Screening with urine *Histoplasma* antigen test in asymptomatic patients starting TNF-alpha inhibitor therapy: a cohort study

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Abstract

Background: Histoplasmosis is the second most frequent granulomatous disease in patients treated with tumor necrosis factor (TNF)-α inhibitors, second only to tuberculosis. However, there is limited information about pre-therapy screening procedures and the need for preventive treatments for patients who will start immunobiologicals.

Methods: This is a cohort study that evaluated the prevalence of histoplasmosis in asymptomatic HIV-negative patients before initiation of TNF-α inhibitors by testing for *Histoplasma* antigen in urine samples. The patients included completed a 180-day follow-up after the initiation of the biologics to assess the onset of symptoms suggestive of histoplasmosis.

Results: From January 2021 to December 2022, 54 patients who were prescribed a TNF-α inhibitor agent for treating autoimmune diseases in centers in southern Brazil were included. In the screening before therapy, the prevalence of a positive urinary *Histoplasma* antigen test was 14.8%. None of the 54 patients developed histoplasmosis after 6 months of immunobiological therapy, including the eight patients who tested positive.

Conclusion: The prevalence of *Histoplasma capsulatum* infection in chronic patients may be higher than expected, but the impact of latent infection in asymptomatic patients is still uncertain, including those starting treatment with immunobiological drugs such as TNF-α inhibitors. Our study did not identify risk factors for the diagnosis of disseminated histoplasmosis in this group, including a positive result in an antigen test performed before immunobiological therapy. To date, there is no evidence to recommend routine antigen-based screening or preventive therapy for histoplasmosis before initiating a TNF-α inhibitor.

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Plain language summary

Using a urine test for fungal infection to screen people without symptoms who are about to start taking immunobiologic medications

This study looked at the prevalence of histoplasmosis, a fungal infection, in asymptomatic patients who were about to start treatment with TNF-α inhibitors, which are medications used for autoimmune diseases. The researchers tested urine samples for Histoplasma antigen before the patients started the treatment and followed them for 180 days after starting the medication to see if they developed any symptoms of histoplasmosis. The study included 54 patients in southern Brazil, and they found that 14.8% of the patients

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tested positive for the Histoplasma antigen before starting the treatment. However, none of the patients, including those who tested positive, developed histoplasmosis during the 6-month follow-up. The researchers concluded that histoplasmosis infection may be more common in these patients than previously thought, but it's still not clear if asymptomatic patients with a positive antigen test will develop the infection when starting TNF-α inhibitor treatment. The study did not find any specific risk factors for developing histoplasmosis in this group of patients, and based on their findings, they did not recommend routine screening or preventive therapy for histoplasmosis before starting TNF-α inhibitor treatment.

Introduction

Treatment of autoimmune inflammatory diseases refractory to traditional medicines has significantly progressed with the development of immunobiological therapies in the past few decades. In particular, therapies for conditions such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD), psoriasis, psoriatic arthritis, and ankylosing spondylitis were greatly enhanced since the approval of the tumor necrosis factor-alpha inhibitors (TNF-α inhibitors, also called Anti-TNF-α), by the Food and Drug Administration (FDA) in the late 1990s.[1–5] However, the potent immunomodulatory effects of these medications may impact the necessary immune response against various infections.[6–9] Consequently, some of the biological therapies have been associated with an increased risk for opportunistic infections, including viral, bacterial, or fungal diseases, requiring individualized screening procedures for each one.[10] The necessity for clinical screening and sometimes treatment of infections that may still be in an asymptomatic phase before initiating a TNF-α inhibitory agent is well established for some conditions, including hepatitis B and C and tuberculosis.[11–13]

Specifically, tuberculosis has its risk increased by treatment with all approved TNF-α inhibitors and has been identified as the most common granulomatous disease complicating this therapy.[14–16] Many of the cases of tuberculosis associated with TNF-α blockade represent reactivation of latent tuberculosis infection (LTBI), therefore, screening with a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) is sought before starting therapy.[17–19] Those patients with a positive TST and IGRA often demand further evaluation with a chest X-ray and generally require initiation of a regimen for LTBI before TNF-α inhibitor initiation.[11,13,20] There is a demand to clarify whether this strategy of screening and prophylactic treatment can be applied to other infectious diseases associated with immunobiologicals, particularly histoplasmosis.

