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Original article

Systemic and localized infection by *Candida* species in patients with rheumatic diseases receiving anti-TNF therapy

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ABSTRACT

Objective: To evaluate the prevalence of systemic and localized infection by *Candida* species and its possible association with demographic, clinical and laboratory manifestations and therapy in patients with rheumatic diseases taking TNF blockers.
Methods: Consecutive patients with rheumatic diseases receiving anti-TNF agents were included. The following risk factors up to four weeks prior to the study were analyzed: use of antibiotics, immunosuppressant drugs, hospitalization and invasive procedures. All subjects were evaluated for clinical complaints; specific blood cultures were obtained for fungi and blood samples were collected for *Candida* spp. detection by polymerase chain reaction.
Results: 194 patients [67 with rheumatoid arthritis (RA), 47 with ankylosing spondylitis (AS), 36 with juvenile idiopathic arthritis (JIA), 28 with psoriatic arthritis and 16 with other conditions] were included. The average age of patients was 42 ± 16 years, with 68 (35%) male and mean disease duration of 15 ± 10 years. Sixty-four (33%) patients were receiving adalimumab, 59 (30%) etanercept and 71 (36%) infliximab. Eighty-one percent of patients were concomitantly taking immunosuppressant drugs. At the time of the study, only one (0.5%) patient had localized fungal infection (vaginal candidiasis). None of the patients included had systemic candidiasis with positive blood cultures for fungi or PCR positive for *Candida* spp. in peripheral blood sample.
Conclusions: This was the first study to assess the prevalence of invasive and localized fungal disease by *Candida* in a significant number of patients with rheumatic diseases on anti-TNF therapy, and demonstrated low risk of candidiasis, despite the high prevalence of immunosuppressive drug use.

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Infecção sistêmica e localizada por *Candida spp.* em pacientes reumatológicos em terapia anti-TNF

Palavras-chave:
Candidíase sistêmica
Candida spp.
Anti-TNF
Artrite reumatoide
Espondilite anquilosante

RESUMO

Objetivo: Avaliar a prevalência de infecção sistêmica e localizada por *Candida spp.* e sua possível associação com dados demográficos, manifestações clínicas e laboratoriais e terapêutica em pacientes com doenças reumatológicas em uso de anti-TNF.
Métodos: Foram incluídos pacientes consecutivos com doenças reumatológicas em uso de agentes anti-TNF. Foram analisados os seguintes fatores de risco até quatro semanas antes do estudo: uso de antibioticoterapia, imunossupressores, hospitalização e procedimentos invasivos. Todos foram avaliados para queixas clinicas, coletaram hemocultura específica para fungos e amostras de sangue para pesquisa de *Candida spp.* por reação em cadeia de polimerase.
Resultados: Foram incluídos 194 pacientes [67 com artrite reumatoide (AR), 47 espondilite anquilosante (EA), 36 artrite idiopática juvenil (AIJ), 28 artrite psoriásica e 16 outros]. A média de idade era de 42 ± 16 anos, com 68 (35%) do sexo masculino e média de duração de doença de 15 ± 10 anos; 64 (33%) pacientes usavam adalimumabe, 59 (36%) etanercepte e 71 (36%) infliximabe; 81% faziam uso concomitante de imunossupressores. No momento do estudo, apenas um (0,5%) paciente apresentou infecção fúngica localizada (candidíase vaginal). Nenhum dos pacientes incluídos apresentou candidíase sistêmica com hemocultura positiva para fungos ou PCR positiva para *Candida spp.* em amostra de sangue periférico.
Conclusões: Este foi o primeiro estudo que avaliou prevalência de doença fúngica invasiva e localizada por *Candida* em um expressivo número de pacientes reumatológicos em terapia anti-TNF e demonstrou baixo risco de candidíase, apesar da alta prevalência de uso de imunossupressores.

Introduction

Anti-TNF therapy has been widely used in patients with rheumatic diseases who do not respond to disease-modifying antirheumatic drugs (DMARDs), with a significant improvement in prognosis. However, at the same time, concerns related to immunosuppression due to such therapy emerge, generating increasing reports of infections by opportunistic fungal agents such as *Candida* spp.[1]

This fungus can produce a wide spectrum of clinical presentations, ranging from superficial mucocutaneous disease to severe invasive infections. Systemic candidiasis affects mainly patients treated with immunosuppressive drugs, including glucocorticoids and broad-spectrum antibiotics, and submitted to invasive procedures, with high mortality rates.[2–5]

Candidemia has previously been reported in small series of patients on biological therapy with TNF blockers, but a clear causal relationship has not been established.[6,7] In addition, there are rare reports of systemic infection by *Candida* spp. in patients with rheumatic diseases.[8,9]

The aim of this study was to determine the prevalence of systemic and localized candidiasis and its possible association with demographics, clinical and laboratory manifestations, and therapy in patients with rheumatic diseases in use of anti-TNF agents.

