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## Association between multiple sclerosis and *Candida* species: evidence from a case-control study

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**Abstract:** Objective. Candida infection among multiple sclerosis (MS) patients has not been studied in depth. We determined whether there is an association between serological evidence of Candida infection and MS.

**Methods.** Blood specimens were obtained from 80 MS patients and 240 matched controls.

Immunofluorescence analysis and ELISA were used to detect Candida species antibodies and slot-blot to detect antigens.

**Results.** Using immunofluorescence analysis, moderate to high concentrations of serum antibodies to Candida famata were present in 30 (37.5%) MS patients vs. 30 (12.5%) controls ( $p < 0.001$ ). Results for Candida albicans were 47.5% (38/80 MS patients) vs. 21.3% (51/240 controls) ( $p < 0.001$ ), Candida parapsilosis 37% (28/80 MS patients) vs. 17.1% (41/240 controls) ( $p < 0.001$ ) and, Candida glabrata, 46.3% (37/80 MS patients) vs. 17.5% (42/240 controls) ( $p < 0.001$ ). After adjusting for age and gender, the odds ratios (95% confidence intervals) for MS, according to the presence of Candida antigens were: 2.8 (0.3-23.1,  $p = 0.337$ ) for Candida famata; 1.5 (0.7-3.4,  $p = 0.290$ ) for Candida albicans; 7.3 (3.2-16.6,  $p < 0.001$ ) for Candida parapsilosis; and 3.0 (1.5-6.1,  $p = 0.002$ ) for Candida glabrata. The results were similar after excluding 10 patients on immunosuppressants.

**Conclusions.** The results of this single study suggest that Candida species infection may be associated with increased odds of MS.

Response to Reviewers: Reviewer 1:

1. "The main objective of this study was to analyze the association between the serological evidence of Candida infection and MS. This is a well designed, written article with clear methodology and results. However, in my opinion it is very confusing to conclude that a prior Candida infection increases the odds for MS, when there is an increasing number of publications looking at the association between multiple viral infections, vaccines...It looks that the background of prior infections is very complex and we still do not know if it is due to a single or multiple infections with an abnormal immunological system. Based on the data of a single study the conclusion of the abstract and article should be changed and just "suggesting it".

Following the suggestions of the reviewer, the conclusions of the abstract and article have therefore been toned down. We now write in the conclusions: "the results of this single study suggest that *Candida* species infection may be associated with increased odds of MS. The clinical importance of this association is uncertain. We therefore propose future studies that should clarify whether and, if so how, yeasts from the genus *Candida* act in the pathogenesis of this disease".

Reviewer 2:

1. This is an interesting and well-written paper on the association between candida species and multiple sclerosis (MS). After adjusting for age and gender, the odds for MS were elevated for several candida species. The rationale for studying this link is provide in the second page of the introduction. The finding appears to be novel. The selection of cases seems reasonable and was not biased. The diagnosis and clinical assessment was reasonable. There was 1:3 matching with controls. The analytic plan is well presented. Several additional analyses were performed in which they excluded cases who were on immuosuppressive therapies. The limitations are discussed appropriately. The results seemed to be dependent to some measure on the assay used and all results were not significant. Perhaps the authors can discuss why this was the case.

We acknowledge the value of this comment. We now write the following paragraph in the discussion section: "In this case-control study we found a significant association between elevated antibodies of four species of *Candida* and MS using indirect immunofluorescence. However, these differences were not observed using ELISA, but for *Candida glabrata*. We feel detection of antibodies by ELISA in this large sample of participants showed poor specificity. Although it is true that antibodies to *Candida* species may occur in normal individuals as a result of commensal colonization of several anatomically distinct sites including skin, oral, gastrointestinal tract, and vagina [27, 28], MS patients had a significant seroprevalence of *Candida* species using indirect immunofluorescence".

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# Association between Multiple Sclerosis and Candida Species: Evidence from a Case-Control Study

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Running Title: Candida species and multiple sclerosis

Key words: Candida; multiple sclerosis; serology; case-control study.

