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Case Report

Blastocystis sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms

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ABSTRACT

Blastocystis is a common unicellular intestinal parasite in humans. Its clinical relevance is still subject to discussion with numerous conflicting reports on its ability to cause disease. A remarkable genetic heterogeneity among isolates suggests an association between distinct subtypes (STs) and pathogenicity, although a clear correlation between symptoms and subtype is lacking. Here, we report on a clinical case which possibly links *Blastocystis* sp. ST2 infection with the simultaneous occurrence of gastrointestinal illness and generalized chronic urticaria. Despite repeated chemotherapy with different antimicrobial drugs, both the gastrointestinal and cutaneous disorders reoccurred after short symptom-free intervals. Eradication of the parasite and permanent resolution of the patient's medical condition was finally achieved with the combined application of metronidazole and paromomycin.

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1. Introduction

Blastocystis is a common enteric parasite of humans with infection rates ranging from approximately 5–20% in developed to more than 50% in developing countries [1,2]. *Blastocystis* has been encountered in patients with gastrointestinal symptoms as well as in asymptomatic individuals. Clinical presentations of *Blastocystis* infection include diarrhea, abdominal discomfort, anorexia, and flatulence [2,3]. Extraintestinal manifestations of *Blastocystis* infection including arthritis or skin disorders have also been described [4,5]. Molecular analysis allows classification of *Blastocystis* isolates into at least ten subtypes (STs) [6]. However, a clear-cut linkage of distinct STs to the presence of disease symptoms is not apparent [7]. More recently, intrasubtype variability with pathogenic and nonpathogenic variants within certain STs [8] as well as intrasubtype difference in morphotypes [9] have been suggested to be related to the pathogenic potential of *Blastocystis*.

2. Case report

A 20-year old man fell ill with diarrhea and nausea during a trip to Shanghai, but recovered within 5 days. Three weeks after the acute disease, he began suffering from generalized urticaria (Fig. 1) and flatulence. An antihistaminic therapy was started with cetirizin without relevant success. Results from a parasitological examination of a stool specimen, as well as *Helicobacter pylori* testing were negative. Three months after onset of the urticaria, the patient presented in our outpatient clinic with persistent urticaria and flatulence. Full blood count including eosinophil count, erythrocyte sedimentation rate, C-reactive protein, cryoglobulins, circulating immune complexes, C3, C4, CH 100, C1-INH, IgE, ANA, MAK, TAK, TRAK, immunoglobulins (IgA, IgM and IgG) and serological investigations (EBV, Coxsackie B1–B6, A9, ECHO virus pool, TSH, T3 and T4) were all within the normal range. Radio allergen sorbent test revealed a sensitization against house dust mite, tree, and grass pollen. A microbiological and parasitological examination was performed on three consecutive stool samples including bacterial enteropathogens, enteric viruses (Astro-, Adeno-, Rota- and Norovirus), Shiga and *Clostridium difficile* toxins, helminths and intestinal protozoans (*Entamoeba histolytica*, *Dientamoeba fragilis*, *Giardia intestinalis*, microsporidia, and cryptosporidia). In all three samples, *Blastocystis* was detected by bright field microscopy at ×400 magnification, applying the criterion of five or more organisms per high-power field as diagnostic index [10]. No other established or potential

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Fig. 1. Widespread urticarial skin lesions on the trunk and extremities.

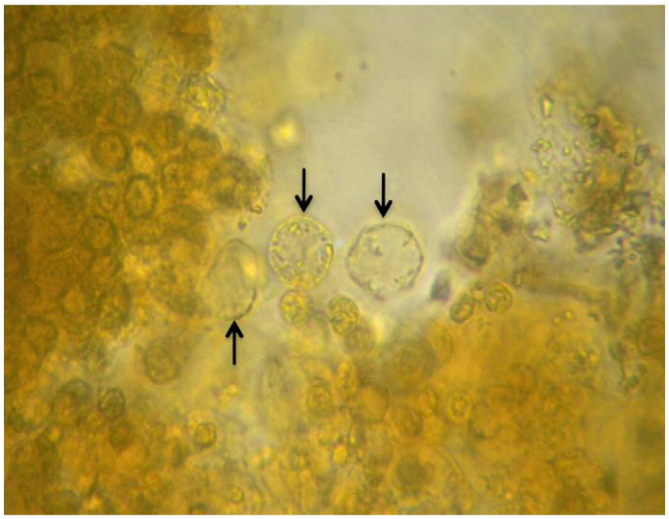

Fig. 2. A light micrograph of vacuolar forms of *Blastocystis* presented in a iodine stained wet mount of a SAF preserved colonic biopsy (×400). Three of the vacuolar forms with marginal nuclei, representative of the structures found in both stool and biopsy specimens, are clearly visible (arrows).

