

CLINICAL SCIENCE

Intestinal microsporidiosis: a hidden risk in rheumatic disease patients undergoing anti-tumor necrosis factor therapy combined with disease-modifying anti-rheumatic drugs?

Nadia Emi Aikawa,^{I,II} Aline de Oliveira Twardowsky,^{II} Jozélio Freire de Carvalho,^I Clovis A. Silva,^{I,III} Ivan Leonardo Avelino França e Silva,^{III} Ana Cristina de Medeiros Ribeiro,^I Carla Gonçalves Schain Saad,^I Julio César Bertacini Moraes,^I Roberto Acayaba de Toledo,^{IV} Eloísa Bonfá^I

^IDivision of Rheumatology, CEDMAC Unit, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP/Brazil. ^{II}Pediatric Rheumatology Unit, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP/Brazil. ^{III}Division of Infectology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP/Brazil. ^{IV}Division of Rheumatology, CEDMAC Unit, Faculdade de Medicina de São José do Rio Preto, SP/Brazil.

OBJECTIVE: Immunosuppressed patients are at risk of microsporidiosis, and this parasitosis has an increased rate of dissemination in this population. Our objective was to evaluate the presence of microsporidiosis and other intestinal parasites in rheumatic disease patients undergoing anti-tumor necrosis factor/disease-modifying anti-rheumatic drug treatment.

METHODS: Ninety-eight patients (47 with rheumatoid arthritis, 31 with ankylosing spondylitis and 11 with psoriatic arthritis) and 92 healthy control patients were enrolled in the study. Three stool samples and cultures were collected from each subject.

RESULTS: The frequency of microsporidia was significantly higher in rheumatic disease patients than in control subjects (36 vs. 4%, respectively; $p < 0.0001$), as well as in those with rheumatic diseases (32 vs. 4%, respectively; $p < 0.0001$), ankylosing spondylitis (45 vs. 4%, respectively; $p < 0.0001$) and psoriatic arthritis (40 vs. 4%, respectively; $p < 0.0001$), despite a similar social-economic class distribution in both the patient and control groups ($p = 0.1153$). Of note, concomitant fecal leukocytes were observed in the majority of the microsporidia-positive patients (79.5%). Approximately 80% of the patients had gastrointestinal symptoms, such as diarrhea (26%), abdominal pain (31%) and weight loss (5%), although the frequencies of these symptoms were comparable in patients with and without this infection ($p > 0.05$). Rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis disease activity parameters were comparable in both groups ($p > 0.05$). The duration of anti-tumor necrosis factor/disease-modifying anti-rheumatic drugs and glucocorticoid use were also similar in both groups.

CONCLUSION: We have documented that microsporidiosis with intestinal mucosa disruption is frequent in patients undergoing concomitant anti-tumor necrosis factor/disease-modifying anti-rheumatic drug therapy. Impaired host defenses due to the combination of the underlying disease and the immunosuppressive therapy is the most likely explanation for this finding, and this increased susceptibility reinforces the need for the investigation of microsporidia and implementation of treatment strategies in this population.

KEYWORDS: Microsporidia; Parasitosis; Anti-TNF; Rheumatoid arthritis; Ankylosing spondylitis.

Aikawa NE, Twardowsky AO, Carvalho JF, Silva CA, França e Silva ILA, Ribeiro ACM, et al. Intestinal microsporidiosis: a hidden risk in rheumatic disease patients undergoing anti-tumor necrosis factor therapy combined with disease-modifying anti-rheumatic drugs? Clinics. 2011;66(7):1171-1175.

Received for publication on January 12, 2011; First review completed on February 14, 2011; Accepted for publication on March 21, 2011

E-mail: nadia.aikawa@gmail.com

Tel.: 005511-3069-8563

Fax: 005511-3069-8503

INTRODUCTION

Microsporidia has emerged in recent years as an opportunistic infectious agent with a ubiquitous distribution.¹⁻⁴ The clinical manifestations of this infection vary widely, from none (asymptomatic) to non-bloody watery diarrhea, abdominal cramps, weight loss, and disseminated disease, especially in immunosuppressed subjects.²⁻⁴

This parasitological disease primarily affects immunosuppressed patients,¹⁻⁴ particularly those with human immunodeficiency virus (HIV), malignancies and diabetes mellitus, as well as patients post heart-lung, liver, and renal transplantations.^{1,2} In addition, this infection also occurs in patients undergoing immunosuppressive drug treatment.^{2,4}

