</td>
<td>Cameroon</td>
<td>154</td>
<td>HIV (+)</td>
<td>5.2%</td>
<td>PCR</td>
</tr>
<tr>
<td>Dworkin et al. (2007)</td>
<td>USA</td>
<td>737</td>
<td>HIV (+)</td>
<td>1.5%</td>
<td>Chromotrope 2R+ Quick hot Gram chromotrope</td>
</tr>
<tr>
<td>Espern et al. (2007)</td>
<td>Niger</td>
<td>228</td>
<td>HIV (+)</td>
<td>10.5%</td>
<td>PCR</td>
</tr>
<tr>
<td>Nkinin et al. (2007)</td>
<td>Cameroon</td>
<td>191</td>
<td>HIV (+), HIV (−) and IC HIV (+) Tbc: 35.7% HIV (−) Tbc: 24% IC: 67.5% global: 58.1%</td>
<td>Calcofluor White M2R</td>
<td></td>
</tr>
<tr>
<td>Wumba et al. (2007)</td>
<td>Congo</td>
<td>50</td>
<td>HIV (+)</td>
<td>2%</td>
<td>Weber’s trichrome + Fungifluor</td>
</tr>
<tr>
<td>Samie et al. (2007)</td>
<td>South of Africa</td>
<td>322</td>
<td>HIV (+) and HIV (−)(^d)</td>
<td>11.2%</td>
<td>PCR</td>
</tr>
<tr>
<td>Norhayati et al. (2008)</td>
<td>Malaysia</td>
<td>893</td>
<td>HIV (+) and HIV (−)(^e)</td>
<td>13%</td>
<td>Gram chromotrope modified</td>
</tr>
<tr>
<td>Anane S. et al. (2009)</td>
<td>Tunisia</td>
<td>119</td>
<td>HIV (+) and HIV (−)(^f)</td>
<td>9.3%</td>
<td>PCR</td>
</tr>
</tbody>
</table>

\(^{a}\) Children with primary immune deficiency.
\(^{b}\) Immunocompetent subjects with diarrhea.
\(^{c}\) Elderly persons.
\(^{d}\) Immunocompetent children, patients hospitalized for diverse conditions.
\(^{e}\) Subjects with diverse conditions (diabetes, malignant hematological diseases), immunocompetent subjects.
\(^{f}\) Immunodepressed subjects.
spore [9,50,58]. In poorly defined circumstances, the polar filament rapidly ejects the spore contents (sporoplasm) into the host cell (Fig. 2). Once in the host, microsporidia proliferate according to species-dependent modes, but in all cases leading to the formation of highly resistant spores [50]. Sources of contamination and the mode of transmission of microsporidia remain imperfectly understood [3,5,39,49,50,59]. Potential reservoirs of microsporidia species that can infect humans include animals, water and humans (diseased individuals and healthy carriers). Considering the clinical symptoms of human microsporidiosis, the most likely mode of transmission would be oral, ocular or respiratory modes, which have been confirmed in vivo in different animal models (rabbits, mice, monkeys) [50].

Zoonotic transmission
To date, there is no formal proof of animal-to-human transmission of microsporidiosis, excepting one case of seroconversion in a 10-year-old child in close contact with a dog infected with *Encephalitozoon cuniculi* [60]. The search for animal reservoirs includes the detection of spores in excrements [4,9]. Spores of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* have been identified in pets and farm animals (dog, cat, pig, goat, donkey, cattle, rabbit). Spores of *Enterocytozoon bieneusi* have been detected in wild mammals (fox, otter, coypu or nutria, raccoon) and birds (industrial poultry, pigeons living in urban areas) [9]. For other species, *Encephalitozoon hellem* has been identified in birds, *Pleistophora* spp. have been isolated in fish and *Encephalitozoon cuniculi* is frequently found in rabbits and dogs [1,4,61]. Gene sequencing has shown evidence of the same strains in humans and animals, which would favor zoonotic transmission [1,9,25]. Furthermore, among the risk factors associated with microsporidiosis in HIV-infected patients, there is the notion of living in close contact with animals. This is another argument favoring the hypothesis of zoonotic transmission [1,9]. Spores are resistant in water and after desiccation, suggesting possible indirect zoonotic transmission via exposure to water, food or aerosols contaminated by spores [1,25,62].