Histoplasmosis is considered the second most frequent granulomatous infection in people treated with TNF-α inhibitors, as well as the most frequent opportunistic fungal infection in endemic areas, with a life-threatening potential.[15,21,22] Similar to what occurs in tuberculosis, previous studies have already demonstrated that reactivation of a latent infection after TNF-α blockade is a possible illness mechanism in histoplasmosis, although it is not clear whether this is the primary process.[23–25] Non-culture-based tests have revolutionized the diagnosis of patients with disseminated forms of histoplasmosis, traditionally challenging by classical technics, but some asymptomatic patients may also present with *Histoplasma capsulatum* antigen detected in body fluids when evaluated using these methods, such as the urine detection assay.[26,27] While routine screening with *Histoplasma* serology or antigen is generally not recommended, it remains unclear

whether those asymptomatic patients who present with a positive test during screening before initiation of a TNF-α inhibitor require a preemptive treatment or are at a particularly increased risk of developing histoplasmosis after initiation of immunobiological therapy.[28,29] Therefore, this study proposes a measurement of the prevalence of *Histoplasma* infection in people who use biological drugs through antigen-based diagnosis, as well as follow-up with these patients after the initiation of TNF-α inhibitor therapy, to assess the prognosis of those infected by the pathogen, and identify a risk profile that could help guide future preventive strategies.

Methods

Study design and setting
This is a prospective cohort study, with recruitment conducted from January 2021 to December 2022, in two reference institutions for patients with autoimmune diseases with indication for infusion of immunobiologicals in a city located in southern Brazil, a region where histoplasmosis is endemic. The research participants were people receiving biological therapies who maintained regular follow-ups at participating care centers. All patients who met the inclusion and exclusion criteria in the proposed period were approached.

Inclusion and exclusion criteria
Patients aged 18 years or older, under medical follow-up due to an autoimmune disease, with a plan to initiate a TNF-α inhibitor drug by the first time (e.g. *infliximab*, *etanercept*, *adalimumab*, *certolizumab pegol* or *golimumab*) within 30 days from the date of the interview and sample collection were considered eligible. Candidates who live with HIV, patients using antifungal agents with activity against *H. capsulatum* (e.g. *amphotericin B*, *itraconazole*, *voriconazole*, *posaconazole*), or patients with recent diagnosis (<1 year) of disseminated histoplasmosis were excluded.

Primary and secondary objectives
The primary objective of this study was to determine the prevalence and risk factors for disseminated histoplasmosis in non-HIV patients receiving biological therapies. Secondary objectives included determining morbidity and mortality in non-HIV patients with histoplasmosis,

documenting exposure factors associated with histoplasmosis, determining clinical variables that can predict the occurrence of disseminated histoplasmosis, as well as prognostic factors for this disease, relating possibly other infectious diagnoses established with the occurrence of the fungal disease, determining risk for disseminated histoplasmosis according to the immunobiological agent used and the disease being, and promoting information for education and prevention actions in people receiving therapies with biologicals.