Materials and methods

Consecutive patients with a diagnosis of rheumatoid arthritis (RA),[10] ankylosing spondylitis (AS),[11] psoriatic arthritis (PsA),[12] juvenile idiopathic arthritis (JIA),[13] and with other conditions (Behcet's disease, Crohn's disease, reactive arthritis, Still's disease, idiopathic uveitis and Takayasu arteritis) were regularly followed in the Rheumatology Outpatient Clinic at the Hospital das Clinicas, Medical School, Universidade de São Paulo. All patients were treated with anti-TNF agents (adalimumab, etanercept or infliximab) at the Center of Dispensation of High Cost Medications (CEDMAC) and were in concomitant use of disease-modifying antirheumatic drugs (DMARDs).

This study was approved by the Institutional Ethics Committee, and informed consent was obtained from all participants.

Evaluation of rheumatic diseases

The disease activity was assessed using standardized instruments, including the Disease Activity Score (DAS28) for patients with RA[14] and JIA, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[15] for AS and PsA. The laboratory parameters were assessed through erythrocyte sedimentation rate (ESR), according to the Westergreen method

(mm/1st hour) and C-reactive protein (CRP) levels by nephelometry (mg/L).

The current treatment (concomitant to anti-TNF agent) with prednisone, DMARDs and immunosuppressant drugs (methotrexate, azathioprine, leflunomide, antimalarial, sulfasalazine and/or cyclosporine) was also evaluated.

Evaluation of candidiasis and risk factors

Patients were clinically evaluated on the day of application of anti-TNF therapy for signs and symptoms of infection with *Candida* spp., including oral candidiasis, genital candidiasis, or characteristic dysuria or leucorrhea. Up to four weeks prior to the study, the following risk factors were analyzed: use of antibiotics, use of immunosuppressant drugs, hospitalization and invasive procedures.

1. *Fungal peripheral blood culture*: Fungal peripheral blood culture in the population using anti-TNF agent was carried out with BD BACTECTM-MYCO/F Lytic Medium (Becton Dickinson, USA) blood cultures flasks.
2. *PCR for Candida spp. in peripheral blood*: Detection of *Candida* spp. DNA was performed by polymerase chain reaction.[16] The analysis of the collected material was held at the Medical Research Laboratory of Mycology (LIM-53) of HC-FMUSP in patients undergoing anti-TNF-alpha therapy.

DNA extractions from whole blood samples of those patients included in this study were performed according to the technique described by Loeffler et al.[17] Blood samples (3–5 ml) were collected in EDTA tube and subjected to initial hemolysis with 1.55 M NH_4Cl, 100 mM $KHCO_3$, and 10 mM EDTA pH 7.4. After obtaining the erythrocyte-free cell pellet, 3 ml of a solution containing 100 mM Tris–HCl pH 8.0, 4 M NaCl, and 20 mM EDTA pH 8.2 were added. Next, the samples were vigorously stirred, and 600 μL of a solution containing TRIS 50 mM, 10 mM EDTA, and lyticase 250 U/ml (L-4276, Sigma, USA) and 5% β-mercaptoethanol were added. The tubes were kept at 37 °C for 2:30 h and after the incubation period, the extraction of total DNA was performed with QIAamp® DNA Mini kit (QIAGEN, Germany), according to the protocol described by the supplier.

As positive controls in amplification reactions, DNA samples of *Candida albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, *C. lusitaniae* and *C. pelliculosa* were obtained.

For amplification of DNA extracted from blood samples from patients and samples of *Candida* spp., the technique of nested-PCR was used. In the first amplification step, ITS1 and ITS4 primers were used, and the second stage was designed for two amplification systems, using species-specific primers: system 1 comprises primers for *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. lusitaniae*; and the system 2, primers for *C. pelliculosa*, *C. parapsilosis* and *C. krusei*.

