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Study on Association of Recent and Past *Chlamydophila pneumoniae* Infection with Classic Multiple Sclerosis

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This work was carried out in collaboration between all authors. Author HH designed the study, performed the statistical analysis and wrote the protocol. Authors MAJM managed the analyses of the study and wrote the first draft of the manuscript. Authors H. Hatamian and AR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: We conducted this study to determine if there is any correlation between Classical Multiple Sclerosis and *Chlamydophilia pneumoniae* infection by ELISA (IgM, IgG, IgA).
Study Design: cross sectional study
Place and Duration of Study: The present study was performed in the Department of Microbiology, Guilan University of Medical Sciences between April 2012 and April 2013
Methodology: *Chlamydophila pneumoniae* infection certified by ELISA in patients (n=46) and control (n=46) using commercial assays (anti- *C. pneumoniae* IgG, anti- C. pneumoniae IgM, and anti- *C. pneumoniae* IgA kits). Data were analyzed by using four statistical tests (Pearson chi square, Kendall's tau, and Spearman's rho).
Results: Seropositivity to anti- *C. pneumoniae* IgG was seen less frequently in patients versus controls (69.0% versus 81.4%; P=0.187). Seropositivity to anti- *C. pneumoniae* IgA was also observed less frequently in patients than in controls (7.2 % versus 11.6%; P= 0.479).However anti- *C. pneumoniae* IgM antibodies were seen more often in classical multiple sclerosis patients than it was in controls (11.9% versus 2.3%; P= 0.085).
Conclusion: We concluded that recent or past *C. pneumoniae* infection has no correlation in initiation or protection of CMS.

Keywords: Chlamydophila pneumonia; multiple sclerosis; anti- C. pneumoniae IgG; anti- C. pneumoniae IgM; and Anti- C. pneumoniae IgA.

1. INTRODUCTION

Autoimmune disorders affect people of all genders, races, and ages, but some have an increased risk of developing autoimmune disorders. Classic multiple sclerosis is a common, exclusively human, chronic demyelinating disease of the brain without opticospinal affection that occurs in nearly all parts of the world. The disease is most commonly diagnosed between ages 20 and 40. It is caused by damage to the myelin sheath, the protective covering that surrounds nerve cells. When this nerve covering is damaged, nerve impulses are slowed down or stopped. Inflammation is not the only cause of nerve damage. Inflammation occurs when the body's own immune cells attack the nervous system. Repeated episodes of inflammation can occur along any area of the brain, optic nerve, and spinal cord [1].

The cause of MS is unknown. In 2006, Zamboni and colleagues proposed that multiple sclerosis is caused by abnormalities in the direction and pathway of cerebral venous flow, leading to deposition of iron in the brain, which triggers an autoimmune reaction [2] They reported that patients with multiple sclerosis had a higher frequency of abnormalities of anatomy and flow in the internal jugular, deep cerebral, vertebral and azygous veins than individuals without multiple sclerosis had [3]. They called this condition chronic cerebrospinal venous insufficiency (CCSVI). Two other leading theories have emerged, one implicating infectious agents, probably viral, and the other implying that the disease is produced by a host immune response to an infectious agent or autoantigen [4]. Infectious agents have been implicated in the pathogenesis of many autoimmune diseases e.g. Group A Streptococcus (GAS) initiates and maintains lesions of chronic plaque psoriasis and clearing of these plaques is observed with specific antibiotic therapy directed towards GAS[2]. In most of these diseases including those in which specific organisms are known to play a role, the details of pathogenesis remain incompletely defined [5].

Chlamydophila pneumoniae is the latest pathogen to be associated with MS. A case of CNS infection with *C. pneumoniae* in a patient with rapidly progressive MS has been reported [6]. Antimicrobial therapy directed against this pathogen was accompanied by marked neurological improvement [6]. Subsequent studies found that *C. pneumoniae* is present in the CSF of patients with newly diagnosed relapsing, remitting MS and in patients with progressive MS, but not in other neurological disease and controls [7].