Data collection and sample processing
The interview to record data for the clinical records and the collection of samples for laboratory tests, as well as the signing of the FICF, were carried out at the participating service center, during their regular medical appointments for screening before starting immunobiological medication. Once the patient accepted to participate, and after the signature of the FICF, clinical information was collected and the patient performed spontaneous urination in a specific bottle for collection, at the assistance site. The test for detection of *Histoplasma* antigen used was the '*Histoplasma galactomannan (HGM) single-monoclonal-antibody sandwich ELISA*' (Immuno-Mycologics, Norman, OK, USA) using monoclonal antibodies against *H. capsulatum*. The test was performed on the urine samples, according to the manufacturer's instructions. Antigen concentration values higher than 0.2 ng/mL were considered positive for the detection of *Histoplasma* antigen in the samples. Clinical variables evaluated in the data collection form included: age, sex, place of residence, occupation/profession, epidemiological information on risk for histoplasmosis (rural activity, contact with chicken coops, exposure to caves, exposure to places with bats, tunnels, civil construction, and places with birds), signs/symptoms of fungal disease, antifungal treatment used, survival. The follow-up was carried out with phone calls and analysis of information from the medical records, in 30 and 180 days after initiation of the biological drug. In the follow-up evaluation, clinical outcome data (including mortality and occurrence of fungal disease) were collected.

Sample size and statistical analysis
In the primary calculations for planning the sample size of the project, in which an estimated prevalence of 3% of *Histoplasma* antigen positivity tests

Table 1. Main characteristics of the patients at the time of inclusion.

Characteristic (n = 54)	Number (%)
Female	34 (63%)
Age, years (mean, range)	46.4 (18–77)
Race	
White	51 (94.5%)
Black	3 (5.5%)
Other/unknown	0
Underlying disorder	
Rheumatoid arthritis (including JRA)	11 (20%)
Inflammatory bowel disease	42 (78%)
Psoriatic arthritis	1 (2%)
TNF-α inhibitor used	
Infliximab	31 (57.4%)
Adalimumab	21 (38.3%)
Etanercept	2 (3.7%)
Additional immunosuppressants	
Corticosteroids	15 (27.7%)
Methotrexate	14 (25.9%)
Azathioprine	22 (40.7%)
Leflunomide	6 (11.1%)
Others	0
None	8 (14.8%)
Two additional immunosuppressants	10 (18.5%)

JRA, juvenile rheumatoid arthritis; TNF, tumor necrosis factor.

was considered in patients candidates for the use of immunobiologicals in the region of the centers participating in the study, with a margin of error of ±3% and a level of 95% confidence, the minimum sample size was calculated at 125 individuals. Considering a safety margin for possible losses, the originally planned sample size was 140 patients. The recruitment stage of our project was largely affected by the COVID-19 pandemic since many patients avoided presential appointments at the centers or had the beginning of therapy with

biologics postponed. However, the prevalence of patients with a positive *Histoplasma* antigen test in the urine sample was considerably higher than in the original proposal. Thus, in a *post hoc* analysis considering a sample size of 54 patients, with an expected percentage for the prevalence of histoplasmosis of 14.8%, it was possible to estimate the proportion of occurrence of the outcome with a margin of error of ±9.5% for the 95% confidence interval.[30] Quantitative variables were treated with Student's *t*-test, Mann–Whitney *U*-test, or the nonparametric Kruskal–Wallis test, when appropriate. Categorical data were evaluated using the Chi-square test or Fisher's exact test, as appropriate. Statistical tests were performed using the SPSS v29 program (Chigago, IL, United States of America). *p* Values equal to or less than 0.05 were considered statistically significant.

Results

During the 2-year period in which this study was developed, 56 patients were evaluated and consented to participate in the research, and of this group, 54 were included in the final analysis after meeting all the inclusion and exclusion criteria and completing the 6-month follow-up. Two patients who were interviewed turned out not to meet the inclusion criteria: one female patient had her treatment changed to tofacitinib, and for the other, the assistant team opted for continued management without biologicals. Four patients approached during the recruitment time did not consent to participate in the research.

The main characteristics of the 54 patients are summarized in Table 1. Most patients were White and middle-aged, with a slightly female majority. Although the searches were conducted in both the Rheumatology and Gastroenterology outpatient clinics, most patients who received a prescription for a TNF-α inhibitor were in treatment for IBD. The preferred drugs were infliximab and adalimumab, and only two patients received etanercept, both for the treatment of RA. Notably, 38 patients (85%) used additional immunosuppressive agents while initiating the TNF-α blocking therapy, with 15 (27.7%) using corticosteroids and 10 (18.5%) requiring two additional agents.