Interhuman transmission
The possibility of interhuman transmission cannot be ruled out. Experimental examples of microsporidia transmission between laboratory animals might suggest that the same infecting species could be transmitted between humans via the same pathways as in animals [25,43]. It is known that microsporidia spores are eliminated into the environment via fecal matter, urine and respiratory secretions [4,25,50]. This would indicate that interhuman transmission would probably involve an oral-fecal pathway, inhalation of contaminated aerosols, or ingestion of contaminated food or water.

Water-borne transmission
Several arguments favor potential water-borne transmission of microsporidia. The spores of intestinal species have been detected in surface water and tertiary effluents of irrigation sources [1,4,9,25,50,61]. In addition, spores of *Enterocytozoon bieneusi* have been identified in fecal matter of fur-bearing animals in contact with surface water [1,4,25]. An increase in the incidence of microsporidiosis has also been reported in one population living near a water distribution system in France [63]. Factors favoring water-borne transmission are: elimination of spores in urine and fecal matter of infected animals and subsequent contamination of water sources; resistance of spores in the exterior environment; the low infecting doses of spores and their...
small size making filtering processes less efficient [1,4,25]. Microsporidiosis is also favored in zones without potable water or a sewage disposal system [43]. Microsporidiosis can be transmitted via swimming pools. Factors favoring this route of transmission include accidental contamination by fecal matter (incontinent subjects, subjects with gastroenteritis, elderly subjects, children), spore resistance to chloride, low infecting dose and high density of swimmers [25,64].

**Food-borne transmission**

Under certain conditions, microsporidia can also be food-borne. One epidemiology study found that eating insufficiently cooked beef at least once a month is associated with a risk of microsporidiosis in HIV-infected subjects [65]. Consumption of insufficiently cooked fish could also explain cases of human microsporidiosis due to *Pleistophora* spp., a species known to affect muscles in fish [25,66]. Food-borne transmission of microsporidia could be a consequence of irrigation water contamination. Spores of microsporidia have been identified not only in irrigation water but also in certain plants (lettuce, parsley, strawberries) [1,5,25].

**Trans-placental transmission**

Mother–infant transmission via the placenta has been described in rodents, rabbits and carnivorous animals but not in humans [1,4,25].

**Sexual transmission**

The data obtained in a Swedish study showed that 33% of homosexual subjects have anti-*Encephalitozoon cuniculi* antibodies, suggesting homosexual practices could contribute to the transmission of microsporidiosis [30]. The identification of microsporidia in the urethra and prostate of AIDS patients with urethritis or prostatic abscess is a further argument favoring the probability of sexual transmission [64,67,68].

**Table 2**

<table>
<thead>
<tr>
<th>Microsporidia</th>
<th>Clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterocytozoon bieneusi</em></td>
<td>Enteritis, cholangitis, cholecystitis, rhinitis, sinusitis, pneumonia, bronchitis</td>
</tr>
<tr>
<td><em>Encephalitozoon intestinalis</em></td>
<td>Enteritis, cholangitis, cholecystitis, peritonitis, nephritis, bronchitis, rhinitis, sinusitis, keratoconjunctivitis, disseminated infection</td>
</tr>
<tr>
<td><em>Encephalitozoon hellem</em></td>
<td>Keratoconjunctivitis, rhinitis, sinusitis, pneumonia, bronchitis, nephritis, urethritis, cystitis, prostatic abscess, urinary tract infection</td>
</tr>
<tr>
<td><em>Encephalitozoon cuniculi</em></td>
<td>Encephalitis, hepatitis, cholecystitis, enteritis, nephritis, rhinitis, sinusitis, keratoconjunctivitis, disseminated infection</td>
</tr>
<tr>
<td><em>Vittaforma cornea</em></td>
<td>Keratitis, nephritis</td>
</tr>
<tr>
<td><em>Pleistophora ronneaei</em></td>
<td>Myositis</td>
</tr>
<tr>
<td><em>Trachipleistophora hominis</em></td>
<td>Myositis, keratoconjunctivitis, sinusitis, rhinitis</td>
</tr>
<tr>
<td><em>Trachipleistophora anthropophthera</em></td>
<td>Keratitis, myositis, encephalitis, disseminated infection</td>
</tr>
<tr>
<td><em>Nosema ocularum</em></td>
<td>Keratoconjunctivitis</td>
</tr>
<tr>
<td><em>Brachiola connori</em></td>
<td>Disseminated infection</td>
</tr>
<tr>
<td><em>Brachiola vesicularum</em></td>
<td>Myositis</td>
</tr>
<tr>
<td><em>Brachiola algerae</em></td>
<td>Keratoconjunctivitis, keratitis, skin ulcer, myositis</td>
</tr>
<tr>
<td><em>Microsporidium africanum</em></td>
<td>Cornea ulcer</td>
</tr>
<tr>
<td><em>Microsporidium ceylonensis</em></td>
<td>Cornea ulcer</td>
</tr>
</tbody>
</table>