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**Abstract**

**Objective.** Candida infection among multiple sclerosis (MS) patients has not been studied in depth. We determined whether there is an association between serological evidence of Candida infection and MS.

**Methods.** Blood specimens were obtained from 80 MS patients and 240 matched controls. Immunofluorescence analysis and ELISA were used to detect Candida species antibodies and slot-blot to detect antigens.

**Results.** Using immunofluorescence analysis, moderate to high concentrations of serum antibodies to Candida famata were present in 30 (37.5%) MS patients vs. 30 (12.5%) controls (p<0.001). Results for Candida albicans were 47.5% (38/80 MS patients) vs. 21.3% (51/240 controls) (p<0.001), Candida parapsilosis 37% (28/80 MS patients) vs. 17.1% (41/240 controls) (p<0.001) and, Candida glabrata, 46.3% (37/80 MS patients) vs. 17.5% (42/240 controls) (p<0.001). After adjusting for age and gender, the odds ratios (95% confidence intervals) for MS, according to the presence of Candida antigens were: 2.8 (0.3-23.1, p=0.337) for Candida famata; 1.5 (0.7-3.4, p=0.290) for Candida albicans; 7.3 (3.2-16.6, p<0.001) for Candida parapsilosis; and 3.0 (1.5-6.1, p = 0.002) for Candida glabrata. The results were similar after excluding 10 patients on immunosuppressants.

**Conclusions.** The results of this single study suggest that Candida species infection may be associated with increased odds of MS.

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## Introduction

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3 Multiple sclerosis [MS) is one of the most common chronic neurological  
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5 disorders among young adults, especially in high northern latitudes, and the  
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7 most common cause of non-trauma related disability in this group of age [1].  
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10 The broad spectrum of symptoms of MS considerably impact upon the quality of  
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12 life experienced by patients and their families to a greater extent than several  
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14 other chronic diseases [2-6]. Although etiological factors are not clearly  
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16 established, there are data which suggest that non-genetic (environmental]  
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18 factors, especially infectious agents [e.g. virus or Chlamydia pneumoniae), may  
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20 play a role in MS [7-10]. Among these, Epstein-Barr virus (EBV) stands out as  
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22 one of the most consistent risk factors [10]. Nevertheless, some features of MS  
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24 epidemiology are not explained by EBV and strongly suggest a key role of other  
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26 infectious agents [9]. For instance, Butcher [11] had suggested a global  
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28 correlation between MS incidence and milk consumption, as taken from data  
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30 provided by the Food and Agriculture Organization of the United Nations (FAO).  
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33 In addition, in an analysis of 27 countries and 29 populations worldwide,  
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36 Malosse et al [12] found a good correlation ( $\rho = 0.836$ ) between MS  
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38 prevalence and fresh cow milk consumption. Milk may contain potentially  
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40 pathogenic yeast species such as *Candida parapsilosis* or *Candida glabrata*,  
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42 both able to grow at 40°C [13].  
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49 In 1993, Gass [14] described a new syndrome, the so-called acute zonal  
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51 occult outer retinopathy (AZOOR) in 13 patients who were predominantly young  
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53 women. This enigmatic syndrome is characterized by rapid loss of one or more  
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55 large zones of outer retinal function, photopsia, minimal fundoscopic changes,  
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57 and electroretinographic abnormalities affecting one or both eyes [14]. Recent  
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1 evidences from case reports and case series suggest a link between AZOOR  
2 and MS [15, 16]. The only available long-term follow-up study of patients with  
3 AZOOR showed the presence of white matter abnormalities on cerebral  
4 magnetic resonance imaging in a substantial subgroup [15]. Both AZOOR and  
5 MS share common characteristics, such as a relapsing course, often followed  
6 by gradual worsening, a skew to female sex, inflammatory lesions localized  
7 predominantly in areas around venules, along with signs of secondary neuronal  
8 cell loss [14-16]. Moreover, for both AZOOR and MS, there seems to be an  
9 association with generalized autoimmune disorders [14-16]. In the last three  
10 years, an association between AZOOR and Candida species infection has been  
11 suggested [17-19].

