A prospective study of *Chlamydia pneumoniae* infection and risk of MS in two US cohorts

K.L. Munger, MSc; G.N. DeLorenze, PhD; L.I. Levin, PhD, MPH; M.V. Rubertone, MD, MPH; J.H. Vogelman, DEE; C.A. Peck, MD; R.W. Peeling, PhD; N. Orentreich, MD; and A. Ascherio, MD, DrPH

Abstract—*Background: Chlamydia pneumoniae* (Cpn) has been proposed as a possible etiologic agent in multiple sclerosis (MS). However, previous studies were cross-sectional and could not assess whether Cpn infection preceded the onset of MS. *Methods:* The authors conducted a prospective nested case-control study among 3 million US Army personnel and 121,466 members of the Kaiser Permanente Medical Care Program (KPMCP) cohort. Serum samples collected prior to onset of MS symptoms were available for 83 MS cases in the Army and 46 in the KPMCP cohort. Two controls were matched to each case on age, sex, and date of blood collection. Microimmunofluorescence was used to measure serum immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody titers to Cpn; IgG titers ≥ 1:16 were considered positive for past Cpn infection. *Results:* Seropositivity for Cpn was not significantly associated with risk of MS in either cohort (Army: OR = 1.0; 95% CI 0.6, 1.8; KPMCP: OR = 1.5; 95% CI 0.7, 3.1) or in the pooled analysis (OR = 1.2; 95% CI 0.8, 1.9). Serum levels of anti-Cpn IgG antibody were also not associated with an increased risk of MS in the Army (OR for a fourfold difference in antibody titers = 0.9; 95% CI 0.7, 1.2) or in the pooled analysis (OR = 1.2; 95% CI 0.9, 1.4), but a significant increase in risk was seen in the KPMCP cohort (OR = 1.7; 95% CI 1.2, 2.5). The difference between these results in the Army and the KPMCP cohort was significant ($p = 0.01$). *Conclusions:* Neither Cpn seropositivity nor serum anti-Cpn IgG antibody titers predicted risk of developing MS. However, due to the heterogeneity of results between cohorts, we cannot exclude the possibility that infection with Cpn may modify the risk of MS.

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Chlamydia pneumoniae (Cpn) has been proposed as an etiologic agent of multiple sclerosis (MS).[1,2] Some support for this hypothesis is provided by the detection of Cpn or oligoclonal bands reactive against Cpn in the CSF of individuals with MS[2-5] and by higher serum anti-Cpn immunoglobulin G (IgG) titers among women with MS compared to controls.[6] Other studies, however, have been unable to corroborate these findings.[4,7-9] Possible reasons for the inconsistencies include differences in laboratory methods for Cpn detection and small sample sizes in most of the studies. Further, all of the studies were cross-sectional in design and could not properly assess whether Cpn infection or the immune response to infection preceded the onset of MS symptoms. Therefore, we used data from two large cohorts to examine if infection with Cpn predicts the risk of developing MS.

Methods. This study has been approved by the Institutional Review Boards of the Harvard School of Public Health, Boston, MA, the Walter Reed Army Institute of Research, Silver Spring, MD, and the Kaiser Foundation Research Institute, Oakland, CA.

Study population. Cases and controls for this study were obtained from two cohorts, one comprised of soldiers in the US Army with blood samples collected and stored by the Department of Defense and the other comprised of participants in the Kaiser Permanente Health Plan who had given blood samples as part of a multiphasic health check-up, as described below.

US Army cohort. Since 1985, all military service members are routinely tested for HIV infection. Residual serum samples from this testing are catalogued and stored by the Department of Defense Serum Repository (DoDSR). Over 7 million military personnel on active and reserve duty, including more than 3 million soldiers in the US Army, have serum samples archived in the DoDSR.[10]

To identify cases of MS occurring in the Army, we searched the US Army Physical Disability Agency database for soldiers granted temporary or permanent disability status due to MS between January 1998 and July 2000.[11] Medical records were identified and reviewed for 146 potential MS cases by two trained abstractors who recorded date of first symptoms attributable to MS (onset), date of MS diagnosis, and clinical and laboratory findings. Cases were considered definite if they had a diagnosis of "definite MS," "clinically definite MS," or "laboratory-supported definite MS" in their records or if they had a history of two or more attacks, MRI findings supportive of MS, and a diagnosis of MS made by a neurologist. Probable MS cases either had a diagnosis of "probable MS," "clinically probable MS," or "laboratory-supported probable MS" in their records, or a diagnosis of MS made by a neurologist