**Vectorial transmission**

Vectorial transmission has been examined since the identification of *Brachiola algerae* (a parasite known to infect mosquitoes) in human hosts [4,25,69]. Three other species of the *Encephalitozoon* genera have been isolated in the epithelial cells of the small bowel and salivary glands of two species of ticks, indicating that microsporidia spores can be transferred to mammalian hosts by blood sucking insects [1,25]. The arthropod vector responsible for this type of transmission has not yet been identified [1,4,25,50,66].

**Clinical study**

The broad spectrum of clinical manifestations is a hallmark of microsporidiosis. Clinical manifestations depend on the causal species, the site of infection and the immune status of the host [1,25] (Table 2). Although there are cases of ocular, naso-sinusal, bronchopulmonary, muscular, cerebral, genito-urinary and disseminated infections, gastrointestinal infections predominate [7,14,50].

**Gastrointestinal infections**

Gastrointestinal microsporidiosis is predominantly observed in HIV-infected subjects, especially those with advanced-stage cellular immunodepression with CD4 counts less than 100/mm³ [4,9]. *Enterocytozoon bieneusi* is the most common infecting species, in 90% the causal agent of chronic diarrhea in AIDS patients. *Encephalitozoon intestinalis* is less frequent [3,13,50,70]. *Encephalitozoon cuniculi* can occasionally cause intestinal microsporidiosis [50].

In the immunodepressed subject the cardinal sign of intestinal microsporidiosis is diarrhea [9,71]. Stools are watery, non-bloody and free of mucus [4,9,12,71]. Nevertheless, diarrhea may be absent. The presence of diarrhea is
directly correlated with the severity of cellular immunodepression as expressed by the low CD4 count. The diarrhea of microsporidiosis develops progressively over several months [4,29,31,50]. It is associated with nausea, loss of appetite, vomiting, abdominal pain, fever and progressive weight loss. In the most severe forms, dehydration leads to cachexia [4,7,9,12,13,29,71,72].

In the immunocompetent subject, symptoms are generally limited to spontaneously regressive diarrhea. It is predominantly observed in travelers returning from a tropical zone [5,9,13,56].

Asymptomatic microsporidiosis has been described in both immunodepressed and immunocompetent subjects. Search for microsporidiosis in stools must therefore be ordered systematically in immunodepressed subjects even without symptoms, not only because of the risk of developing overt symptoms, but also because of the important epidemiological risk of transmission [70].

**Biliary involvement**

Biliary microsporidiosis is rarely described. Most cases are caused by *Enteroctyozoon bieneusi* or *Encephalitozoon intestinalis*. These two species have been isolated from the bile track in HIV-infected subjects with non-lithiasic cholecystitis or cholangitis with or without sclerosis [3,4,73]. The clinical manifestations, in addition to the chronic diarrhea, include abdominal pain predominating in the right hypochondrium associated or not with jaundice [38]. Abdominal ultrasound, endoscopic ultrasound or endoscopic retrograde cholangiopancreatography disclose dilatation of the main bile duct and the intrahepatic ducts, irregularities of the bile duct walls and gallbladder anomalies such as thick walls, distension and sludge. Papillary stenosis can be observed [3,74]. Biological explorations may highlight a cholestasis. Serum alkaline phosphatases are generally elevated (two to three times normal); total bilirubin, aspartate aminotransferase and alanine aminotransferase are generally within the normal range [3].

**Hepatic, pancreatic and peritoneal involvement**

Hepatic, pancreatic and peritoneal involvement is exceptional in human microsporidiosis. Reported cases have been isolated, observed in HIV-infected subjects. Causal species are *Encephalitozoon intestinalis*, *Encephalitozoon cuniculi* and *Trachipleistophora antropophthera* [3,4,12].