26 Although pathogenic yeasts from the genus *Candida* are well recognized  
27 infectious agents [20, 21], they have never been systematically studied in MS.  
28 Demyelinated lesions in the central nervous system have been described in  
29 patients with *Candida* infection [22]. The previous associations encouraged us  
30 to test the hypothesis that some yeast species of medical importance (including  
31 *Candida famata*, *Candida albicans*, *Candida parapsilosis*, and *Candida*  
32 *glabrata*) are associated with MS. Traditional methods of *Candida* species  
33 detection, which include blood culture and biopsy, usually lack both sensitivity  
34 and specificity. Therefore, diverse non-culture-based methods were also  
35 applied in the current study [23].

## Methods

### 2. 1. Study population

MS patients were included in the study if they had met the McDonald criteria [24]. Exclusion criteria included antibiotic therapy within the previous six months, any corticosteroid therapy within the previous 12 months, and major systemic conditions (including hematologic or solid-organ malignancies, recent surgery, gastrointestinal tract surgery, endocrinopathies, and acute or chronic renal failure).

Our sample of patients with MS was derived from the MS association of Madrid (Spain). This association was chosen because a) there was a close relationship between our research group and the association; and b) the association maintained a computer-based registry of MS patients from the community of Madrid. In this computer-based registry, basic demographic data of the members (e.g., age and gender) were recorded. We obtained permission from the association to access this confidential information. The association included more than 1,000 MS patients from the community of Madrid (Spain). We recruited MS patients without regard to age or gender or other demographic factors (i.e., every 5<sup>th</sup> name was selected from patient lists so as to achieve the needed number of recruits) from the MS association. MS patients were recruited between June 2007 and May 2008.

A 20 minute interview was conducted on each MS patient in which information on age of MS onset and disease evolution was also obtained. Three neurologists with expertise in MS (JBL, PC, and MDS) participated in the clinical assessment and applied the EDSS scale to rate the severity of disease (range = 0 – 10) [25]. Three subgroups were formed according to EDSS score: a) low

1 physical disability (EDSS 0-2.5); moderate physical disability (EDSS 3.0-5.5);  
2 and high physical disability (EDSS  $\geq$  6.0).  
3

4 Healthy controls were selected from those subjects who were occasional  
5 blood donors at different health centers in Madrid (Spain) between June 2007  
6 and May 2008. MS patients were 1:3 frequency-matched with control subjects.  
7 Frequency matching was based on age ( $\pm$ 5 years) and gender. Control subjects  
8 were excluded if they had symptoms or a history of a major systemic disease or  
9 MS, and none was being treated with any type of antibiotics.  
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11 A 10-ml venous blood sample was obtained from each individual. In the  
12 case of MS patients, blood samples were obtained just after clinical  
13 assessment. In all analyses, control samples were interspersed with samples  
14 from patients. Laboratory studies (see below) were performed by experienced  
15 scientists (DP, RA and LC) who were blinded to the diagnosis (patient vs.  
16 control). This study was approved by both the research ethics board at  
17 University Hospital "12 de Octubre" and at the Aut3noma University of Madrid.  
18 Signed consent was obtained for all participants.  
19

## 20 2.2. Candida infection detection.

### 21 2.2.1. Candida species growth

22 All the blood samples were cultured for yeasts in a YEPD medium (1%  
23 yeast extract, 2% peptone, and 2% glucose) by incubation at 30°C. The same  
24 medium, containing agar, was used to test growth of individual yeast colonies.  
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## 2.2.2. Antibodies detection