Detection of the amplified systems was carried out in agarose gels, at 2.5%, electrophoresed in horizontal tanks Horizon M-58 (Life Technologies, USA), in 1× TAE buffer (TRIS–acetic acid–EDTA pH 8.0), 80 V for 45 min. After electrophoresis, the gels were stained with GelRedTM (Biotium, USA), with results recorded on a photo documentation system (UVITEC, England).[18]

Statistical analysis

The results were presented as mean ± standard deviation (SD) or median (range) for continuous variables, and as percentage for categorical variables.

Results

Among the 194 patients included, 67 had RA, 47 had AS, 36 had JIA, 28 had psoriatic arthritis, and 16 had other diagnoses. The mean age of patients at the time of the study was 42 ± 16 years, with 68 (35%) male; mean disease duration was of 15 ± 10 years and mean duration of anti-TNF therapy was use 1.9 ± 1.6 years. As to ethnicity, 85% were Caucasian, 8% black, 7% brown and 1% yellow subjects. Sixty-four (33%) patients were receiving adalimumab, 59 (30%) etanercept, and 71 (36%) infliximab. Eighty-one percent of patients were concomitantly taking immunosuppressant drugs. Regarding concomitant medications, 96 (49%) used prednisone, 88 (45%), methotrexate, 46 (24%) leflunomide, 24 (12%) sulfasalazine, 10 (5%) cyclosporine, 6 (3%) azathioprine, and 11 (6%) chloroquine diphosphate (Table 1).

Among patients with RA, mean DAS28 was 3.6 ± 1.5, HAQ 1.1 ± 0.6, ESR 18 ± 16 mm/1st h and CRP 10.1 ± 15.4 mg/L. In patients with AS, mean BASDAI was 3.2 ± 2.6, BASFI 43 ± 32, ASQoL 6.7 ± 5.4, ESR 8 ± 8 mm/1st h, and CRP 4.3 ± 5, 2 mg/L.

Table 1 – Demographics and treatment data of patients with rheumatic diseases on anti-TNF therapy.

Variable	*n* = 194
Demographics	
Current age, years	42 ± 16
Male gender, n (%)	68 (35)
Race	
White, *n* (%)	165 (85)
Black, *n* (%)	16 (8)
Browns, *n* (%)	14 (7)
Yellow, *n* (%)	2 (1)
Disease duration, years	15 ± 10
Time of anti-TNF therapy, years	1.9 ± 1.6
Treatment	
Anti-TNF	
Adalimumab, *n* (%)	64 (33)
Etanercept, *n* (%)	59 (30)
Infliximab, *n* (%)	71 (36)
Glucocorticoids, *n* (%)	96 (49)
Mean dose, mg/day	7.8 ± 4.6
Methotrexate, *n* (%)	88 (45)
Average dose, mg/week	22.2 ± 8.6
Leflunomide, *n* (%)	46 (24)
Sulfasalazine, *n* (%)	24 (12)
Cyclosporine, *n* (%)	10 (5)
Azathioprine, *n* (%)	6 (3)
Chloroquine, *n* (%)	11 (6)

Data are presented as number (%) and mean ± standard deviation.

Table 2 – Clinical and laboratory parameters of disease in patients with rheumatic diseases receiving anti-TNF therapy.

Variables	
Rheumatoid arthritis (n = 67)	
DAS28	3.6 ± 1.5
HAQ	1.1 ± 0.6
ESR, mm/1st hour	18 ± 16
CRP, mg/L	10.1 ± 15.4
Ankylosing spondylitis (n = 47)	
BASDAI	3.2 ± 2.6
BASFI	43 ± 32
ASQoL	6.7 ± 5.4
ESR, mm/1ª hora	8 ± 8
CRP, mg/L	4.3 ± 5.2
Juvenile Idiopathic Arthritis (n = 36)	
DAS28	3.4 ± 1.3
ESR, mm/1st hour	15 ± 18
CRP, mg/L	19.5 ± 55.1
Psoriatic arthritis (n = 28)	
DAS28	2.5 ± 1.3
BASDAI	3 ± 1.7
ESR, mm/1st hour	10.3 ± 7.8
CRP, mg/L	4.2 ± 4.7

DAS28, Disease Activity Score 28; HAQ, Health Assessment Questionnaire; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index.
Data are presented as number (%) and mean ± standard deviation.

Among patients with JIA, mean DAS28 was 3.4 ± 1.3, ESR 15 ± 18 mm/1st h and CRP 19.5 ± 55, 1 mg/L (Table 2).