One case of hepatitis and one of peritonitis caused by *Encephalitozoon cuniculi* have been reported in two HIV-infected patients. The diagnosis was confirmed at autopsy in both [75,76]. *Encephalitozoon* was the cause of fulminating hepatic failure in an AIDS patient with microsporidia diarrhea 2 months earlier. The diagnosis was confirmed at autopsy [77]. Microsporidia peritonitis was reported in another HIV-infected subject following intestinal perforation caused by untreated intestinal *Encephalitozoon intestinalis* infection [78]. An HIV-infected girl developed pancreatic and hepatic microsporidiosis within a context of disseminated *Trachipleistophora antropophthera* infection [79].

**Other localizations**

Before the AIDS pandemic, ocular microsporidiosis was considered rare. The first case was reported in 1973 by Ashton and Wirasinha [80]. At the present time, ocular involvement is the second most common localization in humans after gastrointestinal infections [4,70,81]. Ocular microsporidiosis produces keratoconjunctivitis or deep keratitis [12,17,18,25,51,57,82—89]. Other rare localizations described in humans are: nose and sinus [3,4,12], lung [3,4,12,15,16,90], muscles [3,4,7,12,13,91—93], brain [12,13,50,79,94—96], urine [4,50,81,94] and genital organs [64,67,68].

**Other exceptional manifestations**

Extremely rare cases of tongue ulcerations due to *Encephalitozoon cuniculi* have been reported. Bone (jaw) and skin (nodules) infections have been reported but the causal species was not mentioned [4,12,13,50].

**Disseminated forms**

Disseminated forms of microsporidiosis are rare but should be suspected in immunocompromised patients with multiple-organ failure [53]. Disseminated microsporidiosis has mainly been described in HIV-infected subjects with low lymphocyte counts (CD4 < 50/mm³) and transplant recipients [3,4,53—55,79,94—98]. Most of the incriminated species belong to the *Encephalitozoon* genera: *Encephalitozoon hellem*, *Encephalitozoon cuniculi* and *Encephalitozoon intestinalis* [3,4,95,97]. Other species, *Nosema connori*, *Vittaforma cornea*, *Trachipleistophora anthropophthera* and *Trachipleistophora hominis* have been described in rare cases of disseminated microsporidiosis [4,12,50,94].

The manifestations of disseminated microsporidiosis most commonly reported in the literature are, beyond the gastrointestinal involvement, kidney failure, fever, keratoconjunctivitis, sinusitis, and respiratory and neurological involvement [94]. Other localizations are possible: muscular, ocular, cerebral… [1,13,17].

Disseminated forms have a species-specific tropism. *Enteroctyozoon bieneusi* can disseminate to the hepatobiliary system leading to cholangitis, or to the lungs while *Encephalitozoon intestinalis* disseminates to all organs, but predominantly the kidney [4,50,94]. A urine study is thus indicated in all cases of suspected disseminated microsporidiosis.

**Diagnosis**

**Direct diagnosis**

**Samples**

The diagnosis of microsporidiosis is based on the demonstration of microsporidia spores in biological samples, generally stools, but also other samples (urine, duodenal aspiration, bronchoalveolar lavage fluid, cerebral spinal fluid, conjunctival swab, sputum...) collected according to the clinical expression [1,3—5,12,50]. For intestinal microsporidiosis, a
parasitological examination of the stools is not only readily accessible and repeatable, but also non invasive for the patient. Microsporidia spores are eliminated at intermittent intervals so stool tests should be repeated three times at three-day intervals [3,4,50].

Stainings

Because of the small size of the microsporidia spores, they cannot be identified in fresh samples without specific coloration. Several staining techniques can be used, applied to the swabs realized after concentration or centrifugation. Ritchie concentration is the most widely used method for stool samples. Weber’s trichromic stain and fluorochrome stains are the best staining techniques [1,3,4,12,59]. Weber’s trichromic stain is the most specific and the most widely used to identify microsporidia spores [1,3,4]. The spores appear as rose ovoid elements presenting a colorless posterior vacuole. In some cases stain uptake is stronger at the equator, corresponding to the polar filament (Fig. 3). The background is stained blue or green depending on the counter-stain used (Fast Green, malachite green or aniline blue) [1,4,7]. Fluorochromic stains are fluorescence markers of chitin, a major constituent of the spore walls [3,4,9]. Among the more widely used fluorochromic stains are calcfluor white M2R and uvitex 2B [4,9,12]. The wall of the microsporidian is fluorescent under the fluorescence microscope (350–440 nm excitation filters) independently of the agent, while the spores appear white or turquoise (Fig. 4).