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4 Rabbit antisera against different yeast species were obtained by  
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6 inoculation of 0.5 ml phosphate-buffered saline (PBS) containing 1 or 2 mg  
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8 yeast after autoclaving and lyophilization. Each inoculum had been mixed  
9  
10 previously with the same volume of Freund's adjuvant. Rabbits were inoculated  
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12 up to four times and the antibody titre and specificity of the sera were tested by  
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14 indirect immunofluorescence and Enzyme-Linked ImmunoSorbent Assay  
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16 (ELISA).  
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### 2.2.2.1. Indirect immunofluorescence

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25 For *Candida famata*, 1 ml of culture was placed in 1.5 ml microcentrifuge tubes.  
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27 Cells were washed with PBS, incubated with 50 mM ammonium chloride for 10  
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29 min, and washed three times with PBS-Tween 20. Cells were then treated with  
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31 the different serum samples diluted 1:500 in PBS-Tween 20 at 37°C for 2 hours,  
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33 washed again with PBS-Tween 20, and incubated with the secondary antibody.  
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35 Rabbit anti-human fluorescein-conjugated antibody (immunoglobulin A [IgA]  
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37 plus IgM plus IgG) was added at a dilution of 1:500. The samples were  
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39 incubated at 37 °C for another hour. The cells were then washed, resuspended  
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41 in PBS, and mounted on slides with a drop of Depex (Serva). For the remaining  
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Positivity percentage of each one of the results was compared to the positive control provided by the manufacturer (Euroimmune, Germany). Results were scored as follows: 0% (clearly negative in contrast with the positive control provided by the manufacturer), 20% (low antibody concentrations), 40% and 60% (moderate antibody concentrations), 80% and 100% (clearly positive as the positive control provided by the manufacturer) [17-19].

#### 2.2.2.2. Enzyme-Linked ImmunoSorbent Assay (ELISA).

ELISA was carried out as described previously [17, 18]. Briefly, yeast suspension diluted in PBS was seeded in ELISA microtitre plates (Maxisorp; Nunc). The plates were blocked with PBS containing 3% low-fat dried milk and 0.2 % Tween 20. Sera from the study participants were added at different dilutions and the value obtained at 1:500 dilution was selected for data analysis. Plates were subsequently incubated with goat anti-human (heavy and light chain) Ig horseradish peroxidase-conjugated antibodies (Pierce) and washed five times. Colour development was accomplished by incubation with o-phenylenediamine (Sigma) and measured at 490 nm in a microplate reader (EL340; Bio-Tek Instruments). Results were scored as absolute values [17, 18].

#### 2.2.3. Antigen detection

The presence of Candida species antigens in plasma was determined by slot-blot analyses [18]. Different serum dilutions (200 µl) in TBS were added to each well. Samples were blotted onto a 45 mm nitrocellulose membrane (Bio-Rad) previously hydrated in TBS for 10 min using a Bio-Dot SF apparatus (Bio-Rad). After blotting, the membrane was processed and developed. The primary

1 rabbit polyclonal antibodies against *Candida famata*, *Candida albicans*, *Candida*  
2 *parapsilopsis* and *Candida glabrata* were used at a 1:1000 dilution. A donkey  
3  
4 anti-rabbit IgG horseradish peroxidase-conjugated antibody (Amersham  
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6 Biosciences) at a 1:5000 dilution was used as secondary antibody [18]. Titres  
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8  $\geq 20$  were counted positive and  $\leq 19$ , counted negative or doubtful [18, 19].  
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13 **2.3. Data analysis and Sample Size**