At the time of the study, only one (0.5%) patient had localized fungal infection (vaginal candidiasis) and none had oral thrush. None of the included patients had candidemia with positive blood cultures for fungi or PCR positive for *Candida* spp. in peripheral blood samples.

The analysis of possible risk factors for *Candida* infection revealed that only 14 (7.2%) had received antibiotic therapy up to four weeks before the evaluation, 9 for respiratory tract infection, 3 for cutaneous infection, 1 for urinary tract infection and 1 for odontogenic infection. Furthermore, no patient was hospitalized or was subjected to invasive procedures up to one month prior the study entry.

Discussion

This was the first study to assess the prevalence of invasive and localized fungal disease caused by candida in a significant number of patients with rheumatic diseases treated with anti-TNF therapy, showing low risk of candidiasis, despite the high prevalence of immunosuppressive drugs use.

Epidemiological studies have shown a considerable increase in infections in immunocompromised patients, including *Candida* spp., particularly in nosocomial sepsis, with a high mortality rate. *Candida albicans* is considered as a commensal member of the normal flora of the digestive tract and its pathogenicity is the result of alterations in host defense mechanisms that induce behavioral changes of the fungus.[19,20]

Patients with rheumatic diseases are often exposed to several risk factors associated with a significant increase in fungal infection incidence in recent decades. The use of broad-spectrum antibiotics can cause changes in mucosal flora, leading to the proliferation of candida; corticosteroids can affect the activity of polymorphonuclear cells, macrophages and T cells; surgical procedures and the use of immunosuppressive drugs, particularly biologicals, facilitate the spread of opportunistic pathogens.[2,21] Actually, in the published literature, in 98% of invasive fungal infections such as histoplasmosis, candidiasis and aspergillosis, the use of at least one immunosuppressive agent, particularly corticosteroids, associated with anti-TNF therapy, was reported.[1]

Innate immune response is the dominant mechanism responsible for yeast clearance after an initial systemic infection. Monocytes and macrophages are the cells most associated with response against systemic infection with *C. albicans*. The pathways by which this event occurs are not entirely clear, but appear to involve recognition of pathogen-associated molecular patterns (PAMPs) on the yeast mediated by phagocytic cell receptors, resulting in activation and release of inflammatory cytokines.[19] Furthermore, the spectrum of defenses against mucocutaneous and systemic candidal infection includes cell-mediated immunity which is characterized by the release of cytokines by lymphocytes and by the activation of NK cells and lymphocytes by interleukins. However, there is evidence to support the role of humoral response in the protection against invasive *Candida* infection.[19,20]

It is known that, in cases of systemic infection with *C. albicans*, TNF production is stimulated by the pathogen. Louie et al.[22] demonstrated, in an animal model of systemic candidiasis, that this cytokine has a protective role in infection. The neutralization of TNF activity would lead to suppression of the production of IFN, promotion of monocyte apoptosis and prevention of maintenance of granuloma, allowing fungus growth in several organs.[1] Indeed, in a review of studies on invasive fungal infections associated with anti-TNF therapy (infliximab, adalimumab, etanercept) in various diseases (graft vs. host disease, inflammatory bowel disease and RA) between 1966 and 2007, Tsiodras et al.[1] found 281 cases. Candidiasis was responsible for 23% of infections and, of these, only 3 occurred in patients with RA. Although evidence in the literature suggests that there may be differences regarding the risk of infection among different anti-TNF drugs,[23] the present study showed low frequencies of candidiasis for the three agents used.

Moreover, among patients with rheumatic diseases there are only two septic arthritis reports by *Candida* spp. in patients with rheumatoid arthritis in anti-TNF therapy.[8,9] However, in both cases, the concomitant use of other immunosuppressive agents may have contributed to the development of candida infection.

The PCR methodology used for the detection of *Candida* spp. in this study does not appear to justify the absence of positivity, since this is considered one of the most sensitive modalities for diagnosing candidemia, compared to traditional methods.[24] In a study of mice with induced candidemia, the sensitivity of this technique was 79%.[25] On the other hand,

the fungal isolation in culture of sterile fluid such as blood and peritoneal fluid is associated with a low recovery rate, reaching only a positivity of 40–60% in patients with systemic candidiasis confirmed by autopsy.[26]

From this study, we concluded that invasive and localized fungal disease by candida did not represent a common infection in patients with rheumatic diseases with immunosuppressive therapy associated with anti-TNF agents.

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Conflicts of interest

The authors declare no conflicts of interest.

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