After consent and application of the questionnaire, all 54 patients included in this research had a urine sample collected for the HGM

single-monoclonal-antibody sandwich ELISA test. Of these, eight patients (14.8% of the total) had a positive result in the *Histoplasma* antigen test in the urine sample. All tests were collected during the screening period preceding the beginning of immunobiological medication, and none of the patients had fever or symptoms of fungal disease at the time. The comparison between the baseline characteristics and environmental factors of patients with a positive *Histoplasma* antigen test in urine and those with a negative result is exhibited in Table 2. Patients in both groups were similar in general, although some trends were observed. Patients who had a positive *Histoplasma* antigen test tended to be more immunosuppressed and to present more affirmative answers when asked about environmental exposure to birds or chicken coops. The main baseline characteristics of patients with a positive urinary *Histoplasma* antigen test are summarized in Table 3.

There was no diagnosis of histoplasmosis in any of the 54 patients during the follow-up period of the study. The patients were interviewed by telephone on days 30 and 180 after starting the use of the TNF-α inhibitor and asked about symptoms compatible with histoplasmosis, diagnosis of any infectious disease, and the necessity of antifungal treatments. The patients' medical records at each center were reviewed to assess whether there were hospitalizations due to opportunistic disease, or description of symptoms or diagnosis of histoplasmosis at the medical appointments during the corresponding period. There was no record of hospitalization for investigation of fungal disease or clinical suspicion of histoplasmosis in the medical evaluations of all the participating patients up to the 6-month observation period. Likewise, there were no deaths from any cause in both groups, making it impossible to evaluate the mortality rate or prognostic factors for harsh outcomes.

Discussion

One of our objectives was to estimate the overall prevalence of *H. capsulatum* infection in patients with autoimmune diseases, with no suspected fungal disease. Large prevalence inquiries of infectious diseases in a population were always challenging to accomplish. Historically, the histoplasmin skin test was used to establish areas where histoplasmosis was endemic since a positive result was often interpreted as previous

contact with *H. capsulatum*.[31] Estimates are that in the United States, between 60% and 90% of people living in areas around the Ohio and Mississippi River valleys were exposed to the fungus at some point in their lives.[32,33] In Brazil, where the fungus is widespread across the territory, the overall positive reactivity in the histoplasmin skin test identified in historical series was 19.3%, with some regions reaching 90%.[34] Meanwhile, prevalence studies with molecular tests are scarce, and their interpretation is complex. Many of those who inhale *Histoplasma* conidia experience asymptomatic hematogenous spread of the fungus through the reticuloendothelial system *via* infected macrophages and therefore detection of circulating antigen is commonly considered infection.[31,35] Although molecular tests are highly specific for the diagnosis of disseminated histoplasmosis in the immunosuppressed patient, the clinical significance of a positive antigen detection test for an immunocompetent asymptomatic patient, especially at low levels, remains unclear.[36]

As mentioned, our recruitment stage was compromised by the COVID-19 pandemic. Nevertheless, the prevalence of a positive test for histoplasmosis was surprising. The *Histoplasma* antigen positivity rate found in asymptomatic patients before biological therapy was 14.8%, almost five times higher than the proposed estimate of 3%. This finding can be considered compatible with the high rates of positive histoplasmin skin tests found in classic studies conducted in the Brazilian state in which the participating centers are located, and the assisted patients usually reside.[37] Furthermore, there is an elevated prevalence of immunosuppressed individuals, mainly HIV/AIDS patients, who present at emergency services in this same region and are diagnosed with histoplasmosis.[38] It is important to emphasize that the patients participating in our cohort had no previous history of use of immunobiological medication and did not have symptoms or clinical suspicion of fungal infection at the time of collection of the urine sample for antigen testing.