In fact, patients with rheumatic diseases who are taking disease-modifying anti-rheumatic drugs (DMARD) and anti-tumor necrosis factor (TNF) therapy have a high risk for general infections,⁵⁻⁷ including intestinal pathogenic parasite infestations such as *Strongyloides stercoralis*.^{8,9} However, to our knowledge, there are no data regarding the risk of microsporidiosis in these patients.¹⁰⁻¹³

Therefore, the objective of this study was to evaluate the frequency and clinical significance of microsporidiosis in patients with rheumatic diseases who are undergoing anti-TNF/DMARD treatment compared to an age- and socio-economic condition-matched healthy population.

MATERIALS AND METHODS

We evaluated 89 consecutive patients at our hospital who were diagnosed with rheumatoid arthritis (RA) (American College of Rheumatology criteria),¹⁴ ankylosing spondylitis (AS) (New York criteria),¹⁵ or psoriatic arthritis (PsA) (European spondyloarthropathy Study Group – ESSG criteria and Moll and Wright classification).¹⁶ All patients were undergoing anti-TNF therapy (adalimumab, etanercept, or infliximab) combined with DMARDs. The control group included 92 healthy employees of our hospital who were matched by age and socio-economic status. Prior to this study, no specific routine existed in our Infusion Center with regard to stool examination for parasites and fecal leukocytes before anti-TNF therapy. The prophylactic use of anti-helminthic drugs was recommended for patients under concomitant glucocorticoid therapy.

This study was approved by the local ethics committee, and informed consent was obtained from each participant or his or her legal guardian.

Demographic data, the number of school years attended, and the socio-economic status (according to *Associação Brasileira dos Institutos de Pesquisa de Mercados*)¹⁷ were recorded. Background information and parasitosis symptoms in patients and control participants were acquired via interview and included the following: general (loss of appetite, weight loss, and adynamia), gastrointestinal (abdominal pain, abdominal distension, flatulence, diarrhea, dysentery, tenesmus, obstipation, nausea, vomiting, hematochezia, and worm elimination), cutaneous (exanthema), and pulmonary manifestations (wheezing, thoracic pain, and hemoptysis).

Disease activity and laboratory evaluation of rheumatic diseases

Disease activity was evaluated by the following: the duration of morning stiffness, the number of swollen and/or painful joints, and the disease activity score (DAS28) for RA patients¹⁸ and the number of swollen and/or painful joints and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)¹⁹ for AS and PsA patients.

The laboratory parameters assessed included the erythrocyte sedimentation rate (ESR) according to Westergreen (mm/^{1st} hour) and C-reactive protein (CRP) level determined by nephelometry (mg/L). Data concerning current

treatment with prednisone, DMARDs, anti-TNF and immunosuppressive drugs (methotrexate, azathioprine, leflunomide, chloroquine, sulfasalazine and/or cyclosporine) were collected.

Parasitological assessment

Three stool samples were collected from rheumatic disease patients and control subjects on different days and placed into plastic vials without preservatives. The stool samples were microscopically analyzed for the detection of protozoan oocysts, cysts, helminth eggs, and larvae by a qualified technician blinded to the groups using the techniques described by Faust et al, Rugai and Lutz (modified), and Hoffman, Pons and Janer.²⁰

Microsporidia positivity was defined as the isolation of at least one parasite using the GRAM-Chromotrope²¹ technique.

Intestinal coccidia were evaluated by the GRAM-Chromotrope²¹ technique; Leishman staining²² was used to identify *Blastocystis hominis* and fecal leukocytes; the Kinyoun procedure²³ and capture enzyme-linked immunosorbent assay (capture ELISA) were used to detect *Cryptosporidium sp.*, *Cyclospora cayetanensis*, and *Isospora belli*; and Kato-Katz was used to detect *Schistosoma mansoni*.²⁴ Coproculture was also performed using SS agar, MacConkey agar or Karmali plate agar.²⁵

Giardia lamblia, *Strongyloides stercoralis*, *Ancilostoma duodenali*, *Ascaris lumbricoides*, *Entamoeba histolytica*, *Microsporidia* (positive stool leukocytes), *Entamoeba dispar*, *Dientamoeba fragilis*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isospora belli*, and *Blastocystis hominis* (positive stool leukocytes) were defined as pathogenic parasites in immunocompromised patients.¹ All participants with positive pathogenic parasites were treated with the appropriate recommended antiparasitic drugs.²⁶

Statistical analysis

Results are presented as the mean \pm standard deviation or the median (range) for continuous variables and the number (%) for categorical variables. Continuous variables were compared using the *t* test and the Mann Whitney test to evaluate differences between patients with and without intestinal microsporidia and other parasitoses. For categorical variables, differences in proportions were assessed by Fisher's exact test. For all statistical tests, significance was set at a *p*-value <0.05 .