bacterial, viral, or parasitic pathogen or any toxins could be detected. Phylogenetic analysis, performed as described previously [7], revealed that the isolate belonged to *Blastocystis* sp. ST2 according to the consensus terminology by Stensvold et al. [11]. Treatment with metronidazole 800 mg/d for 10 days was initiated after which the patient was asymptomatic for the first time since disease onset. A control examination of stool specimens for the presence of *Blastocystis* was not performed after cessation of therapy. Six months later, flatulence and constipation recurred. Microscopic examination of three consecutive stool samples again revealed *Blastocystis* and a second course of metronidazole therapy (800 mg/d) followed. One month later, stool sample controls were again positive for *Blastocystis*. As the patient reported no improvement, a third therapy cycle with metronidazole (800 mg/d) was undertaken. In the following stool samples, the parasite was detected in low concentration. Due to persistence of clinical symptoms, therapy was switched to trimethoprim–sulfamethoxazole (TMP/SMX; 960 mg/d for 1 week) which had no effect. Therefore, treatment with paromomycin 3 g/d was started. Afterwards, the patient was asymptomatic for 1 month. Stool controls 1 month later revealed *Blastocystis* and the patient experienced relapse of both gastrointestinal and urticarial symptoms. A colonoscopy with mucosa biopsy was performed, which demonstrated a discrete unspecific mucosal inflammation of the colon and a heavy colonization of the colonic mucosa with *Blastocystis* structures (Fig. 2). Histological examination of a colonic biopsy showed no invasive *Blastocystis*. No evidence for a malabsorption syndrome, tumor or inflammatory bowel disease was found. After a symptom-free period of 5 months, the patient again experienced flatulence and urticaria. Finally, a combination therapy with paromomycin 3 g/d plus metronidazole 1.8 g/d for 10 days was administered, after which the patient has remained symptom-free for 18 months. Microscopic stool examination of three samples 2 weeks later and of two samples 4 weeks later were negative for *Blastocystis*. Eradication of the parasite was confirmed by PCR testing, as described previously [12], of a stool specimen 5 months later.

3. Discussion

We report on a rare case of *Blastocystis* sp. ST2 infection which is possibly associated with the simultaneous occurrence of both a gastrointestinal and cutaneous disorders. In our patient, multiple serological, microbiological, and allergy tests did not disclose any abnormality, except for the detection of *Blastocystis* sp. ST2 from stool specimens as the only potentially pathogenic organism. Subtype analysis was only conducted on primary isolate upon patient presentation to our clinic due to symptom persistence. Thus, disease relapses could also be due to strains different from the primary isolate or due to reinfection with another subtype. Due to the parasite's genetic heterogeneity—ten subtypes are currently recognized [6]—, it has been hypothesized that the parasite's pathogenic properties might be subtype-dependent. However, conclusions from attempts to link potential pathogenicity to subtype/isolate remained equivocal [7,13,14]. Recent studies have related ST3 to pathogenic potential [15,16]. By contrast, ST2 was not significantly linked to symptomatic infection [17]. Moreover, in an animal model ST2 was not associated with elevated pathogenicity [8]. Conversely, ST2 was correlated with higher parasite abundance and infection intensity than other subtypes [7]. The small subunit (SSU) rDNA sequence identified from our patient isolate was identical to a sequence generated from a *Blastocystis* isolate of a Danish patient (GenBank accession no. AM275371) with gastrointestinal symptoms and urticaria (data not shown). Genetic and/or morphologic variation within ST2 may explain the different pathogenic behavior. Pathogenic and nonpathogenic variants within *Blastocystis* STs have been shown in an experimental setting [8]. Intrasubtype differences in morphology may contribute to pathogenicity, since amoeboid forms were related to a symptomatic status [9,18,19]. In a colonic biopsy of our patient, only vacuolar forms of the parasite were detectable (Fig. 2). Amoeboid forms were not visible, stool samples included, although *in vitro* cultivation of the patient isolate was not conducted. Recent findings of ST2 chronic infection in both symptomatic and asymptomatic individuals [20] suggest expression of symptoms to be contingent on host factors such as specific IgA production [21]. Several case studies present evidence of implication of *Blastocystis* in skin disorders such as urticaria [18,22–26]. However, these reports often lack detailed analysis of the isolated strain such as molecular typing and *Blastocystis* morphology. So far, onset of both gastrointestinal illness and skin symptoms like rash and urticaria, has only been documented for ST3 infections [15,18]. The role of subtypes/morphotypes in the etiology of extra-intestinal disorders is currently unknown. In our case, laboratory and allergy investigations excluded any underlying systemic disease, such as malabsorption or endocrinological, autoimmune, and rheumatological diseases. IgE-mediated allergic reactions due to antigen shedding may cause *Blastocystis* associated urticaria [23]. Apart from a sensitization against common allergens, our patient had no allergic disposition, nor did the urticarial symptoms respond to antihistaminic treatment. The coincidence of symptom resolution and parasite eradication is indicative of *Blastocystis* as the etiologic agent, although symptoms may have resolved spontaneously or due to elimination of a