Chlamydiae are known to infect macrophages and monocytes as well as epithelial cells. *Chlamydophila pneumoniae* also infects endothelial and smooth muscle cells of blood vessels. One of the hallmarks of chlamydial infection is its tissue persistence and the development of chronic infection. Tissue injury in all chlamydial diseases appears to be immune mediated [8]. As a large number of individuals are infected with the organism, it is possible that *C. pneumoniae* is the inciting agent of MS in genetically susceptible individuals. Activation of perivascular and parenchymal microglial cells along with the attendant biochemical mediators would lead to destruction of the surrounding myelin [6].

The findings about isolation of *C. pneumoniae* from the cerebrospinal fluid (CSF) of MS patients and the detection of both *Chlamydophila* -specific DNA and antibody in MS CSF are controversial. Other analyses of brain and CSF have shown no significant difference in *C. pneumoniae*-specific DNA or antibody between MS and control subjects [9]. Recent work has revealed intrathecal production of *C. pneumoniae*-specific IgG in only 24% of MS patients compared with 5% of control patients. More importantly, the major CSF oligoclonal bands from MS patients did not react to *C. pneumonia* [10].

We aimed to investigate any correlation between Classical Multiple Sclerosis and latent or active *Chlamydophila pneumoniae* infection by investigating humoral response to *C. pneumonia* in patients with CMS using ELISA to detect IgM, IgG and IgA

2. MATERIALS AND METHODS

This cross sectional prospective study was conducted in MS patients who were under supervision of MS society of Guilan province, northern Iran and age and gender matched healthy individuals as controls. This study was performed along April, 2012 to April, 2013. MS patients had been diagnosed by magnetic resonance imaging (MRI) and Evoked Potential (EP) assays and also McDonald criteria were recruited. Serum samples were collected by standard methods. All Specimens were stored at -70ºC until the experiment was performed. Required data were collected by filling out a questioner including main information such as age, gender, duration of the disease, number of crisis, duration of interferon intake, score of EDSS (Expanded Disability Status Scale is a method of quantifying disability in multiple sclerosis), result of MRI, result of Evoked Potential Test and type of antiviral therapy (if prescribed).

A group of 46 subjects with CMS along with 46 healthy controls were examined with ELISA for the presence of antibodies (IgG, IgM, IgA) against *C. pneumoniae* whole antigen. All patients and control were residents of the area (more than 6 months) with age range 10-50, affected by classic MS (not opticospinal MS) whose diagnosis was confirmed by MRI and Evoked Potential tests.

Serological tests were performed using commercial non quantitative ELISA assays (Anti- *C. pneumoniae* IgG, Anti- *C. pneumoniae* IgM, and Anti- *C. pneumoniae* IgA kits, Euroimmune, Germany). All stages of the test were carried out by following the instruction of

the company. Seropositivity to anti- *C. pneumoniae* IgM or anti- *C.pneumoniae* IgA was considered as recent infection and seropositivity to anti- *C. pneumoniae* IgG was regarded as past infection.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 16 was used for statistical analysis. Pearson chi square, Spearman's rho correlation, Kendall's tau correlation and odd ratio tests were applied to analyze all variables. All P-values were regarded two-tailed and significant at $P<0.05$.

The study conformed to the Helsinki declaration and was reviewed and approved by the local research committee and ethics committee written informed consent was obtained from all subjects and control group.

3. RESULTS AND DISCUSSION

Age range of patients (n=46) were 13-51 (most frequent around 28-38 and average 32.6). Duration of the disease was more frequent between 2 to 9 years (76.3%). Most patients were women (71.4%). Expanded Disability Status Scale (EDSS) of most patients was between 2.5 to 3.5 (45.2%) and 28.6% of them had EDSS lower than 2.5 (Fig. 1). Evoked potential test and MRI were positive in all patients. Number of disease crisis in most patients (57.2%) was 1-2.