From the Departments of Nutrition (K.L. Munger and Dr. Ascherio) and Epidemiology (Dr. Ascherio), Harvard School of Public Health, Boston, MA; Division of Research (Dr. DeLorenze), Kaiser Foundation Research Institute, Oakland, CA; Division of Preventive Medicine (L.I. Levin), Walter Reed Army Institute of Research, Washington, DC; Army Medical Surveillance Activity (Dr. Rubertone), US Army Center for Health Promotion and Preventive Medicine, Washington, DC; Orentreich Foundation for the Advancement of Science, Inc. (Drs. Vogelman and Orentreich), Cold-Spring-on-Hudson, NY; US Army Physical Disability Agency (Dr. Peck), Washington, DC; National Laboratory for Sexually Transmitted Diseases (Dr. Peeling), National Microbiology Laboratory, Winnipeg, Canada; and Channing Laboratory (Dr. Ascherio), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

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Address correspondence and reprint requests to Dr. Alberto Ascherio, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115; e-mail: alberto.ascherio@channing.harvard.edu

and either a history of two or more attacks or positive MRI results. Cases were classified as primary progressive if they had a diagnosis of primary progressive or chronic progressive MS with no relapses, as stated in the medical record, or as relapsing-remitting if they had a diagnosis of relapsing-remitting MS or their records clearly outlined at least two distinct exacerbations, or as secondary progressive MS if stated in their records. Of the 146 cases reviewed, 118 had definite or probable MS; of these cases, 83 (53 definite, 30 probable) had at least one serum sample stored in the DoDSR collected before onset of MS symptoms. For each case, we selected the earliest serum sample (baseline sample) and up to two additional samples collected before onset, and one sample collected after onset. Two samples before onset were available for 40 of the cases and three samples for 29; serum collected after onset was available for 68 cases. MS course was primary progressive in 15 (18%) cases, relapsing-remitting in 47 (57%), secondary progressive in 3 (4%), and unknown in 18 (22%). Two controls were randomly selected and matched to each case by age (±1 year), sex, race/ethnicity (white, African American, Hispanic, other) and dates of blood collection (±30 days). Controls were on active duty in the Army on the date of MS diagnosis of the matched case.

Kaiser Permanente. Between 1964 and 1971, 121,466 Kaiser Permanente Medical Care Program (KPMCP) members in Northern California participated in a multiphasic health check-up, a comprehensive health evaluation program. Participants received a check-up that involved a blood collection and completion of a comprehensive health questionnaire. Serum samples from this cohort have been catalogued and stored by the Orentreich Foundation for the Advancement of Science, Inc. (OFAS) in Cold Spring-on-Hudson, NY.[12]

All outpatient visits at Kaiser Permanente hospitals, medical centers, and medical offices since 1995 are recorded in a computerized database, the Outpatient Summary Clinical Record (OSCR). We searched the OSCR for the records of patients who had received any outpatient care including neurology, physical medicine, rehabilitation, or neuropsychiatry services between 1995 and 1999 and identified over 2,000 potential MS cases. Linking these cases with the OFAS serum bank, we identified 93 with an available serum sample. Using the chart abstraction form designed for the Centers for Disease Control and Prevention's Demyelinating Diseases Study, a trained abstractor reviewed their medical records, recording dates of various neurologic symptoms (e.g., gait ataxia, diplopia, optic neuritis, transient weakness/paraesthesia of limb, Lhermitte's sign), date of MS diagnosis, MRI and CSF results, and type of MS (relapsing-remitting or progressive). Of the 93 cases reviewed, 72 had a diagnosis of definite MS. Onset date was set as the earlier date of any symptom attributable to MS, as outlined by Poser,[13] or a diagnosis of optic neuritis. Using this method, we determined onset dates for 58 cases. One serum sample collected before onset was available for 46 of these cases. MS course was progressive (includes both primary and secondary) for 26 (57%), relapsing-remitting for 7 (15%), and other or unknown for 13 (28%). Unlike in the Army cohort, we were not able to distinguish between primary and secondary progressive MS in this cohort. For each case, two controls matched by age (±30 days), sex, and date of blood collection (±1 year) were randomly selected from individuals who had an available serum sample. Controls were active KPMCP members at the time the case was diagnosed.