so far unrecognized pathogen. Whether the patient was infected with *Blastocystis* prior to disease onset remained unknown. Metronidazole, TMP/SMX, and paromomycin are among the most widely used drugs for the treatment of *Blastocystis* infection, although several therapy failures have been reported [27–30]. In our patient, monotherapy with these drugs only attained a temporary cessation of symptoms along with a reduction of stool parasite numbers. Treatment failures may be due to drug resistance, noncompliance, or poor drug efficacy. Drug susceptibility testing was not done for the isolated strain, and the impact of different *Blastocystis* subtypes/morphotypes on drug sensitivity is unclear. Noncompliance is an unlikely cause since our patient was highly cooperative. Inaccessibility of the pathogen to the drug and insufficient drug levels due to drug inactivation also contribute to treatment failure.

To conclude, we report a case of *Blastocystis* sp. ST2 infection possibly associated with the recurrence of gastrointestinal and urticarial symptoms which was successfully treated with metronidazole and paromomycin. Future studies on the role of morphotypes, variation between *Blastocystis* STs, and host interaction for symptom manifestation are encouraged.

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References

[1] Stenzel DJ, Boreham PF. *Blastocystis hominis* revisited. Clin Microbiol Rev 1996;9:563–84.
[2] Boorom KF, Smith H, Nimri L, Viscogliosi E, Spanakos G, Parkar U, et al. Oh my aching gut: irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. Parasit Vectors 2008;1:40.
[3] Stensvold CR, Lewis HC, Hammerum AM, Porsbo LJ, Nielsen SS, Olsen KE, et al. *Blastocystis*: unravelling potential risk factors and clinical significance of a common but neglected parasite. Epidemiol Infect 2009;137:1655–63.
[4] Lakhanpal S, Cohen SB, Fleischmann RM. Reactive arthritis from *Blastocystis hominis*. Arthritis Rheum 1991;34:251–3.
[5] Kick G, Rueff F, Przybilla B. Palmoplantar pruritus subsiding after *Blastocystis hominis* eradication. Acta Derm Venereol 2002;82:60.
[6] Stensvold CR, Alfellani MA, Norskov-Lauritsen S, Prip K, Victory EL, Maddox C, et al. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol 2009;39:473–9.
[7] Ozyurt M, Kurt O, Molbak K, Nielsen HV, Haznedaroglu T, Stensvold CR. Molecular epidemiology of *Blastocystis* infections in Turkey. Parasitol Int 2008;57:300–6.
[8] Hussein EM, Hussein AM, Eida MM, Atwa MM. Pathophysiological variability of different genotypes of human *Blastocystis hominis* Egyptian isolates in experimentally infected rats. Parasitol Res 2008;102:853–60.
[9] Vassalos CM, Spanakos G, Vassalou E, Papadopoulou C, Vakalis N. Differences in clinical significance and morphologic features of *Blastocystis* sp. subtype 3. Am J Clin Pathol 2010;133:251–8.
[10] Tan KS. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 2008;21:639–65.
[11] Stensvold CR, Suresh GK, Tan KS, Thompson RC, Traub RJ, Viscogliosi E, et al. Terminology for *Blastocystis* subtypes—a consensus. Trends Parasitol 2007;23:93–6.
[12] Stensvold R, Brillowska-Dabrowska A, Nielsen HV, Arendrup MC. Detection of *Blastocystis hominis* in unpreserved stool specimens by using polymerase chain reaction. J Parasitol 2006;92:1081–7.