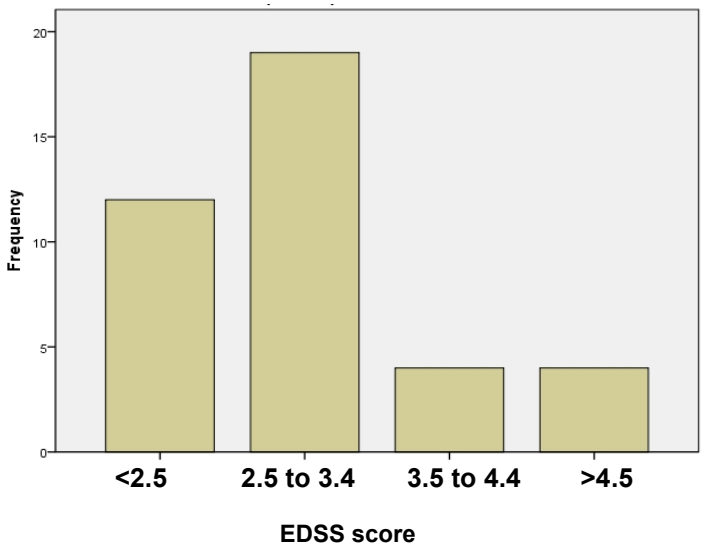

Fig. 1. Distribution of EDSS score in patients

Seropositivity to anti-*C. pneumoniae* IgG (indicating past infection) in controls was higher than that it was in patients (81.4% versus 69.0%) (Fig. 2) but this difference was not significant and did not indicate a protective role against developing the disease (Table 1).

Seropositivity to anti- *C. pneumoniae* IgA (indicating active infection) in controls was also higher than that it was in patients (11.6% versus 7.2% consequently) (Fig. 3) but this difference was not significant and did not indicate a protective role against developing the disease (Table 1).

Seropositivity to anti-*C. pneumoniae* IgM (indicating active infection) in patients was higher than that it was in controls (11.9% and 2.3% consequently) (Fig. 4) but this difference was not significant indicating a causative role favor to developing the disease (Table 1).
Seropositivity to both anti- *C. pneumoniae* IgM and anti-*C. pneumoniae* IgG (indicating recent active infection) was higher in patients than that it was in controls (7.2% versus 2.3%) but this difference was not significant indicating a causative role for developing MS (P=0.219 and correlation coefficient[1]=-0.135).

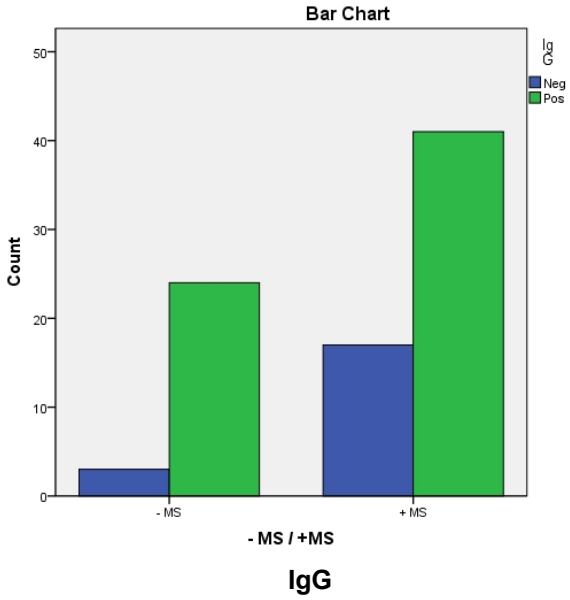

Fig. 2. Seropositivity rate to anti-*C. pneumoniae* IgG in patients and control

Seropositivity to both anti- *C. pneumoniae* IgA and anti-*C. pneumoniae* IgG, indicating chronic infection was higher in controls than that it was in patients (11.6% versus 2.4%) but this difference was not significant and did not indicate a protective role against developing MS (P=0.192 and correlation coefficient[2] = -0.144).