Despite the final sample size limiting the statistical power for the analysis of characteristics detailed in Table 2, the trends pointed out are relevant. Patients with a positive *Histoplasma* antigen test tended to be more immunosuppressed, and the use of leflunomide reached statistical

Table 2. Comparison between patients with positive and negative *Histoplasma* antigen test in urine samples.

Characteristic	Positive urinary HAg (*n* = 8)	Negative urinary Hag (*n* = 46)	*p* Value
Female	5 (62.5%)	29 (63%)	1.0
Age, years (median, IQ range)	55.5 (39.2–71.7)	47.0 (32.1–61.8)	0.188
Race			
White	6 (75%)	45 (97.8%)	0.054
Black	2 (25%)	1 (2.2%)	
Underlying disorder			
Rheumatoid arthritis (including JRA)	3 (37.5%)	8 (17.4%)	0.192
Inflammatory bowel disease	5 (62.5%)	37 (80.4%)	0.260
Psoriatic arthritis	0 (0%)	1 (2.2%)	1.0
Diagnosis time, years (median, IQ range)	9.0 (2.5–15.5)	5.0 (1–9)	0.510
Additional immunosuppressants			
Corticosteroids	3 (37.5%)	12 (26%)	0.506
Methotrexate	2 (25%)	12 (26%)	0.948
Azathioprine	3 (37.5%)	19 (41.3%)	0.840
Leflunomide	3 (37.5%)	3 (6.5%)	0.036
Two additional immunosuppressants	3 (37.5%)	7 (15.2%)	0.134
Any immunosuppressants	8 (100%)	38 (82.6%)	0.201
Environmental factors	2 (25%)	8 (17.4%)	0.609
Rural residence	4 (50%)	25 (54.3%)	0.820
Urban residence	2 (25%)	13 (28.3%)	0.849
Suburban residence	3 (37.5%)	10 (21.7%)	0.336
Exposure to chicken coops	5 (52.5%)	16 (34.8%)	0.138
Exposure to birds	5 (62.5%)	14 (30.4%)	0.080
Exposure to bats	0 (0%)	5 (10.9%)	0.587
Exposure to caves	1 (12.5%)	5 (10.9%)	1.0
Exposure to tunnels	1 (12.5%)	3 (6.5%)	1.0
Exposure to civil construction locations	4 (50%)	26 (56.5%)	0.732
Negative for all exposures	1 (12.5%)	9 (19.6%)	0.635

HAg, urinary *Histoplasma* antigen test; IQ range, interquartile range; JRA, juvenile rheumatoid arthritis.

Table 3. Baseline characteristics of patients with a positive *Histoplasma* antigen test in urine samples.

Case	Gender	Age	Race	Residence	Underlying disorder	Diagnosis time, years	Additional immunosuppressants	TNF-α inhibitor prescribed
1	Male	62	White	Urban area	RA	2	LEF	ADA
2	Female	54	Black	Urban area	IBD	1	AZA	IFX
3	Male	63	White	Urban area	RA	4	MTX, LEF	ADA
4	Male	57	Black	Urban area	IBD	20	AZA	IFX
5	Female	45	White	Urban area	IBD	17	AZA	IFX
6	Female	45	White	Rural area	IBD	0.8	CORT	ADA
7	Female	60	White	Urban area	RA	12	CORT, MTX	ADA
8	Female	46	White	Rural area	IBD	10	CORT, LEF	ADA

ADA, adalimumab; AZA, azathioprine; CORT, corticosteroids; IBD, inflammatory bowel disease; IFX, infliximab; LEF, leflunomide; MTX, methotrexate; RA, rheumatoid arthritis.

significance when compared to the group with a negative antigen test. Patients with a positive test also tended to have greater environmental exposure to birds and chicken coops, known sources of exposure to *Histoplasma*.[31]

None of the 54 patients included in the cohort were diagnosed with histoplasmosis during the 6-month follow-up period of the study, including the eight patients with a positive result for the urinary *Histoplasma* antigen. Multiple strategies were employed to maintain track of each case and avoid missing the record of an event, including telephone calls to report symptoms, review of medical appointment registries with the assisting teams, and review of medical records at the centers to verify admissions for any cause or need of emergency care. The results of *Histoplasma* antigen tests were kept confidential to the assisting teams, so they were not informed about the positive tests in asymptomatic patients and were not encouraged to investigate fungal disease before or after the initiation of biological therapy.