RESULTS

A total of 89 patients with rheumatic diseases (47 RA, 31 AS and 11 PsA) and 92 healthy control subjects were included in this study. The two groups were comparable with regard to the mean current age (47.2 ± 10.9 vs. 43.8 ± 14.3 years, respectively; $p=0.094$), percentage who were Caucasian (80 vs. 73%, respectively; $p=0.3$) and socio-economic distribution, with a similar predominance of the middle and lowest Brazilian socio-economic classes (C, D or E)¹⁷ based on scholarship and ownership of household items (84 vs. 82%, respectively; $p=0.7$). There were a greater proportion of females in the control group than in the patient group (93 vs. 62%, respectively; $p<0.0001$). The mean disease duration for all patients was 15.5 ± 8.6 years.

Table 1 - Intestinal parasites in rheumatic disease patients undergoing anti-TNF/DMARD therapies and healthy controls.

Intestinal parasites	Rheumatic disease patients (n = 89)	Control subjects (n = 92)	p-value
Pathogenic parasites			
Microsporidia	34 (38)	4 (4)	<0.0001
Microsporidia with leukocytes	28 (31)	4 (4)	<0.0001
<i>Entamoeba histolytica/dispar</i>	0 (0)	4 (4)	0.12
<i>Dientamoeba fragilis</i>	3 (3)	0 (0)	0.12
<i>Giardia lamblia</i>	3 (3)	0 (0)	0.12
<i>Strongyloides stercoralis</i>	3 (3)	2 (2)	0.68
<i>Cryptosporidium parvum</i>	3 (3)	5 (5)	0.72
<i>Cyclospora cayetanensis</i>	0 (0)	0 (0)	1.0
<i>Isoospora belli</i>	0 (0)	0 (0)	1.0
<i>Ancilostoma duodenale</i>	1 (1)	0 (0)	0.49
<i>Ascaris lumbricoides</i>	0 (0)	0 (0)	1.0
<i>Blastocystis hominis</i> with leukocytes	46 (52)	42 (46)	0.46
Non-pathogenic parasites			
<i>Endolimax nana</i>	17 (19)	25 (27)	0.22
<i>Entamoeba coli</i>	6 (7)	12 (13)	0.21
<i>Entamoeba hartmanni</i>	1 (1)	1 (1)	0.5

Results are presented as n (%); DMARDs - disease-modifying anti-rheumatic drugs.

Intestinal parasites

The overall frequency of pathogenic parasites was similar in both the patient and control groups (63 vs. 58%, respectively; $p=0.54$). Positive tests for microsporidia were significantly higher in all types of rheumatic disease patients compared with the control subjects (38 vs. 4%,

respectively; $p<0.0001$), and microsporidia plus positive fecal leukocytes were also more frequently detected in patients than in control subjects (31 vs. 4%, respectively; $p<0.0001$). Of note, 81.6% of the positive microsporidia patients had concomitant fecal leukocytes. In contrast, no differences were detected for other pathogenic parasites (*Entamoeba histolytica/dispar*, *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Isoospora belli*, *Ancilostoma duodenale*, *Ascaris lumbricoides*, and *Blastocystis hominis* with leukocytes) and non-pathogenic parasites (*Endolimax nana*, *Entamoeba coli*, and *Entamoeba hartmanni*) ($p>0.05$; Table 1). Among the 28 patients with microsporidia and positive stool leukocytes, 36% had other concomitant pathogenic parasites, particularly *Blastocystis hominis* [8/28 (29%) patients]; however, no association of microsporidia and *Blastocystis hominis* was observed ($p=0.28$). Regarding the prevalence of pathogenic parasites in each disease compared to the control group, microsporidiosis was higher in RA (32 vs. 4%, respectively; $p<0.0001$), AS (45 vs. 4%, respectively; $p<0.0001$) and PsA patients (40 vs. 4%, respectively; $p<0.0001$). Moreover, there were no statistical differences in microsporidia positivity between the specific diseases (RA vs. AS and PsA; AS vs. PsA; all $p>0.05$). *Giardia lamblia* was significantly more common in AS patients vs. control subjects (10% vs. 0%, respectively; $p=0.0149$), whereas no differences were observed for the other pathogenic parasites ($p>0.05$).