Table 1. Statistical relation of seropositivity to anti-*C. pneumoniae* IgG, anti- *C. pneumoniae* IgA and anti- *C. pneumoniae* IgM with occurrence of MS

Statistical test ELISA test	Seropositivity rate patients	control	Statistical analysis					
			P1	FET	P2	P3	LR	LLA
Anti-*C. pneumoniae* IgG	69.0%	81.4%	0.187	0.216	0.190	0.191	0.185	0.190
Anti- *C. pneumoniae* IgA	7.2%	11.6%	0.479	0.713	0.482	0.485	0.477	0.482
Anti- *C. pneumoniae* IgM	11.9%	2.3%	0.085	0.110	0.087	0.087	0.073	0.087

P1: pearson chi square Asymp. Sig. (2-sided); FET : Fisher's Exact Test Exact Sig. (2-sided); P2: Kendall's tau test Sig. (2-tailed); P3: Spearman's rho correlation test Sig. (2-sided)
LR: Likelihood Ratio Asymp. Sig. (2-sided); LLA: Linear-by-Linear Association Asymp. Sig. (2-sided)

[1 ,2] *Partial correlation test*

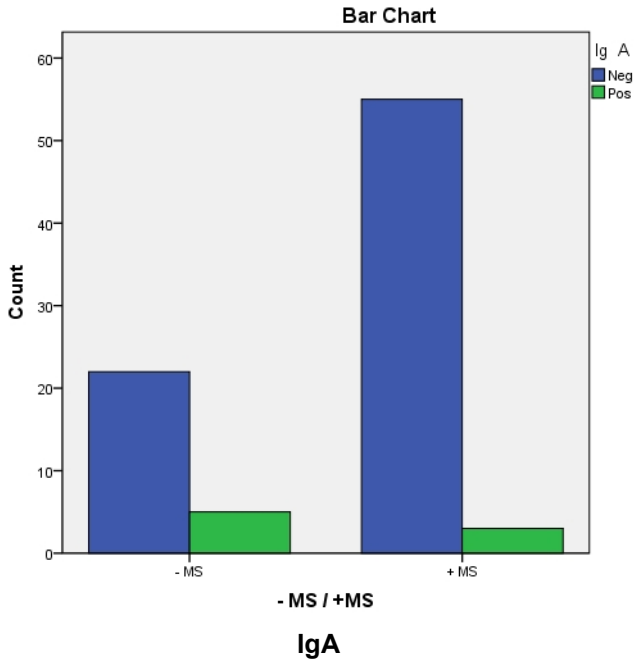

Fig. 3. Seropositivity rate to anti-*C. pneumoniae* IgA in patients and control

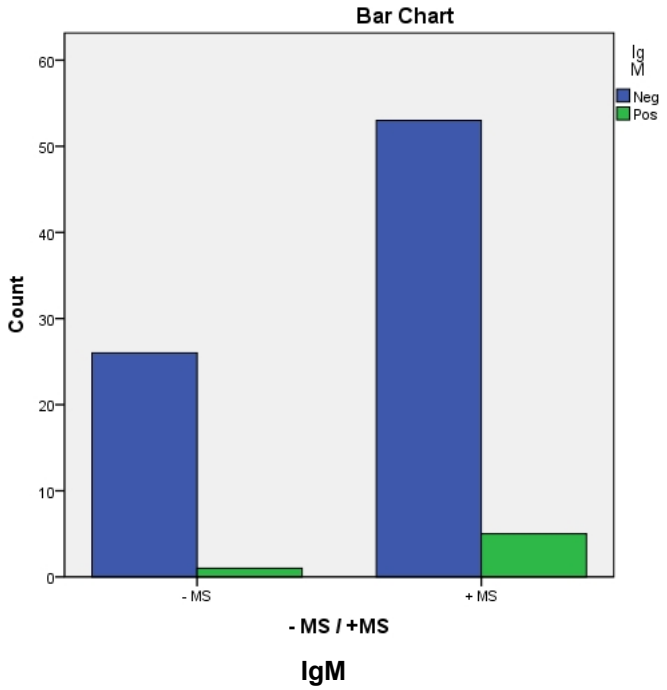

Fig. 4. Seropositivity rate to Anti-*C. pneumoniae* IgM in patients and control

Autoimmunity is a consequence of the breakdown of self-tolerance; the result is an attack of the immune system on various organs and tissues as if they were foreign invaders [11]. In recent years, the compound interplay between infections and autoimmunity has been studied extensively [12,13] the mechanisms proposed include: (1) Lysis of oligodendrocytes (which produce myelin) by a replicating pathogen; (2) Destruction of infected cells by the immune system; (3) Non-specific (bystander) damage to myelin by an activated immune system; and (4) Autoimmunity related or unrelated to infection [9].

Tissue injury in all chlamydial diseases appears to be immune mediated [14]. Retrospective serological studies provides evidence that *C. pneumoniae* was active for years before its isolation. Much of the knowledge of the epidemiology of *C. pneumoniae* infection has been derived from serologic studies utilizing the *C. pneumoniae*-specific microimmunofluorescence test, ELISA, and PCR [15].

The association between *C. pneumoniae* and MS was intensively investigated with controversial results. Isolation techniques failed to detect *C. pneumoniae* in CSF and brain tissue of MS patients [16]. In some studies, *C. pneumoniae* were identified in the cerebrospinal fluid by polymerase chain reaction in patients with definite MS and were not identified in other studies. Analysis of data supports the hypothesis that *C. pneumoniae* persistence in some MS patients may be the result of an impaired clearance within the central nervous system [17].