The diagnosis of microsporidiosis can be established on the basis of the parasitological examination of several samples, but the genus and the species cannot be identified [3,7,59]. This identification is necessary to adapt treatment and determine the prognosis. Treatment and risk of dissemination are species-dependent. Species identification can be achieved with electron microscopy, molecular biology or indirect immunofluorescence [3,7,59].

Species identification

Electron microscopy

Electron microscopy can be used for biopsy tissue, biological fluids (urine, bronchoalveolar lavage fluid, duodenal aspiration fluid, cerebrospinal fluid, sputum...) and stool samples [4]. Study of the characteristic structural features of the spores can provide the genus and species: of particular importance are the structure and organization of the polar filament, the modalities of cytoplasmic and nuclear division, the different stages of the parasite cycle and the nature of the host-parasite interface. Species identification is generally possible except for two morphologically identical species infecting humans (Encephalitozoon cuniculi and Encephalitozoon hellem) [4,12].

For stool samples and biological fluids, species differentiation is difficult because most of the proliferative stages are absent; these types of samples contain only spores [7,20]. For tissue biopsy material, electron microscopy is a specific technique allowing the detection of different stages of the parasite development. The sensitivity of this method is nevertheless limited because of the small amount of material examined. In addition, electron microscopy is a complex method requiring rigorous, and invasive, sampling in addition to technical expertise. Results cannot be obtained rapidly and are difficult to interpret [1,4,51]. Because of these drawbacks, electron microscopy is not used in routine practice to achieve the species differentiation essential for adapting treatment [4,51].

Molecular methods

Molecular methods provide a precious tool for detecting microsporidia and differentiating species in biological samples of infected patients [12,59].
Conventional PCR. The amplification targets are mainly genes coding for the small subunit of ribosomal RNA [4,7,9,13,35,75–77]. Certain authors have however used other genes such as the large subunit of ribosomal RNA or the internal transcribed spacer (ITS) [3,4,12]. PCR is a sensitive, specific and reproducible method allowing species identification with a couple of specific probes [9,25,32,49,50]. It is thus an attractive alternative to electron microscopy and plays an important role in therapeutic decision-making. The PCR detection threshold for microsporidia is 10^2 spores/g fecal matters, much lower than for optical microscopy where the cutoff is around 10^4 to 10^5 spores/g [4,12,25,50,99]. Conventional PCR has however several drawbacks. First, it is a long, expensive technique performed by specialized laboratories. There is also risk of contamination and the parasite load cannot be quantified [99].

Quantitative PCR. Over the last few years, quantitative PCR, particularly with real-time procedures, has revolutionized the diagnosis of certain infectious diseases, including microsporidiosis. Quantitative PCR is a specific highly sensitive method with a detection threshold of less than 40 spores/ml in stool suspensions [99]. In patients with microsporidiosis, it allows a quantification of the parasite load in different biological samples: stools, urine, sputum, blood, serum, biological tissues [99]. The advantage is double. First, this method enables a close follow-up of the kinetics of microsporidia eradication in patients under treatment, enabling documented assessment of treatment efficacy. In addition, it establishes cutoff levels for positive results, facilitating the interpretation of results by differentiating microsporidiosis infection from asymptomatic carriage [99,100]. Real-time PCR offers the supplementary advantage of eliminating all post-PCR manipulation, reducing the reaction time and limiting the risk of contamination, and consequently of false positives [99,100].

Multiplex PCR. This technique is designed to use two couples of probes specific for two species simultaneously. Multiplex PCR is a sensitive specific technique for the detection of Enterocytozoon bieneusi and Encephalitozoon spp. [101].

Indirect immunofluorescence

Indirect immunofluorescence using monoclonal antibodies is effective both for the diagnosis of microsporidiosis and for species differentiation [102,103]. It is a simple and rapid technique, which does not require costly reagents or equipment. Sensitivity and specificity are excellent (100%) [102]. Yield is comparable with PCR, which remains the most widely used technique despite its inconveniences (complex, costly, reserved for research laboratories) [103]. Thus, the use of monoclonal antibodies could replace PCR for the specific diagnosis of microsporidiosis [103]. Specific monoclonal antibodies are currently available in the form of laboratory kits furnished by several firms (Bordier Affinity Products SA, Meridian diagnostics). These kits are however not widely marketed [59,102,103].