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15 Statistical analyses were performed in SPSS Version 15.0 (SPSS, Inc.,  
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17 Chicago, IL). All tests were two sided, and significance was accepted at the 5%  
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19 level ( $\alpha = 0.05$ ). The continuous variables age and ELISA were not normally  
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21 distributed, and thus Mann-Whitney test was used to calculate differences  
22  
23 between the patients and controls. Chi square was used for categorical data  
24  
25 (gender, immunofluorescence and slot-blot analyses). Spearman's correlation  
26  
27 coefficient was used to determine the correlations between the clinical and  
28  
29 serological parameters. Odds ratios (ORs) and 95% confidence intervals (CIs)  
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31 assessing the risk of MS associated with infection by each type of *Candida*,  
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33 using indirect immunofluorescence and slot-blot, were estimated by use of  
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35 binary logistic regression, adjusted for age and gender.  
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42 The targeted sample size (80 participants in the patient group and 240  
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44 participants in the control group) had 100% power to detect a 30% difference in  
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46 slot-blot analysess between patients and controls (assuming  $\alpha = 0.05$ ).  
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52 **Results**

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55 Of 85 eligible patients with MS, 5 (5.9%) were excluded due to  
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57 insufficient volume of blood drawn. None were excluded because they had  
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59 major comorbidities or had received antibiotic or corticosteroid therapy. The  
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1 final study sample consisted of 80 patients with MS and 240 controls who were  
2 frequency-matched by age and gender. Forty (50%) MS patients were taking  
3 immunomodulatory (beta-interferons and glatiramer acetate) or  
4 immunosuppressant (azathioprine, methotrexate and mitoxantrone) medications  
5  
6 immunosuppressant (azathioprine, methotrexate and mitoxantrone) medications  
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8 (table 1). The remaining 33 (41.25%) had never been on immunomodulatory or  
9 immunosuppressant medications or their drugs had been withdrawn due to  
10 inefficacy at least one year before clinical assessment (7/80, 8.75%). The  
11 patients with MS and controls were similar in age and gender (table 1). Disease  
12 duration ranged from 1 to 39 years (median = 10.5 years) (table 1). No yeasts  
13 were cultured from the blood samples of patients and controls.  
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25 Moderate to high concentrations of serum antibodies to *Candida famata*  
26 were present in 30 (37.5%) MS patients vs. 30 (12.5%) controls ( $p < 0.001$ ),  
27 using indirect immunofluorescence analysis (table 2). In a regression analysis  
28 that adjusted for age and gender, subjects with moderate to high antibody  
29 concentrations were more than four times more likely to have MS than were  
30 those with negative or low concentrations of antibodies ( $OR = 4.4$ ,  $95\% CI = 2.4$   
31  $- 8.1$ ,  $p < 0.001$ ) (table 3). However, the median concentrations of antibodies  
32 using ELISA were similar in the group of patients with MS compared with the  
33 control group [ $0.5 \pm 0.5$  (mean  $\pm$  SD), 0.3 (median) vs.  $0.5 \pm 0.5$  (mean  $\pm$ SD),  
34 0.4 (median),  $p = 0.101$ ] (table 2). Similarly, the proportion of MS patients with  
35 positivity for *Candida famata* antigens, detected by slot-blot analysis, was  
36 similar to that in the control group (79 MS patients, 98.8% vs. 232 controls,  
37 96.7%,  $p = 0.459$ ) (adjusted  $OR = 2.8$ ,  $95\% CI = 0.3 - 23.1$ ,  $p = 0.337$ ) (Tables 3  
38 and 4).  
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1 Similar results were obtained for *Candida albicans* and *Candida*  
2 *parapsilosis* (Tables 2 to 4), except that the proportion of MS patients with  
3  
4 positivity for *Candida parapsilosis* antigens, detected by slot-blot analysis, was  
5  
6 significantly higher than that of the control group (73 MS patients, 91.3% vs.  
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8 142 controls, 59.2%) (adjusted OR = 7.3, 95% CI = 3.2 - 16.6,  $p < 0.001$ )  
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11 (Tables 3 and 4).  
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15 For *Candida glabrata*, moderate to high concentrations of serum  
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17 antibodies were detected by indirect immunofluorescence in 37 of 80 patients  
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19 with MS compared with 42 of 240 controls (46.3% versus 17.5%,  $p < 0.001$ ;  
20  
21 Table 2) (adjusted OR = 4.2, 95% CI = 2.4 - 7.4,  $p < 0.001$ ) (Table 3). Similarly,  
22  
23 the concentrations of antibodies, using ELISA, were significantly higher in the  
24  
25 patient group than in the control group [ $0.4 \pm 0.3$  (mean  $\pm$  SD), 0.3 (median) vs.  
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27  $0.3 \pm 0.1$  (mean  $\pm$  SD), 0.3 (median),  $p = 0.001$ ]. In addition, the proportion of  
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29 MS patients with positivity for *Candida glabrata* antigens, detected by slot-blot  
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31 analysis, was significantly higher than in the control group (18 MS patients,  
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33 22.5% vs. 21 controls, 8.7%) (adjusted OR = 3.0, 95% CI = 1.5 - 6.1,  $p = 0.002$ )  
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36 (Tables 3 and 4).  
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42 No correlations were found between the clinical and the serological  
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44 parameters with any type of *Candida* species. The above results were also  
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46 similar after excluding 10 MS patients who were on immunosuppressant  
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48 therapies or after excluding 47 MS patients who were receiving (40 cases) or  
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50 ever received (7 cases) immunosuppressant or immunomodulatory therapies  
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52 (data not shown). In addition, after excluding 40 MS patients whose MS  
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54 duration was  $> 10$  years, the results remained similar (data not shown).  
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## Discussion