Cpn serology. Serum anti-Cpn IgG and immunoglobulin M (IgM) antibody levels were measured using microimmunofluorescence (MIF).[14,15] The samples were assayed in triplets (case plus two matched controls in random order), and laboratory technicians were blinded to the disease status of the samples. Anti-Cpn IgG and IgM titers were measured at dilutions of 1:16, 1:32, and 1:64, and samples positive at 1:64 were titered to endpoint. The serum IgG and IgM titers were defined as the highest dilution with a positive test result. Samples with IgG titer values ≥1:16 were considered positive for Cpn infection.[15] All negative samples and those positive at a dilution of 1:512 or higher were retested for confirmation. Positive and negative control samples were included in each run, and *Chlamydia trachomatis* and *C psittaci* antigens were included in the antigen panel to monitor cross-reactivity of antibodies. Quality control (QC) triplets were included with the case/control triplets to monitor the reliability of the MIF assay. Twenty QC triplets were randomly distributed throughout the Army samples and 10 among the KPMCP samples. The intra-assay coefficient of variation for anti-Cpn IgG was 4% in the Army samples and 16% in the KPMCP samples.

Covariates. In the Army cohort information was also available on latitude of residence at time of entry into the Army (Northern tier: north of 41°; Southern tier: south of 37°; and middle tier) and education level (high school, some college, completed college/graduate school). In the KPMCP cohort, participants in the multiphasic health check-up were not asked to self-report race; instead, race was assigned to participants by clinicians on the basis of skin color (white, black, yellow, other). Skin color was available for all cases and controls and had high agreement with race that was self-reported by 34 cases and 61 controls in visits after the multiphasic health check-up (whites: 93%, African Americans: 83%, and Asians: 100%); therefore, we used skin color as a proxy for race. Information on smoking status (never, past, current) was available from the health questionnaire completed by the participant during the multiphasic health check-up.

Statistical analysis. Analyses were done separately in each cohort as well as in both cohorts combined (pooled). Conditional logistic regression was used to estimate risk of MS associated with *Chlamydia* seropositivity in baseline samples. The reciprocal of the dilution of serum antibody titers was log-transformed as titers were measured in increasing twofold dilutions, and conditional logistic regression was used to estimate the risk of MS for a fourfold difference in serum antibody titers. Linear regression was used to compare serum anti-*Chlamydia* IgG antibody titers in pre-onset baseline samples with those in post-onset samples among cases with both samples in the Army cohort. All p values were two-tailed; a p value of 0.05 was considered significant. SAS version 8.2 was used for the analysis.

Results. The distributions of selected characteristics in cases and controls are shown in table 1. Over 60% of cases and controls from the Army were men, as compared with 13% in the KPMCP cohort. Furthermore, because of differences in the source populations, age at MS onset was lower in the Army (mean 27 years), and the interval between blood collection and MS onset was shorter (mean 4 years) than in the KPMCP (mean age at onset 46 years; mean interval 15 years) (see table 1).

Serum anti-Cpn IgM antibody levels were positive (≥1:32) in the baseline sample of one Army control and none of the cases and negative for all cases and controls in the KPMCP cohort. Cpn IgG seropositivity prior to MS onset was not associated with an increased risk of developing MS. In the Army, 67% of both cases and matched controls were positive for anti-Cpn IgG in baseline samples (OR = 1.0; 95% CI = 0.6 to 1.8), as were 63% of cases and 53% of controls in the KPMCP cohort (OR = 1.5; 95% CI = 0.7 to 3.1; pooled OR = 1.2; 95% CI = 0.8 to 1.9). Cpn seropositivity also did not predict risk of having relapsing-remitting (OR = 0.8; 95% CI = 0.4 to 1.7) or primary progressive (OR = 1.0; 95% CI = 0.3 to 3.7) MS in the Army. Further adjustment in the Army cohort for latitude of residence at time of entry into active duty and education level or in the KPMCP cohort for race and smoking status did not change these results (Army: OR = 1.0; 95% CI = 0.6 to 1.9; KPMCP: OR = 1.4; 95% CI = 0.7 to 3.0). Results in the Army cohort were similar when the analysis was restricted to definite MS cases (all: OR = 0.96; 95% CI = 0.47 to 2.0; relapsing-remitting: OR = 0.8; 95% CI = 0.4 to 1.8; primary progressive: OR = 1.0; 95% CI = 0.1 to 11). This analysis was not possible in the KPMCP cohort as cases were defined only as "MS" or "not MS."