Actually *C. pneumoniae* has been linked to multiple sclerosis from three decades ago. Sriram and colleagues reported on a multiple sclerosis patient, who failed to respond to immunosuppressive treatment, but had *C. pneumoniae* in the CSF and improved dramatically after antibiotic treatment [18]. In a larger cross-sectional study, the same group reported that *C. pneumoniae* could be cultured from 64% of multiple sclerosis patients versus 11% of controls, and polymerase chain reaction (PCR) allowed the detection of the *C. pneumoniae* genome in 97% of multiple sclerosis patients versus 18% of controls with other neurological diseases [19].

Several contradictory brief reports, mostly in the form of letters, have appeared in the meantime, shedding doubt on the *Chlamydia* hypothesis. The number of patients studied in these scattered reports was small, and data were based mainly on PCR analysis [10,20-24]. However, the PCR detection of *C. pneumoniae* in the CSF (not in blood sample) is not standardized and the contradictory results might be explained by different PCR protocols, different strategies to extract DNA, different handling of the CSF, the amount of CSF drawn, the cell number in the CSF and other variations.

Chronic intrathecal immunoglobulin (Ig) production is a hallmark of multiple sclerosis characterized by the presence of oligoclonal IgGs. The presence of intrathecal IgGs to *C. pneumoniae* was independent of the duration of disease and relatively stable over time [25]. Because many studies however could not clarify whether *C. pneumoniae* is present in brain tissue and CSF of MS patients. Other studies focused on the humoral immune response against *C. pneumonia.* This is why we conducted the present study to investigate humoral response to *Chlamydophila pneumoniae* by using ELISA to detect IgM, IgG and IgA in sera.

In the study of Kaufman, Cerebrospinal fluid samples from controls and patients with MS were split and sent to laboratories with different experiences for the detection of *C. pneumoniae* by polymerase chain reaction. Conflicting reports of *C. pneumoniae* detection in the some samples from patents with MS highlight the need to exchange

detection techniques among laboratories involved in this controversy [26]. It should be noted even more recent complex molecular assays could not solve the problem. For example, a nested-PCR assay to detect ompA and a PCR-Enyzme immunoassay depending on streptavidin-biotin capture and dig detection of the PCR products to detect omp9, was designed and performed by Budak et al but *C. pneumoniae* DNA was not detected by those assays in patient samples [27].