Several *Candida* species are widely distributed throughout the human population as both commensal organisms and as intermittent pathogens [26, 27]. In the clinical arena, blood cultures are the main assay for routine detection of candidiasis [26]. However, microbiological confirmation is difficult because blood cultures can be negative in up to 50% of autopsy-proven cases of deep-seated candidiasis or may only become positive late in the infection [26]. Thus, different approaches are needed to demonstrate disseminated fungal infection. Given the lack of a single definitive test to detect *Candida* species, different assays were carried out in the current study. First, search for antibodies against *Candida* species in blood by means of immunofluorescence and ELISA tests, and second, by detection of *Candida* species proteins in blood by means of slot-blot analyses [17-19].

To our knowledge, this is the first large epidemiologic study to test for an association between MS and infection with *Candida* species. In this case-control study we found a significant association between elevated antibodies of four species of *Candida* and MS using indirect immunofluorescence. However, these differences were not observed using ELISA, but for *Candida glabrata*. We feel detection of antibodies by ELISA in this large sample of participants showed poor specificity. Although it is true that antibodies to *Candida* species may occur in normal individuals as a result of commensal colonization of several anatomically distinct sites including skin, oral, gastrointestinal tract, and vagina [27, 28], MS patients had a significant seroprevalence of *Candida* species using indirect immunofluorescence. However, the most striking result of

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1 our study is a near-complete (91.3%) presence of *Candida parapsilosis*  
2 antigens in patients with MS compared with only 59.2% of subjects in the  
3 control group. Subjects with positivity for *Candida parapsilosis* antigens were  
4 seven times more likely to have MS than were those with negative or doubtful  
5 concentrations of antigens. The basis for the case-control differences we  
6 observed is not clear. The prevalence hypothesis, proposed by Kurtzke [29],  
7 suggests that MS is caused by a pathogen, which may be more common in  
8 regions of high MS prevalence. This pathogen would be widespread and, in  
9 most individuals, would cause an asymptomatic persistent infection and, only  
10 rarely and years after the primary infection, does this agent would cause MS.  
11 Milk consumption has been correlated with higher worldwide prevalence rates  
12 of MS [11, 12] and pathogenic *Candida* species may be isolated from milk  
13 products [13]. One could hypothesize that chronic or reactivated latent infection  
14 by pathogenic *Candida* species drives or initiates autoimmunity. However,  
15 longitudinal studies of incident MS patients are required to assess whether the  
16 observed associations are due to a pathogenic effect of *Candida* species on the  
17 risk of developing incident MS or a susceptibility on the part of patients with MS  
18 to be infected by yeasts.