Disease activity parameters at the study onset were alike in patients with and without microsporidia infections for all diseases (RA, AS, and PsA) ($p>0.05$). The analysis of the duration of anti-TNF/DMARD therapy showed no statistical difference between patients with and without microsporidia infection ($p=0.55$). The frequencies of glucocorticoid use in patients with and without microsporidiosis [44% (95% IC 0.27-0.62) vs. 64% (95% IC 0.49-0.76); $p=0.08$] were alike, as was the number of DMARDs used (Table 2).

Table 2 - Disease activity parameters and treatments in patients with rheumatic diseases undergoing anti-TNF and DMARD therapies with and without microsporidia infection.

Parameters	Patients with microsporidia (n = 34)	Patients without microsporidia (n = 55)	p-value
Rheumatoid arthritis (n = 47)			
Morning stiffness, minutes	13 ± 17.9	26.9 ± 43.2	0.24
Number of painful joints	6.5 ± 5.8	11 ± 10	0.12
Number of swollen joints	4.8 ± 5.1	5.1 ± 4.1	0.83
DAS28	3.8 ± 1.6	4.6 ± 1.3	0.10
ESR, mm/1 st hour	26.6 ± 26.1	27.1 ± 15.5	0.94
CRP, mg/L	8.49 ± 9.5	15.4 ± 17.9	0.19
Ankylosing spondylitis (n = 31)			
Number of painful joints	5.6 ± 13.4	3.6 ± 4.2	0.59
Number of swollen joints	1.4 ± 1.4	1.7 ± 2.3	0.68
BASDAI	3.48 ± 2.01	3.47 ± 2.43	0.99
ESR, mm/1 st hour	23 ± 27	14 ± 16	0.27
CRP, mg/L	12.6 ± 14.5	13.2 ± 21.1	0.94
Psoriatic arthritis (n = 11)			
Morning stiffness (minutes)	0 (0-20)	17.5 (0-120)	0.14
Number of painful joints	5 (0-8)	3.5 (0-12)	0.93
Number of swollen joints	1 (0-2)	1 (0-2)	0.77
BASDAI	2.33 (0-6.98)	4.06 (0.27-5.16)	0.48
ESR, mm/1 st hour	11.5 (3-76)	23 (1-46)	0.73
CRP, mg/L	2 (0.2-19.6)	6.8 (1.5-32.5)	0.55
Rheumatic patients treated (n = 89)			
Anti-TNF and DMARD therapy duration (months)	15 ± 11.7	13.6 ± 9.7	0.55
Glucocorticoid use	15 (44)	35 (64)	0.08
Methotrexate use	26 (76)	33 (60)	0.17
Number of DMARDs	1.1 ± 0.7	0.9 ± 0.7	0.33

Table 3 - Clinical manifestations in patients with rheumatic diseases undergoing anti-TNF and DMARD therapies with and without microsporidia infection.

Clinical manifestations	Patients with microsporidia (n = 34)	Patients without microsporidia (n = 55)	p-value
Abdominal pain	10 (29)	10 (18)	0.296
Diarrhea	10 (29)	7 (13)	0.094
Weight loss	2 (6)	8 (15)	0.306
Nausea	8 (24)	14 (25)	1.0
Vomit	6 (18)	8 (15)	0.768
Flatulence	6 (18)	19 (35)	0.096
Dysentery	2 (6)	0 (0)	0.143
Tenesmus	0 (0)	4 (7)	0.294
Obstipation	6 (18)	9 (16)	1.0
Loss of appetite	7 (21)	6 (11)	0.231
Hematochezia	2 (6)	5 (9)	0.704
Worm elimination	0 (0)	5 (9)	0.152
Wheezing	8 (24)	5 (9)	0.072
Thoracic pain	8 (24)	6 (11)	0.139
Hemoptysis	2 (6)	2 (4)	0.635
Exanthema	2 (6)	10 (18)	0.121
Abdominal distension	2 (6)	15 (27)	0.013
Adynamia	11 (32)	34 (62)	0.009
Any gastrointestinal symptom	29 (85)	47 (85)	1.0
Systemic dissemination	0 (0)	0 (0)	1.0

Results are presented as n (%); DMARDs - disease-modifying anti-rheumatic drugs.