Derfuss et al found that an intrathecal IgG production to *C. pneumoniae* is indeed more common in multiple sclerosis patients than controls. In their study all PCR results were negative [28]. It should be noted that measuring intrathecal IgG production to *C. pneumoniae* also could not solve the problem and controversial results are obvious in the performed studies. Intrathecal synthesis of anti- *C. pneumoniae* IgG have been undetectable in MS patients in some studies or, if present, were not selectively associated with MS, but were shared by several inflammatory neurological conditions [29]. More importantly, the major CSF oligoclonal bands from MS patients did not react to *C. pneumonia* [11].

For all above mentioned causes we decided to design the present study through detecting anti *C. pneumoniae* antibodies (IgG, IgA, IgM) in sera of patients and matched control to investigate any significant differences including positive or negative correlation between Classical Multiple Sclerosis and *Chlamydophila pneumoniae* infection.

We found higher seropositivity rate of anti-*C. pneumoniae* IgM in patients but it was not significant indicating a causative role for active infection favor to developing the disease. We also found less seropositivity rate of anti-*C. pneumoniae* IgA in patients but it was not significant indicating a protective role against developing the disease. In addition we found that seropositivity to anti-*C. pneumoniae* IgG in patients was less than that it was in control but this difference was also not significant. It indicates lack of protective role for past infection against developing the disease. Seropositivity to both anti-*C.pneumoniae* IgM and anti-*C. pneumoniae* IgG and also to both anti-*C. pneumoniae* IgA and anti-*C. pneumoniae* IgG did not show significant difference in two groups. Results of the present study showed that recent or past *C. pneumoniae* infection has no correlation in initiation or protection of CMS. Lack of any correlation as we found in our study, in consistent with other studies [10,23] is another indication of lacking causal role of active and chronic *C.pneumoniae* infection in developing of CMS.

4. CONCLUSION

The growing body of data does not support a central role for *C. pneumoniae* as a candidate in MS pathogenesis, but suggests that, in a subset of MS patients, *C. pneumoniae* could induce a chronic persistent brain infection acting as a cofactor in the development of the disease. Thus, the actual involvement of *C. pneumoniae* in MS still remains to be elucidated. Further work exploring the role of *C. pneumoniae* in inflammatory demyelination is required. This may be accomplished either by developing an animal model or in a therapeutic trial in patients with MS and it is also necessary investigated the cellular aspects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Scolding NJ, Zajicek JP, Wood N, Compston DAS. The pathogenesis of demyelinating disease. Progr Neurobiol. 1994;43:143–173.
2. Zamboni P. The big idea — Iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. J R Soc Med. 2006;99:589–93.
3. Zamboni P, Galeotti R, Menegatti E, et al. Chronic cerebrospinal venous insufficiency in patients with multiple sclerosis. J Neurol Neurosurg Psychiatry. 2009;80:392–9.
4. Saxena VN, Dogra J. Long term oral azithromycin in chronic plaque psoriasis: A controlled trial. Eur J Dermatol. 2010;20(3):329-33.
5. Fairweather D, Kaya Z, Shellam GR, Lawson CM, Rose NR. From infection to autoimmunity. J Autoimmun. 2001;16:175–86.
6. Fujita M, Hatachi S, Yagita M. Acute *Chlamydia pneumoniae* infection in the pathogenesis of autoimmune diseases. Lupus. 2009;18(2):164-168.
7. Sinha AA, Lopez MT, McDevitt HO. Autoimmune diseases: The failure of self tolerance. Science. 1990;248:1380-88.
8. Virok D, Kis Z, Karai L, et al. *Chlamydia pneumoniae* in artherosclerotic middle cerebral artery. Stroke. 2001;32:1973–1976.
9. Tsai JC, Gilden DH. *Chlamydia pneumoniae* and multiple sclerosis: No significant association. Trends in Microbiology. 2001;9(4):152–154.
10. Boman J, Roblin PM, Sundstrom P, Sandstrom M, Hammerschlag MR. Failure to detect *Chlamydia pneumoniae* in the central nervous system of patients with MS. Neurology. 2000;54:265.
11. Ercolini AM, Miller SD. The role of infections in autoimmune disease. Clin Exp Immuno. 2009;155(1):1–15.
12. Sherbet G. Bacterial infections and the pathogenesis of autoimmune conditions. BJMP. 2009;2(1):6-13.
13. Shoenfeld Y, et al. The mosaic of autoimmunity: hormonal and environmental factors involved in autoimmune diseases. Isr Med Assoc J. 2008;10: 8-12.
14. Grayston JT. Infections caused by *Chlamydia pneumoniae* strain TWAR. Clin Infect Dis. 1992;15:757–763.
15. Koskiniemi M, Gencay M, Salonen O, et al. *Chlamydia pneumoniae* associated with central nervous system infections. Eur J Neurol. 1996;36:160–163.
16. Fainardi E, Castellazzi M, Seraceni S, Granieri E, Contini C. Under the microscope: Focus on *Chlamydia pneumoniae* infection and multiple sclerosis. Curr Neurovasc Res. 2008;5(1):60-70.
17. Sotgiu S, Piana A, Pugliatti M, Sotgiu A, Deiana GA, Sgaramella E, et al. *Chlamydia pneumoniae* in the cerebrospinal fluid of patients with multiple sclerosis and neurological controls. Mult Scler. 2001;7(6):371-4.
18. Sriram S, Mitchell W, Stratton C. Multiple sclerosis associated with *Chlamydia pneumoniae* infection of the CNS. Neurology. 1998;50:571–2.
19. Sriram S, Stratton CW, Yao S, Tharp A, Ding L, Bannan JD, et al. *Chlamydia pneumoniae* infection of the central nervous system in multiple sclerosis. Ann Neurol. 1999;46:6–14.
20. Hammerschlag MR, Ke Z, Lu F, Roblin P, Boman J, Kalman B. Is *Chlamydia pneumoniae* present in brain lesions of patients with multiple sclerosis? J Clin Microbiol. 2000;38:4274–6.
21. Layh-Schmitt G, Bendl C, Hildt U, Dong-Si T, Juttler E, Schnitzler P, et al. Evidence for infection with *Chlamydia pneumoniae* in a subgroup of patients with multiple sclerosis. Ann Neurol. 2000;47:652–5.