The baseline geometric mean serum anti-Cpn IgG titers were unrelated to length of sample storage and age at blood collection among controls in either cohort (data not shown). Titers were similar between cases and controls in

Table 1 *Selected characteristics of multiple sclerosis cases and matched controls*

	US Army				KPMCP	
	Cases					
Characteristics	All	RR	PP	Controls	Cases	Controls
No.	83	47	15	166	46	92
Age at collection of baseline blood, y, mean*	24	24	27	24	31	31
Male, %*	65	62	73	65	13	13
Race/ethnicity, %†						
White	60	62	60	60	72	70
African American	35	34	33	35	24	17
Hispanic	1	2	0	1	—	—
Other/unknown	4	2	7	4	4	13
Smoking status at collection of baseline blood,‡ %						
Never					24	32
Past					13	11
Current					41	45
Unknown					22	13
Residing in North tier at entry into Army, %	30	32	33	25	—	—
Age at MS onset, y, mean (range)	27 (18–41)	27	31	—	46 (24–79)	—
Time between baseline blood collection and MS onset, y, mean (range)	4 (<1–11)	4	4	—	15 (<1–32)	—
Seropositive for Cpn IgG, §%	67	62	60	67	63	53

* Matching variable.
† Matching variable in Army only.
‡ Smoking status at blood collection was not available in the Army cohort.
§ Serum anti-Cpn immunoglobulin G (IgG) titers ≥ 1:16.

KPMCP = Kaiser Permanente Medical Care Program; RR = relapsing-remitting multiple sclerosis; PP = primary progressive multiple sclerosis.

the Army but were higher in KPMCP cases compared to controls (table 2). No increased risk of MS was seen in the Army cohort with a fourfold difference in serum anti-Cpn IgG levels overall (OR = 0.9; 95% CI = 0.7 to 1.2) or for risk of having a relapsing-remitting or primary progressive course at onset (see table 2). In the KPMCP cohort, however, a fourfold difference in serum anti-Cpn IgG levels was associated with a significant increase in risk of MS (OR = 1.7; 95% CI = 1.2 to 2.5). The difference in relative risk between the cohorts was significant ($p = 0.01$). In the pooled analysis, a fourfold difference in serum anti-Cpn IgG levels was not significantly associated with risk of developing MS (see table 2). When this analysis was restricted to anti-Cpn IgG seropositive cases and controls (i.e., IgG ≥1:16), there remained no increased risk of MS in the Army (OR = 0.7; 95% CI = 0.4 to 1.2), but the OR for a fourfold difference in IgG titers increased to 5.1 (95% CI = 1.5 to 17) in the KPMCP cohort. Results among seropositive cases and controls were not materially changed after adjusting for latitude of residence at entry and education level in the Army or race and smoking in the KPMCP cohort or when restricted to definite MS cases in the Army (OR = 0.8; 95% CI = 0.4 to 1.6).

Results were virtually unchanged after excluding five cases with onset after age 60 in KPMCP or five cases in the Army and three in KPMCP with positive serum IgG antibody levels to all three Chlamydia species (Cpn, *C trachomatis*, *C psittaci*), which may be indicative of a nonspecies specific immune response. Further, neither seropositivity for nor IgG serum levels against *C trachomatis* or *C psittaci* were associated with risk of MS in either cohort.

Cases in the KPMCP were predominately female, nearly 20 years older on average at onset, and had a longer period of time between blood collection and onset than the Army cohort; therefore, we also examined the association between serum anti-Cpn IgG levels and risk of MS by sex, age at onset, and time between blood collection and onset. In the Army, results of analyses restricted to women were similar to those of men; the number of men (6 cases) in the KPMCP cohort was too small for analysis. OR for a fourfold difference in serum anti-Cpn IgG levels did not change with age at onset within each cohort (Army cohort: <20 to 24: OR = 0.85; 25 to 29: OR = 1.0; 30 to 34: OR = 1.2; ≥35: OR = 0.72; KPMCP cohort: <40: OR = 1.7; 40 to 49: OR = 1.4; ≥50: OR = 1.8). In the KPMCP cohort, the OR for a fourfold difference in titers were similar among samples collected less than 10 years before onset (OR = 1.7; 95% CI = 0.80 to 3.5) and 10 or more years (OR = 1.7; 95% CI = 1.1 to 2.6) before onset.