No differences were detected in the frequencies of other infections that required antibiotic therapy in patients with and without microsporidiosis, such as acute lower respiratory infection (2.9 vs. 14.5%, respectively; $p=0.14$), urinary tract infection (29.4 vs. 30.9%, respectively; $p=1.0$) cutaneous infection (20.6 vs. 23.6%, respectively; $p=0.8$) and tuberculosis (2.9 vs. 1.8%, respectively; $p=1.0$).

The comparison of rheumatic disease patients with and without microsporidiosis revealed that 85.4% of the patients had gastrointestinal symptoms such as diarrhea (19%), abdominal pain (22%), and weight loss (11%), but these symptoms occurred just as frequently in patients with and without this infection ($p>0.05$). Other signs and symptoms related to parasitoses were also comparable in the two groups, with the exception of abdominal distension ($p=0.013$) and adynamia ($p=0.009$), both of which occurred less often in patients with microsporidiosis (Table 3). No patient or control group had a disseminated infection.

DISCUSSION

This study is the first to identify a high frequency of microsporidia infections associated with intestinal mucosa disruption in rheumatic disease patients undergoing anti-TNF and DMARD therapies.

The study design has several advantages, including the inclusion of patients with well-established rheumatic disease criteria.¹⁴⁻¹⁶ In addition, the Gram-Chromotrope stain methodology applied in this study is considered to be one of the most specific detection techniques with the best sensitivity for detecting microsporidia in fluids, including feces.² Moreover, external factors such as socio-economic conditions may influence parasitosis prevalence. For example, the

frequency of microsporidia in HIV-infected patients was higher in Venezuela (13.6%), an underdeveloped country, than in Italy (1.8%).²⁷⁻²⁸ This potential bias was greatly reduced by the matching of our patients with a control group with the same socio-financial distribution. One limitation of our study was the absence of a control group of rheumatic disease patients treated only with DMARDs, due to the impossibility of achieving an adequate matching for disease duration and severity in these patients. In fact, anti-TNF therapy is now being indicated very early for patients with these diseases. There are, however, no data on the frequency of microsporidia infection in rheumatic disease patients without anti-TNF therapy.

Microsporidia has emerged as an important cause of infectious complications in severely immunocompromised patients with HIV, recipients of solid organ transplant and patients with hematological malignancies.¹⁻³ Cell-mediated immunity appears to be critical for protection against microsporidia through the T helper cell 1 (Th1) cytokine response.³ The importance of a Th1 response in the resistance to microsporidial infection has been demonstrated by *in vitro* studies showing that knockout animals for Th1 cytokines such as interferon and interleukin-12 could not clear microsporidia infections.²⁹ In fact, more severe microsporidia infections were observed in HIV-infected patients with declining CD4+ and CD8+ T-cell numbers.²⁹ Accordingly, the inhibition of TNF-alpha, a cytokine well known to be related to the Th1 response,³⁰ could possibly facilitate the microsporidia infestation observed in the present study. Moreover, experimental studies have reported a decrease in the specific protective IgG against microsporidia in animals treated with immunosuppressive drugs.³¹ We have confirmed that microsporidia infestation is more prevalent in immunosuppressed patients¹⁻⁴ and have now extended this observation to rheumatic disease patients undergoing anti-TNF/DMARD treatment.

Microsporidiosis and intestinal parasitosis may present with diverse clinical manifestations, depending on the host immune status and the microsporidium species.¹⁻⁴ Diarrhea and wasting syndromes are the most common complaints;¹⁻⁴ however, these parasite infections can be asymptomatic. In fact, the parasitosis in our study was frequently associated with gastrointestinal manifestations and concomitant stool leukocytes, indicating a possible intestinal mucosa disruption,³² which is a known risk for intestinal dissemination.³³

Infection is a major co-morbidity in rheumatic conditions, and therapies such as conventional DMARDs and anti-TNF are known to enhance the risk of infection.^{34,35} In this regard, previous reports have suggested that susceptibility to infection may be distinct in different underlying rheumatic diseases. The main explanation for this finding seems to be a disease-associated genetic background and immunosuppressive therapy.^{36,37} However, in the present study, no difference was observed in microsporidiosis frequency between the specific rheumatic diseases, suggesting that anti-TNF/DMARD treatment is a more relevant risk factor for this infestation than is the specific rheumatic disease itself.