Treatment

Several treatments have been proposed for the management of intestinal microsporidiosis. Efficacy has been variable depending on the causal species. The criteria of therapeutic success are the resolution of the clinical manifestations and negative samples (absence of spores) [8,70]. At the present time, albendazole and fumagillin are the most effective compounds against Encephalitozoon intestinalis and Enterocytozoon bieneusi, respectively. Other therapeutic alternatives are under trial [6,25,98,104].

Albendazole (Zentel®, ZZole®)

Albendazole is a benzimidazole derivative with wide antihelminthic activity [8,25,70]. The target of benzimidazoles is the beta subunit of tubulin [12,20,105–107]. The mechanism of action of albendazole consists in the inhibition of microsporidia division by blocking the synthesis of tubulin, a major constituent of the mitosis spindle [6,8,9]. Clinical studies have demonstrated the efficacy of albendazole against species of the Encephalitozoon genus in HIV-infected patients for whom it is the treatment of choice for intestinal, ocular and disseminated microsporidiosis [25,70]. It has on the other hand very little efficacy against Enterocytozoon bieneusi since it yields only a decline in the parasite load and degenerative alterations of the spores. Clinically, stool frequency and volume decrease in infected patients whose body weight stabilizes. Relapse is however common after treatment withdrawal [6,108]. Consequently, albendazole has a parasitostatic effect on Enterocytozoon bieneusi by incomplete inhibition of replication; stool and duodenal biopsy samples remain positive [1,6,8,13,25,70,108,109].

Albendazole is absorbed well after oral intake when associated with fat-rich food. It is metabolized in the liver where a sulfoxide metabolite, which is more active and less toxic than albendazole itself, is formed [8,13,104,108]. The oral dose of albendazole is 400 mg b.i.d. for adults and 7.5 mg/kg b.i.d for children with total dose of 15 mg/kg bid for 2 to 4 weeks [6,9,13,104,107].

Albendazole is well tolerated and does not require special surveillance. Rare adverse effects have been described: digestive disorders (abdominal pain, diarrhea), generally minor elevation of the serum transaminases, which is reversible at withdrawal, proteinuria, neurological manifestations, exceptional cases of reversible alopecia, malaise with vertigo, skin rash, fever, pruritus, and very exceptionally, hematological disorders (neutropenia, pancytopenia) [6,13,104].

Albendazole is contraindicated for patients with known hypersensitivity to albendazole and for pregnant or lactating women [13,104]. Drug interactions exist with cimetidine, dexamethasone and praziquantel, leading to increased serum levels of albendazole [13,104].

Other benzimidazole derivatives

Certain benzimidazole derivatives have been studied in terms of efficacy for the treatment of microsporidiosis. Certain compounds have been found to be active against Encephalitozoon intestinalis in vitro but are poorly absorbed after oral administration (mebendazole) or present toxic effects limiting their use (nocodazole and parbendazole). Other derivatives such as thiabendazole are well absorbed but poorly active [8,104]. Fenbendazole may be of potential...
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interest because of its rapid absorption after oral intake and its metabolism into oxfendazole. Fenbendazole and oxfendazole are very active against *Encephalitozoon intestinalis* and are non toxic *in vitro*. These compounds appear to be promising for the treatment of microsporidiosis [6,8].

Fumagillin (Fumidil B®, Fumadil®, Fugillin®, Fumagillin®, Flesinct®)

Fumagillin was identified 1949. It is an antibiotic extracted from *Aspergillus fumigatus*. Fumagillin was used in 1953 by beekeepers against encephalitozoonosis in bees caused by *Noosea apis* and in human medicine for the treatment of amibiasis prior to the development of more effective amoebidical agents [6,8,9,110,111].