The follow-up time of 180 days after the start of immunobiological therapy can be considered adequate, since, in studies involving TNF-α inhibitors, a considerable part of the diagnoses of disseminated histoplasmosis occurs within this period. In the first case series of severe disease caused by *H. capsulatum* in patients using TNF-α inhibitors, published in 2002 by Lee *et al.*, 10 cases of histoplasmosis were reported, nine associated with the use of infliximab and one associated with etanercept.[23] In those treated with infliximab, manifestations of histoplasmosis occurred within 1 week to 6 months after the beginning of therapy, mostly, including fever, malaise, cough, dyspnea, and interstitial pneumonitis. The only patient using etanercept developed symptoms after 11 months of treatment.

Whether the occurrence of histoplasmosis is due to the reactivation of latent infection after initiation of the TNF-α inhibitor or whether it is the emergence of a new infection, or reinfection, that could not be contained by an immunosuppressed host has been debated since the emergence of the first cases in this population. Nakelchik and Mangino, in a report published in 2002, describe the case of a 52-year-old woman who developed severe histoplasmosis only 9 weeks after starting therapy with infliximab plus low-dose prednisone for RA, despite having a long history of treatment with other immunosuppressants, such as mycophenolate, methotrexate and high dose corticoid.[24] The patient was a lifelong resident of Ohio, with no relevant travel history. Examining the temporality of events, she was considered to have experienced reactivation of histoplasmosis, even though she lived in an endemic area for the infection. The first clear report documenting the reactivation of latent histoplasmosis after initiation of a TNF-α inhibitor was published in 2006

by Jain *et al.*[25] A male patient developed symptoms approximately 1 year after starting treatment with infliximab plus intermittent corticosteroids and was diagnosed after the growth of *H. capsulatum* in bronchoalveolar lavage culture. Although he denied travel in the last 5 years outside of California, a US state where *Histoplasma* is infrequent, he had traveled 5 years before to an area endemic for the fungus, suggesting reactivation of a previous infection. In a large review on granulomatous infections in patients on TNF-α blockers, by Wallis *et al.* in 2004, the authors compared the number of cases of granulomatous infections in US patients treated with infliximab or etanercept, as reported to the FDA Adverse Event Report System from January 1998 to September 2002.[15] Although the occurrence of 72% of infliximab-associated granulomatous infections within the first 90 days of treatment may be considered compatible with reactivation of latent infection, compared with 28% for etanercept, there was no specification of how many of these cases were of histoplasmosis.[15] In our cohort, infliximab was the most frequent agent, corresponding to 57.4% of TNF-α inhibitor prescriptions, and only two patients used etanercept.

While reactivation of latent lesions is considered the chief mechanism observed in patients diagnosed with tuberculosis after initiation of TNF-α inhibitors, this may not be the case for histoplasmosis.[16] On the one hand, reactivation was suggested by the studies addressed, and it is argued that a much higher incidence of histoplasmosis would be expected in users of these biological therapies in the endemic areas if reactivation were the major mechanism. Such perspective takes into account the difference observed in the number of people with a positive reaction in the histoplasmin skin test, the marker of prior contact with *Histoplasma* which can achieve 90% of adults in endemic areas, and the nonproportional increase in the incidence of histoplasmosis in patients after the beginning of TNF-α inhibitory therapy, which reaches up to 18.78 cases per 100,000 infliximab users as described by Wallis *et al.*, that is, an incidence of 0.00018% of adults.[15,32,33] Another analysis that averts reactivation as the primary mechanism after immunosuppression is a retrospective chart review of 586 patients who underwent solid organ or bone marrow transplantation from January 1994 to December 1996 in Indiana, an endemic area where 50–80% of the population has evidence of previous *Histoplasma* infection as per skin tests.[39] Despite 18% of patients having positive pretransplant histoplasmosis serologic tests and 4% having chest radiographs consistent with previous granulomatous disease, none developed histoplasmosis during a 3-year follow-up period.