19 We acknowledge several limitations of this study. First, we recruited a  
20 group of patients with MS (i.e., patients seen in a patient-based association)  
21 and, as a consequence, our results might not be generalized to population-  
22 dwelling MS patients. Therefore, we acknowledge the need to replicate these  
23 findings in a population-based survey of unselected patients. Second, while we  
24 frequency-matched patients and controls based on age and gender we were not  
25 able to adjust for other potential unmeasured confounders, such as

1 socioeconomics status, dietary intakes or hygiene habits. Third, there is also  
2 evidence that immunosuppressant therapies could interfere with the generation  
3 of antibodies [30]. However, after excluding 10 MS patients who were receiving  
4 immunosuppressant therapies, the concentrations of Candida species  
5 antibodies remained significantly higher in MS patients than controls. Fourth,  
6 therapeutic use of repeated injectable beta-interferons or glatiramer acetate  
7 (immunomodulatory therapies) may prone to skin colonization by Candida  
8 species that could be followed by candidemia. However, the results were similar  
9 after excluding 47 MS patients who were receiving (40 cases) or ever received  
10 (7 cases) immunosuppressant or immunomodulatory therapies. In addition, we  
11 enrolled 40 MS patients whose MS duration was > 10 years. Patients with long-  
12 lasting disease duration (> 10 years) may be exposed to corticosteroids or they  
13 could have a higher frequency of urinary tract infections and hence antibiotic  
14 treatment. However, the results were similar after excluding these 40 MS  
15 patients whose MS duration was longer than 10 years. This supports that the  
16 reported association is present early in MS course.

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40 In summary, the results of this single study suggest that Candida species  
41 infection may be associated with increased odds of MS. The clinical importance  
42 of this association is uncertain. We therefore propose future studies that should  
43 clarify whether and, if so how, yeasts from the genus Candida act in the  
44 pathogenesis of this disease.  
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### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

JB-L and LC conceived, coordinated and designed the original study. JB-L conducted the analysis of data and drafted the manuscript. JBL, DP, RA, PC, and MDS collected data and samples. DP and RA conducted the serological testing. All authors offered critical input into the manuscript and all have read and approved the final version.

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Table 1. Characteristics of study population.

	MS Patients (N = 80)	Controls (N = 240)	<i>p</i> value
Age in years	42.4 ± 7.2 (42.5)	42.0 ± 4.6 (43.0)	0.328 <sup>a</sup>
Gender (female)	56 (70.0%)	169 (70.4%)	1.0 <sup>b</sup>
Mean duration in years	13.2 ± 7.9 (10.5)		
Disease course at time of serum acquisition			
<i>Relapsing-remitting</i>	44 (55.0%)		
<i>Secondary progressive</i>	26 (32.5%)		
<i>Primary progressive</i>	10 (32.5%)		
EDSS scores			
0-3.5	23 (28.8%)		
4.0-5.5	23 (28.8%)		
≥ 6.0	34 (42.5%)		
Immunomodulating or immunosuppressive medications at time of serum acquisition			
<i>Beta-interferons</i>	23 (28.8%)		
<i>Glatiramer acetate</i>	6 (7.5%)		
<i>Azathioprine</i>	5 (6.3%)		
<i>Methotrexate</i>	3 (3.8%)		
<i>Mitoxantrone</i>	2 (2.5%)		
<i>Fingolimod</i>	1 (1.3%)		
None	40 (50.0%)		

Mean ± SD (median) and frequency (%) are reported. <sup>a</sup> Mann–Whitney U test and <sup>b</sup> chi-square test. EDSS = expanded disability status scale.

Table 2. Seroprevalence of Candida species antibodies in patients and controls, using indirect immunofluorescence and ELISA tests.