The target of fumagillin is a cellular metalloprotease, methionine aminopeptidase-2 (MetAP2). This enzyme is indispensable for microsporidia metabolism and survival. It is essential for eliminating methionine on the terminal end of proteins, necessary for post-translational and functional modifications [111,112]. The mechanism of action of fumagillin consists in the inhibition of microsporidia replication by irreversible blockade of the site of action of MetAP2 and by the inhibition of RNA synthesis [8]. Fungal death is the consequence [6,8,110–112]. Fumagillin has been used successfully against species of the *Encephalitozoon* genus and against *Vittaforma cornea in vitro* and in humans for the treatment of *Enterocytozoon bieneusi* intestinal microsporidiosis [111,112]. The drug is prescribed for oral intake 20 mg t.i.d for a total dose of 60 mg/d for 14 days.

The efficacy of fumagillin is counterbalanced by its adverse effects. When administered orally, the drug exhibits bone marrow toxicity by its direct effect on the megakaryocytic line and myeloid progenitors [110]. Thrombocytopenia and neutropenia are the most common adverse effects requiring regular medical surveillance for the entire duration of treatment [1,6,8,9,110–112]. In addition, abdominal pain, diarrhea, vomiting and hyperlipasemia have been noted with the use of fumagillin. This drug is contraindicated in the event of hypersensitivity [13,104].

Other drugs used

Nitazoxanide (Cryptaz®)

Nitazoxanide is a broad-spectrum anti-parasite agent effective against protozoa such as amoeba, nematodes, cestodes and trematodes [6,113]. It is also used for the treatment of cryptosporidiosis [6,104,113]. The drug inhibits the action of pyruvate ferrodoxine oxydoreductase of the electron transport system [104]. It is prescribed at the dose of 1 g b.i.d for 60 days [6,104]. Nitazoxanide has proven efficacy *in vivo* on cell cultures of *Encephalitozoon intestinalis* and *Vittaforma cornea*. It is also effective for the treatment of human microsporidiosis caused by *Enterocytozoon bieneusi* in AIDS patients [113]. This compound appears to be promising for the treatment of human microsporidiosis [113].

Other drugs

Other drugs have been tried for the treatment of microsporidiosis but with variable results, which have been difficult to reproduce: thalidomide (Thalomid®) [8,13,70,114,115], metronidazole (Flagyl®) [6,8,74,116–119], atovaquone (Wellvone®) [8,6,119,120], nifedipin (Adalate®) [8], azithromycin (Zithromax®) [120–122], furazolidone (Furoxone®) [6,8,119,123].

Drugs on trial

TNP-470

TNP-470 is a semi-synthetic analog of fumagillin, O-(chloracetyl-carbamoyl) fumagilloid, also called AGM-1470 or fumagil® [1,6,25,104]. It is an angiogenic inhibitor with the same mechanism of action as fumagillin, inhibition of MetAP2 [8,112]. TNP-470 is as effective as fumagillin against several microsporidia species in cell cultures (*Encephalitozoon intestinalis*, *Vittaforma cornea*) and in athymic mice (*Enterocytozoon cuniculi*, *Vittaforma cornea*) [1,25,110]. TNP-470 is less toxic than fumagillin and appears to be promising for the treatment of microsporidiosis [1,25,111]. This compound has been shown to prolong survival longer in mice after intraperitonial administration than after subcutaneous administration. This might be related to its short half-life [111]. No trial on the treatment of human microsporidiosis with this derivative has been published [111].

Ovalicin

Ovalicin is an antibiotic synthesized by *Pseudorotium ovalis*. Ovalicin inhibits MetAP2 [111] and can inhibit *Encephalitozoon intestinalis* and *Vittaforma cornea* replication *in vitro* and prolong the survival of mice infected with *Vittaforma cornea* [111].

Fluoroquinolones

Fluoroquinolones are a class of antibiotics. Trials on the treatment of microsporidiosis are being conducted [1,88,124]. Their mechanism of action against microsporidia results from the inhibition of topoisomerase IV, an enzyme implicated in cell division [1,124]. Didier et al. tested the activity of 15 fluoroquinolones *in vitro* against *Encephalitozoon intestinalis* and *Vittaforma cornea* and in athymic mice against *Vittaforma cornea* [124]. The *in vitro* results showed that only six compounds can inhibit microsporidia replication. In athymic mice, none of the fluoroquinolones tested had proven efficacy for clearance of *Vittaforma cornea*. Four compounds did however prolong mouse survival and might be effective in combination with other drugs such as fumagillin, which, in this case, could be administered at non-toxic doses [124].