We have identified microsporidiosis to be a frequent infection that is associated with mucosal lesions in patients undergoing concomitant anti-TNF/DMARD treatment. This finding supports the notion that the recommendation for the prophylactic use of anti-helminthic drugs in patients on

glucocorticoid therapy could be extended to those initiating anti-TNF therapy.

Competing interest: This study was supported by grants from FAPESP (2009/51897-5 to EB), CNPQ (3300665/2009-1 to JFC, 300248/2008-3 to CAS and 301411/2009-3 to EB), Federico Foundation (to JFC, CAS and EB) and Wyeth (to NEA).

REFERENCES

1. Stark D, Barratt JL, van Hal S, Marriott D, Harkness J, Ellis JT. Clinical significance of enteric protozoa in the immunosuppressed human population. *Clin Microbiol Rev.* 2009;22:634-50, doi: 10.1128/CMR.00017-09.
2. Didier ES. Microsporidiosis: an emerging and opportunistic infection in humans and animals. *Acta Trop.* 2005;94:61-76, doi: 10.1016/j.actatropica.2005.01.010.
3. Didier ES, Weiss LM. Microsporidiosis: current status. *Curr Opin Infect Dis.* 2006;19:485-92, doi: 10.1097/01.qco.0000244055.46382.23.
4. Franzen C, Müller A. Cryptosporidia and microsporidia—waterborne diseases in the immunocompromised host. *Diagn Microbiol Infect Dis.* 1999;34:245-62, doi: 10.1016/S0732-8893(99)00003-6.
5. Rychly DJ, DiPiro JT. Infections associated with tumor necrosis factor- α antagonists. *Pharmacotherapy.* 2005;25:1181-92, doi: 10.1592/phco.2005.25.9.1181.
6. Bernatsky S, Hudson M, Suissa S. Anti-rheumatic drug use and risk of serious infections in rheumatoid arthritis. *Rheumatology.* (Oxford) 2007;46:1157-60, doi: 10.1093/rheumatology/kem076.
7. Furst DE. The risk of infections with biologic therapies for rheumatoid arthritis. *Semin Arthritis Rheum.* 2010;39:327-46, doi: 10.1016/j.semarthrit.2008.10.002.
8. Krishnamurthy R, Dincer HE, Whittmore D. *Strongyloides stercoralis* hyperinfection in a patient with rheumatoid arthritis after anti-TNF- α therapy. *J Clin Rheumatol.* 2007;13:150-2, doi: 10.1097/RHU.0b013e3180690933.
9. Boatright MD, Wang BW. Clinical infection with *Strongyloides stercoralis* following etanercept use for rheumatoid arthritis. *Arthritis Rheum.* 2005;52:1336-7, doi: 10.1002/art.20882.
10. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med.* 2000;343:1594-602.
11. Keystone EC, Kavanaugh AF, Sharp JT, Tannenbaum H, Hua Y, Teoh LS, et al. Radiographic, clinical, and functional outcomes of treatment with adalimumab (a human anti-tumor necrosis factor monoclonal antibody) in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum.* 2004;50:1400-11, doi: 10.1002/art.20217.
12. Punzi L, Podswiadek M, Sfriso P, Oliviero F, Fiocco U, Todesco S. Pathogenetic and clinical rationale for TNF-blocking therapy in psoriatic arthritis. *Autoimmun Rev.* 2007;6:524-8, doi: 10.1016/j.autrev.2006.12.003.
13. Lovell DJ, Giannini EH, Reiff A, Cawksell GD, Silverman ED, Nocton JJ, et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N Engl J Med.* 2000;342:763-9.
14. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 1988;31:315-24, doi: 10.1002/art.1780310302.
15. Bennett PH, Wood PHN. Population Studies of the Rheumatic Diseases. New York, Excerpta Medica. 1968, p 456.
16. Dougados M, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum.* 1991;34:1218-27, doi: 10.1002/art.1780341003.
17. Almeida PM, Wickerrhauser H. Critério de classe econômica da Associação Brasileira de Anunciantes (ABA) e Associação Brasileira dos Institutos de Pesquisa de Mercado (ABIPEME). 1991, pp 1-129.