22. Li WP, Ming X, Cook B, Blumberg B, Dowling P. *Chlamydia pneumoniae* sequence frequently present in both MS and control spinal fluid [abstract]. Neurology. 2000;54(Suppl 3):A165.
23. Pucci E, Taus C, Cartechini E, Morelli M, Giuliani G, Clementi M, et al. Lack of Chlamydia infection of the central nervous system in multiple sclerosis [letter]. Ann Neurol. 2000;48:399–400.
24. Treib J, Haass A, Stille W, Maass M, Stephan C, Holzer G, et al. Multiple sclerosis and *Chlamydia pneumoniae* [letter]. Ann Neurol. 2000;47:408–11.
25. Derfuss T, Hohlfeld R, Meinl E. Multiple sclerosis. Chlamydia hypothesis in debate. ervenarzt. 2001 Oct;72(10):820-3.
26. Kaufman M, Gaydos CA, Sriram S, Boman J, Tondella ML, Norton HJ. Is *Chlamydia pneumoniae* found in spinal fluid samples from multiple sclerosis patients? Conflicting results Mult Scler. 2002;8(4):289-94.
27. Budak F, Keçeli S, Efendi H, Budak F, Vahaboğlu H. The investigation of *Chlamydophila pneumoniae* in patients with multiple sclerosis. Int J Neurosci. 2007;117(3):409-15.
28. Derfuss T, Gürkov R, Then Bergh F, Goebels N, Hartmann M, Barz C, Wilske B, et al. Intrathecal antibody production against *Chlamydia pneumoniae* in multiple sclerosis is part of a polyspecific immune response. Brain. 2001;124(Pt 7):1325-35.
29. Fainardi E, Castellazzi M, Seraceni S, Granieri E, Contini C. Under the microscope: Focus on *Chlamydia pneumoniae* infection and multiple sclerosis. Curr Neurovasc Res. 2008;5(1):60-70.

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