[13] Bohm-Gloning B, Knobloch J, Walderich B. Five subgroups of *Blastocystis hominis* from symptomatic and asymptomatic patients revealed by restriction site analysis of PCR-amplified 16S-like rDNA. Trop Med Int Health 1997;2:771–8.
[14] Kaneda Y, Horiki N, Cheng XJ, Fujita Y, Maruyama M, Tachibana H. Ribodemes of *Blastocystis hominis* isolated in Japan. Am J Trop Med Hyg 2001;65:393–6.
[15] Jones MS, Whipps CM, Ganac RD, Hudson NR, Boorom K. Association of *Blastocystis* subtype 3 and 1 with patients from an Oregon community presenting with chronic gastrointestinal illness. Parasitol Res 2009;104:341–5.
[16] Tan TC, Suresh KG, Smith HV. Phenotypic and genotypic characterisation of *Blastocystis hominis* isolates implicates subtype 3 as a subtype with pathogenic potential. Parasitol Res 2008;104:85–93.
[17] Dogruman-Al F, Dagci H, Yoshikawa H, Kurt O, Demirel M. A possible link between subtype 2 and asymptomatic infections of *Blastocystis hominis*. Parasitol Res 2008;103:685–9.
[18] Katsarou-Katsari A, Vassalos CM, Tzanetou K, Spanakos G, Papadopoulou C, Vakalis N. Acute urticaria associated with amoeboid forms of *Blastocystis* sp. subtype 3. Acta Derm Venereol 2008;88:80–1.
[19] Tan TC, Suresh KG. Predominance of amoeboid forms of *Blastocystis hominis* in isolates from symptomatic patients. Parasitol Res 2006;98:189–93.
[20] Dogruman-Al F, Kustimur S, Yoshikawa H, Tuncer C, Simsek Z, Tanyuksel M, et al. *Blastocystis* subtypes in irritable bowel syndrome and inflammatory bowel disease in Ankara, Turkey. Mem Inst Oswaldo Cruz 2009;104:724–7.
[21] Mahmoud MS, Saleh WA. Secretory and humoral antibody responses to *Blastocystis hominis* in symptomatic and asymptomatic human infections. J Egypt Soc Parasitol 2003;33:13–30.
[22] Gupta R, Parsi K. Chronic urticaria due to *Blastocystis hominis*. Australas J Dermatol 2006;47:117–9.
[23] Pasqui AL, Savini E, Saletti M, Guzzo C, Puccetti L, Auteri A. Chronic urticaria and *blastocystis hominis* infection: a case report. Eur Rev Med Pharmacol Sci 2004;8:117–20.
[24] Biedermann T, Hartmann K, Sing A, Przybilla B. Hypersensitivity to non-steroidal anti-inflammatory drugs and chronic urticaria cured by treatment of *Blastocystis hominis* infection. Br J Dermatol 2002;146:1113–4.
[25] Armentia A, Mendez J, Gomez A, Sanchis E, Fernandez A, de la Fuente R, et al. Urticaria by *Blastocystis hominis*. Successful treatment with paromomycin. Allergol Immunopathol (Madr) 1993;21:149–51.
[26] Cassano N, Scoppio BM, Loviglio MC, Vena GA. Remission of delayed pressure urticaria after eradication of *Blastocystis hominis*. Acta Derm Venereol 2005;85:357–8.
[27] Haresh K, Suresh K, Khairul Anus A, Saminathan S. Isolate resistance of *Blastocystis hominis* to metronidazole. Trop Med Int Health 1999;4:274–7.
[28] Markell EK, Udkow MP. *Blastocystis hominis*: pathogen or fellow traveler? Am J Trop Med Hyg 1986;35:1023–6.
[29] Moghaddam DD, Ghadirian E, Azami M. *Blastocystis hominis* and the evaluation of efficacy of metronidazole and trimethoprim/sulfamethoxazole. Parasitol Res 2005;96:273–5.
[30] Stensvold CR, Smith HV, Nagel R, Olsen KE, Traub RJ. Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? J Clin Gastroenterol 2009;44:85–90.