Our results do not favor the reactivation of latent histoplasmosis after initiation of TNF-blocking therapy as the leading mechanism of illness. Despite the small number of patients compared to larger population studies, there were eight patients with evidence of current *Histoplasma* infection at the moment of initiation of immunosuppression, and none developed disseminated histoplasmosis or another form of histoplasmosis after therapy.

TNF-α is one of the most important inflammatory mediators of organisms, especially in acute processes of immune response to pathogenic microorganisms, including *fungi*.[40,41] TNF-α local effects include the recruitment of neutrophils and macrophages to the site of infection, through chemosignaling and stimulation of the production of adhesion molecules in the vascular endothelium, among other functions.[42,43] Systemic effects include participation in hypothalamic signaling for the generation of fever, production of acute phase proteins by the liver, and promotion of cell differentiation in the bone marrow.[42,44] The effects of TNF-α inhibition on the control of acute or latent infections are still being understood.

In addition to TNF, classical studies have already identified that other cytokines are crucial for the host response against *Histoplasma*, including interferon-gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor, and interleukin (IL)-1β, demonstrating that depletion of any of these in mice converts a nonlethal infection into a lethal one.[45] Specifically for the *H. capsulatum*-induced granuloma, IFN-γ, IL-17, and IL-22, whose primary local source is both CD4+ and CD8+ T cell subsets, together with TNF-α produced by the local macrophages, appear to play a major role in the pathogen control.[46,47] Modern studies have characterized the regulatory function of IL-17 in the balance between Th1 and Th2 responses, as well as demonstrated the existence of an IL-17/IL-23 regulatory axis in the

defense against *Histoplasma*.[47,48] Thus, the compensatory mechanisms of the immune response may be sufficient to maintain the stability of the granuloma during the blockade of TNF-α, preventing reactivation of the *Histoplasma* present in latent lesions, but insufficient in containing an acute exposure to novel inhaled conidia.

Considering that higher vulnerability to a new exposure may be a more important mechanism than reactivation, the need for screening for histoplasmosis before initiating TNF-α inhibitory therapy is uncertain. In those who are starting therapy, specialists recommend the investigation of symptoms of active or recent histoplasmosis, as well as arguing about activities with potential risk of exposure to the fungus after starting the medication.[28] Hage *et al.*[28] also recommend that patients with suspected histoplasmosis in the 2 years before initiation of TNF-α inhibitor therapy undergo chest radiography. Routine screening with *Histoplasma* serology or antigen is generally not recommended by specialists or guidelines.[11,28,29,49,50] Accordingly, our findings do not suggest the adoption of routine screening for *Histoplasma* antigen in urine. In the study involving patients undergoing solid organ or bone marrow transplantation, serological or radiographic evidence of prior histoplasmosis infection was also not predictive of disease in the immunocompromised population.[39] Since the risk of reactivation of histoplasmosis is not established, prophylactic or preemptive therapy has not been recommended either. The same question remains open regarding the care of people living with HIV, where there is no evidence to guide preemptive therapy for asymptomatic patients with positive *Histoplasma* antigen test, and such a strategy is not advised.[38]

Limitations of our study certainly include our final sample size, since it was not possible to reach the originally proposed number of participants. Despite the impact on accuracy, however, the sample size was sufficient to estimate the prevalence of *Histoplasma* antigen positivity rate in the population. Antigen concentration values higher than 0.2 ng/mL were considered positive for detection of *Histoplasma* antigen in the samples. Unfortunately, we were unable to retrieve the antigen concentration value for some of the eight patients with a positive test, which is an important limitation of our study. The number of

included patients also limited the statistical power to identify the environmental factors that are more related to a higher probability of a positive screening for *Histoplasma* infection or occurrence of disseminated histoplasmosis due to therapy. Finally, there is an inherent limitation to the diagnostic method used, since, although the antigen test is reasonably specific, there is the possibility of false positive results for *Histoplasma* detection due to the presence of other endemic mycoses, such as *Blastomyces*, *Coccidioides*, *Penicillium*, *Paracoccidioides*, *Aspergillus*, and antithymocyte globulin as part of antirejection treatment transplant patients.[51,52] Nevertheless, none of the participating patients was diagnosed with another mycosis during follow-up.