	MS Patients (N = 80)	Controls (N = 240)	p value
Indirect immunofluorescence			
Candida famata antibodies <i>Moderate to high concentrations</i>	30 (37.5%)	30 (12.5%)	< 0.001 <sup>a</sup>
Candida albicans antibodies <i>Moderate to high concentrations</i>	38 (47.5%)	51 (21.3%)	< 0.001 <sup>a</sup>
Candida parapsilosis antibodies <i>Moderate to high concentrations</i>	28 (35.0%)	41 (17.1%)	0.001 <sup>a</sup>
Candida glabrata antibodies <i>Moderate to high concentrations</i>	37 (46.3%)	42 (17.5%)	< 0.001 <sup>a</sup>
ELISA *			
Candida famata antibodies	0.5 ± 0.5 (0.3)	0.5 ± 0.5 (0.4)	0.101 <sup>b</sup>
Candida albicans antibodies	1.0 ± 0.8 (0.7)	0.9 ± 0.6 (0.8)	0.824 <sup>b</sup>
Candida parapsilosis antibodies	0.5 ± 0.4 (0.4)	0.4 ± 0.3 (0.3)	0.110 <sup>b</sup>
Candida glabrata antibodies	0.4 ± 0.3 (0.3)	0.3 ± 0.1 (0.3)	0.001 <sup>b</sup>

<sup>a</sup> Chi-square test and <sup>b</sup> Mann–Whitney U test. \* Mean ± SD (median) are reported.

Table 3. Candida species antibodies (detected by indirect immunofluorescence) and antigens (detected by slot-blot analyses) and odds of multiple sclerosis.

	Unadjusted			Adjusted for age and gender		
	Odds ratio*	95% CI	p value	Odds ratio *	95% CI	p value
Indirect immunofluorescence *						
Candida famata antibodies <i>Moderate to high concentrations</i>	4.2	2.3-7.6	< 0.001	4.4	2.4-8.1	< 0.001
Candida albicans antibodies <i>Moderate to high concentrations</i>	3.3	2.0-5.7	< 0.001	3.3	1.9-5.7	< 0.001
Candida parapsilosis antibodies <i>Moderate to high concentrations</i>	2.6	1.5-4.6	0.001	2.8	1.6-5.0	0.001
Candida glabrata antibodies <i>Moderate to high concentrations</i>	4.0	2.3-7.0	< 0.001	4.2	2.4-7.4	< 0.001
Slot-blot analysis **						
Candida famata antigens <i>Positive</i>	2.7	0.3-22.1	0.337	2.8	0.3-23.1	0.337
Candida albicans antigens <i>Positive</i>	1.5	0.7-3.2	0.318	1.5	0.7-3.4	0.290
Candida parapsilosis antigens <i>Positive</i>	7.2	3.2-16.3	< 0.001	7.3	3.2-16.6	< 0.001
Candida glabrata antigens <i>Positive</i>	3.0	1.5-6.0	0.002	3.0	1.5-6.1	0.002

\* The reference category was negative / low concentrations. \*\* The reference category was negative / doubtful concentrations.

Table 4. Candida species antigens in patients and controls, using slot-blot analyses.

	MS Patients (N = 80)	Controls (N = 240)	p value
Indirect immunofluorescence			
<i>Candida famata</i> antigens			
Negative / Doubtful concentrations	1 (1.3%)	8 (3.3%)	0.459 <sup>a</sup>
Positive	79 (98.8%)	232 (96.7%)	
<i>Candida albicans</i> antigens			
Negative / Doubtful concentrations	9 (11.3%)	38 (15.8%)	0.366 <sup>a</sup>
Positive	71 (88.8%)	202 (84.2%)	
<i>Candida parapsilosis</i> antigens			
Negative / Doubtful concentrations	7 (8.8%)	98 (40.8%)	< 0.001 <sup>a</sup>
Positive	73 (91.3%)	142 (59.2%)	
<i>Candida glabrata</i> antigens			
Negative / Doubtful concentrations	62 (77.5%)	219 (91.3%)	0.003 <sup>a</sup>
Positive	18 (22.5%)	21 (8.7%)	

<sup>a</sup> Chi-square test.