Anti-mitotics

Two anti-mitotic compounds, pancratistatin and 7-deoxynarciclasin have been tested *in vitro* against *Encephalitozoon intestinalis* in order to determine their therapeutic effect in microsporidial infections. Early results have been promising. The target of these compounds remains to be identified. They act by inhibiting the development of cell division in microsporidia [125].
Polyamine analogs
Polyamines are quaternary ammonia compounds, which play an important role in the proliferation and differentiation of microorganisms since they are indispensable for the synthesis of protein and nucleic acids [25,126]. The mechanism of action of polyamine analogs results from the clearance of cell polyamines deregulating cell metabolism. Parasite growth is thus inhibited [1,5,25,126]. Polyamine analogs have proven efficacy in vivo and in vitro against Encephalitozoon cuniculi. They have not been tried in humans but appear to be promising despite the absence of data on Enterocytozoon bieneusi and Encephalitozoon intestinalis [8,126].

Interferon gamma
Interferon-γ is a cytokine produced by CD4 lymphocytes whose protective effect is partially mediated by the synthesis of this cytokine. Interferon-γ activates macrophages and potentiates the cytotoxic effect of natural killer cells. A recent study on the efficacy of interferon-γ in mice infected with Encephalitozoon cuniculi showed the drug could be useful for the treatment of microsporidiosis, especially in patients with very low CD4 counts. It cannot however be used in subjects submitted to immunosuppressive regimens (e.g. transplant recipients) [23].

Anti-retroviral therapy
With anti-retroviral therapy, HIV-infected patients have a lower viral load and improved CD4 counts with reconstitution of their immune defense. Consequently, anti-retroviral therapy reduces the prevalence of opportunistic infections, including microsporidiosis, and reduces the morbidity and mortality related to HIV infection [1,25,42,70,127,128]. It also enables the eradication of microsporidia infection without use of a specific treatment. It considerably reduces the risk of recurrent microsporidiosis observed after treatment withdrawal in subjects with severe immune deficiency [128].

Prophylaxis
A detailed description of the sources and modes of transmission of human microsporidiosis remains to be established, compromising the potential efficacy of preventive measures.

Individual prophylaxis
The most likely transmission mode being orofecal, high-risk subjects (HIV-positive subjects and organ transplant recipients) should abide by strict rules of hygiene [1,5,25]. Besides hand washing, advice includes washing fresh vegetables, drinking bottled (or boiled) water, eating well-cooked meat and seafood and limiting contact with animals susceptible of transmitting the disease [1,13,25]. At the present time, no chemoprophylaxis is available for microsporidiosis. For HIV-infected subjects, reconstitution of immune defenses with antiretroviral triple therapy is the key to prevention.

Population-based prophylaxis
Several procedures have proven efficacy for reducing the viability and infectious capacity of microsporidia belonging to the Encephalitozoon genus, including treatment of drinking water sources with chloride or ozone [5,25]. Other processes such as water disinfection (quaternary ammonia, 70% ethanol, phenol derivatives, hydrogen peroxide, sodium hydroxide) [3,5,25,72] are lethal in vitro for Encephalitozoon cuniculi. These methods have not been tried in humans. Further research is needed to clarify the epidemiology of microsporidiosis and to institutes preventive measures not only for the population in general but also for subjects at risk.

Conclusion
Microsporidiosis is an emerging pathology, particularly prevalent in immunodepressed subjects. Clinically, manifestations are protean, with a predominance of gastrointestinal disorders. The diagnosis requires identification of microsporidia spores necessitating specifically designed biological tests. It is up to clinicians to order these specific tests and provide pertinent clinical information. The treatment of human microsporidiosis has been the topic of several clinical trials, but with various success. Therapeutic efficacy depends on the infecting species and the therapeutic agent. Species identification, generally using molecular biology techniques, is thus indispensable. At the present time, albendazole is the most effective compound against Encephalitozoon intestinalis. Fumagillin and its analogs are the best choice for Enterocytozoon bieneusi. The risk related to use of these compounds must however not be overlooked. Further research is needed to develop an effective anti-microsporidia agent free of adverse effects, particularly for Enterocytozoon bieneusi the most commonly incriminated species. Advanced studies on the microsporidia genome and biochemical structure will be useful for identifying compounds effective against all pathogenic species. Better knowledge of the epidemiology of microsporidiosis is needed for instituting preventive measures.

Conflict of interest statement
None.

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