18. Prevo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum.* 1995;38:44-8, doi: 10.1002/art.1780380107.
19. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gaisford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol.* 1994;21:2286-91.
20. Koga K, Kasuya S, Khamboonruang C, Sukavat K, Nakamura Y, Tani S, et al. An evaluation of the agar plate method for the detection of *Strongyloides stercoralis* in northern Thailand. *J Trop Med Hyg.* 1990;93:183-8.
21. Ryan NJ, Sutherland G, Coughlan K, Globan M, Doultree J, Marshall J, et al. A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. *J Clin Microbiol.* 1993;31:3264-9.
22. Price DL. Comparison of three collection-preservation methods for detection of intestinal parasites. *J Clin Microbiol.* 1981;14:656-60.
23. Henriksen SA, Pohlenz JF. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Vet Scand.* 1981;22:594-6.
24. Pereira DB Jr. Utilization of the Kato-Katz-method (thick-smear technique) the diagnosis of *Isospora* (author's transl). *Rev Bras Pesqui Med Biol.* 1979;12:351-2.
25. Oyarzabal OA, Macklin KS, Barbaree JM, Miller RS. Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. *Appl Environ Microbiol.* 2005;71:3351-4, doi: 10.1128/AEM.71.6.3351-3354.2005.
26. Farthing MJ. Treatment options for the eradication of intestinal protozoa. *Nat Clin Pract Gastroenterol Hepatol.* 2006;3:436-45, doi: 10.1038/ncpgasthep0557.
27. Chacin-Bonilla L, Panunzio AP, Monsalve-Castillo FM, Parra-Cepeda IE, Martinez R. Microsporidiosis in Venezuela: prevalence of intestinal microsporidiosis and its contribution to diarrhea in a group of human immunodeficiency virus-infected patients from Zulia State. *Am J Trop Med Hyg.* 2006;74:482-6.
28. Marangi A, Maggi P, Panaro MA, Angarano G, Pastore G, Lisi S, et al. Intestinal microsporidiosis in AIDS patients with diarrhoeal illness in Apulia (south Italy) New Microbiol. 1995;18:435-9.
29. Khan IA, Didier ES. Insights into the immune responses to microsporidia. In: Lindsay DS, Weiss LM. World Class Parasites. Vol. 9. Toxoplasma, sarcocystis, and microsporidia. Boston MA: Kluwer Academic Publishers. 2004. 135-157.
30. Elenkov IJ. Glucocorticoids and the Th1/Th2 balance. *Ann N Y Acad Sci.* 2004;1024:138-46, doi: 10.1196/annals.1321.010.
31. Galván AL, Agudelo Sdel P, Restrepo JG, Toro F, Galviz LA, Botero J. Cyclosporine A effect in mice C57BL/6 infected with *Encephalitozoon intestinalis*. *Biomedica.* 2006;26:126-37.
32. Jindal N, Arora S. Role of faecal leucocytes in the diagnostic evaluation of acute diarrhoea. *Indian J Med Sci.* 1991;45:261-4.
33. Gupta DN, Saha DR, Sengupta PG, Mondal SK, Ghosh S, Saha MR, et al. Value of faecal leucocyte count as an indicator of invasiveness in mucoid diarrhoea. *J Commun Dis.* 1997;29:329-32.
34. Haroon N, Inman RD. Infectious complications of biological therapy. *Curr Opin Rheumatol.* 2009;21:397-403, doi: 10.1097/BOR.0b013e32832c792d.
35. Furst DE. The risk of infections with biologic therapies for rheumatoid arthritis. *Semin Arthritis Rheum.* 2010;39:327-46, doi: 10.1016/j.semarthrit.2008.10.002.
36. Burmester GR, Mease P, Dijkman BA, Gordon K, Lovell D, Panaccione R, et al. Adalimumab safety and mortality rates from global clinical trials of six immune-mediated inflammatory diseases. *Ann Rheum Dis.* 2009;68:1863-9, doi: 10.1136/ard.2008.102103.
37. Schiff MH, Burmester GR, Kent JD, Pangan AL, Kupper H, Fitzpatrick SB, et al. Safety analyses of adalimumab (HUMIRA) in global clinical trials and US postmarketing surveillance of patients with rheumatoid arthritis. *Ann Rheum Dis.* 2006;65:889-94, doi: 10.1136/ard.2005.043166.