Up to three samples collected before onset and one after onset were available for the Army cohort. There was no

Table 2 *Geometric mean titers (GMT) of anti-Cpn immunoglobulin G (IgG) antibody in baseline serum samples of multiple sclerosis cases and matched controls and odds ratios associated with a four-fold difference in Cpn IgG antibody titers*

	Cases		Controls			
Group	n	GMT	n	GMT	OR* (95% CI)	*p* Value
Army						
All	83	37	166	40	0.9 (0.7, 1.2)	0.61
RR	47	36	94	41	0.9 (0.6, 1.3)	0.63
PP	15	35	30	33	1.1 (0.6, 2.0)	0.87
KPMCP	46	50	92	24	1.7 (1.2, 2.5)	0.007
Pooled	129	41	258	34	1.2 (0.9, 1.4)	0.19

* US Army adjusted through matching for age (± 1 year), sex, race, and date of blood collection (± 30 days); KPMCP adjusted through matching for age (± 30 days), sex, and date of blood collection (± 1 year).

KPMCP = Kaiser Permanente Medical Care Program; RRMS = relapsing-remitting multiple sclerosis; PPMS = primary progressive multiple sclerosis.

significant change over time in mean serum anti-Cpn IgG levels among all MS cases or relapsing-remitting or primary progressive cases prior to MS onset. Among 68 cases with a serum sample collected after onset (average: 1 year, range: <1 to 6 years), age-adjusted geometric mean serum anti-Cpn IgG levels were higher in post-onset than in baseline samples among all cases (48 vs 37, $p = 0.25$) as well as among 38 relapsing-remitting MS cases (52 vs 39, $p = 0.03$) but not 14 primary progressive cases (28 vs 30, $p = 0.22$). Neither *C trachomatis* nor *C psittaci* IgG titer levels differed between baseline and post-onset samples.

Discussion. In this prospective study, we found that the presence of detectable serum antibodies against Cpn did not predict the risk of developing MS. Overall, serum levels of anti-Cpn IgG also did not predict risk; however, in one of the two cohorts investigated, a fourfold difference in anti-Cpn IgG serum levels was associated with a 70% increased risk of MS. Further, Cpn was not specifically associated with an increased risk of a relapsing-remitting or primary progressive course at onset.

Anti-Cpn IgG titers are usually elevated following infection, but in some individuals titers may fall to undetectable levels within a few years.[16] Thus, titers less than 1:16 do not exclude the possibility of past infection. Our results, however, suggest that recent or current infection or an increased immune response to Cpn are not associated with MS. The reasons we observed an increase in MS risk associated with pre-onset anti-Cpn IgG serum levels in the KPMCP cohort, but not in the Army, are not clear. The anti-Cpn IgG titer levels in samples from both cohorts were measured by the same laboratory using the same methods.[15] Additionally, the samples were assayed in triplets with the case and two matched controls in random order, and the laboratory technicians were blinded to the case/control status of the samples. Although anti-Cpn IgG antibody degradation in the samples is possible as they were stored for up to 12 (Army) or 32 (KPMCP) years, we saw no association between serum anti-Cpn IgG antibody levels and length of sample storage among the controls in either cohort. Further, degradation would be an unlikely explanation of the differences in titers by disease state because samples of cases and matched controls within each cohort were collected at the same time and handled identically.

Differences in the sex composition of the two cohorts also do not explain these results, as within the Army cohort results were similar in men and women. The most conspicuous differences between the two cohorts are that the Army cases were on average 20 years younger at onset than the KPMCP cases, and the time interval between blood collection and MS onset was substantially shorter in the Army. The unusually late age at onset in the KPMCP cohort is an artifact as in this cohort we could only detect patients with MS utilizing Kaiser Permanente health care services in 1995 or later, which is about 30 years after the blood collection. Thus, two hypotheses could explain the different results between the two cohorts. One is that infection with Cpn increases the risk of MS only after a long incubation period; the other is that Cpn is only related to risk of MS in older individuals. Neither of these interpretations is supported by other data and therefore these results should be taken cautiously. We also cannot exclude the possibility that some other unknown factor varies between the two cohorts and may explain the inconsistent results.

In serial analyses in the Army cohort, mean anti-Cpn IgG titers did not significantly change over time between blood collection and MS onset. Overall, mean anti-Cpn IgG levels after onset were not significantly higher than pre-onset levels. However, mean titers after onset were significantly higher compared to pre-onset levels among the relapsing-remitting cases, suggesting that Cpn reactivation or reinfection, events that are associated with increased anti-Cpn IgG titer levels,[16] may be a common occurrence after relapsing-remitting MS onset. Serum titers of IgG against *C trachomatis* and *C psittaci* were similar in pre- and post-onset samples.