Conclusion
The positivity rate of a *H. capsulatum* antigen test in samples of asymptomatic patients may be higher than what is currently considered, including people with chronic diseases or non-HIV immunosuppressed patients. The impact of a latent or asymptomatic *Histoplasma* infection in patients treated with immunobiological drugs such as TNF-α inhibitors is uncertain, still, there is no evidence to recommend routine screening with molecular methods or preemptive therapy at the moment. Our study could not identify risk factors for the diagnosis of disseminated histoplasmosis in patients who initiate TNF-α blocking therapy, including a positive *Histoplasma* antigen test during pretreatment screening. Larger studies in endemic areas may contribute to identifying risk factors for *Histoplasma* infection or disease in at-risk populations, especially related to environmental exposure.

Declarations

Ethics approval and consent to participate
The project was approved by the Ethical Committees of the hospitals included in the study (Hospital de Clinicas de Porto Alegre, Hospital Sao Lucas da PUCRS; CAAE: 17893019.2.0000.5327). All eligible patients approached had to sign the Free and Informed Consent Form (FICF) before participating in the study. A unique identifier number was assigned to each participant, which was used to code samples and test results. Confidentiality regarding the identity of the patients was always preserved. The results

of the diagnostic tests performed in our study protocol were not communicated to the assisting medical teams. All decisions regarding the investigation of fungal disease before or after biological therapy, the use of antifungal therapy, or even the investigation of fungal disease by classical methods (culture, serology, histopathology) at any time during follow-up were the responsibility of the assisting teams.

Consent for publication
Not applicable.

Author contributions
Murillo M. Cipolat: Data curation; Formal analysis; Methodology; Project administration; Writing – original draft; Writing – review & editing.

Débora R. R. Rodrigues: Investigation.

Letícia G. Silveira: Investigation; Resources; Writing – review & editing.

Inês G. Silveira: Investigation; Resources; Writing – review & editing.

Mahara S. V. Nothaft: Investigation.

Claiton V. Brenol: Conceptualization; Resources; Writing – review & editing.

Larissa R. da Silva: Investigation; Resources; Writing – review & editing.

Alessandro C. Pasqualotto: Conceptualization; Data curation; Resources; Writing – review & editing.

Diego R. Falci: Conceptualization; Data curation; Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Visualization; Writing – original draft; Writing – review & editing.

Competing interests
Dr. Falci has received research grants and consulting fees from Pfizer, MSD, and Gilead Sciences; also reports travel support from Pfizer, United Medical, Janssen, and Merck; participation on Data Safety Monitoring or Advisory Boards for Gilead Sciences, Merck, and GlaxoSmithKline (GSK); has given paid lectures on behalf of Astra-Zeneca, Pfizer, Janssen, GSK, Merck, Gilead Sciences, Knight Pharmaceuticals; and received nonfinancial research support from IMMY. Dr. Pasqualotto has received research grants from Gilead, Pfizer, and IMMY; he reports travel support from Pfizer, Knight Pharmaceuticals, and Merck; participation on a Data Safety Monitoring or Advisory Board for Gilead; and payment or honoraria for talks on behalf of Gilead, United Medical, Pfizer, Merck, Sharp & Dohme (MSD), IMMY, Astra-Zeneca, and Astellas Pharma. Other authors have no conflicts of interest to declare.

Availability of data and materials
Due to the nature of the research, and ethical reasons, supporting data is not available.

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