There are currently no other prospective studies on Cpn infection and risk of MS. Among the cross-sectional studies, some have found evidence in support of an association[2-6] whereas others have not.[4,7-9] The inconsistencies have been attributed to the lack of sensitivity and standardization of assays for Cpn detection, use of heterogeneous control groups, and small sample sizes.[17,18] A few studies[9,19,20] have found elevated anti-Cpn IgG titers in CSF, but not serum, suggesting that normal serum titers do not exclude a potential role of Cpn in MS.

In a previous study[6] we found that women with progressive MS were more likely to be seropositive for anti-Cpn IgG and have higher anti-Cpn IgG levels compared with controls; no distinction, however,

could be made between primary or secondary progressive disease. Other cross-sectional studies[2,4,21] and one follow-up study of MS patients[22] have provided some evidence suggesting that Cpn may play a role in disease activity and promotion of a progressive disease course. Some support for a deleterious effect of Cpn on MS progression is also provided by a study among mice with experimental autoimmune encephalomyelitis (EAE).[23] Intraperitoneal and systemic Cpn infection increased the severity of EAE, and severity was attenuated with antibiotic therapy, whereas *C trachomatis* infection had no effect on EAE severity. In the current study, we did not find an association of Cpn infection with an increased risk of primary progressive MS. Because our study was not designed to assess the association between Cpn infection and development of a secondary progressive course, prospective studies examining this association are warranted.

Disclaimer. The views expressed are those of the authors and should not be construed to represent the positions of the Department of the Army or Department of Defense.

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References

1. Sriram S, Mitchell W, Stratton C. Multiple sclerosis associated with Chlamydia pneumoniae infection of the CNS. Neurology 1998;50:571–572.
2. Sriram S, Stratton CW, Yao S, et al. Chlamydia pneumoniae infection of the central nervous system in multiple sclerosis. Ann Neurol 1999;46:6–14.
3. Layh-Schmitt G, Bendl C, Hildt U, et al. Evidence for infection with Chlamydia pneumoniae in a subgroup of patients with multiple sclerosis. Ann Neurol 2000;47:652–655.
4. 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;8:289–294.
5. Yao SY, Stratton CW, Mitchell WM, Sriram S. CSF oligoclonal bands in MS include antibodies against Chlamydophila antigens. Neurology 2001;56:1168–1176.
6. Munger KL, Peeling RW, Hernan MA, et al. Infection with Chlamydia pneumoniae and risk of multiple sclerosis. Epidemiology 2003;14:141–147.
7. 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.
8. 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;38:4274–4276.
9. Krametter D, Niederwieser G, Berghold A, et al. Chlamydia pneumoniae in multiple sclerosis: humoral immune responses in serum and cerebrospinal fluid and correlation with disease activity marker. Mult Scler 2001;7:13–18.
10. Rubertone MV, Brundage JF. The Defense Medical Surveillance System and the Department of Defense serum repository: glimpses of the future of public health surveillance. Am J Public Health 2002;92:1900–1904.
11. Levin LI, Munger KL, Rubertone MV, et al. Multiple sclerosis and Epstein-Barr virus. JAMA 2003;289:1533–1536.
12. Friedman GD, Blaner WS, Goodman DS, et al. Serum retinol and retinol-binding protein levels do not predict subsequent lung cancer. Am J Epidemiol 1986;123:781–789.
13. Poser CM. Onset symptoms of multiple sclerosis (letter). J Neurol Neurosurg Psychiatry 1995;58:253–254.
14. Wang S. Serology for Chlamydia pneumoniae (TWAR). In: Allegra L, Blasi F, eds. Chlamydia pneumoniae, the lung and the heart. Milan: Springer-Verlag, 1999;16–23.
15. Dowell SF, Peeling RW, Boman J, et al. Standardizing Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). Clin Infect Dis 2001;33:492–503.
16. Grayston JT, Campbell LA, Kuo C-C, et al. A new respiratory tract pathogen: Chlamydia pneumoniae strain TWAR. J Infect Dis 1990;161:618–625.
17. Tompkins LS, Schachter J, Boman J, et al. Collaborative multidisciplinary workshop report: detection, culture, serology, and antimicrobial susceptibility testing of Chlamydia pneumoniae. J Infect Dis 2000;181 Suppl 3:S460–461.
18. Bashir K, Kaslow RA. Chlamydia pneumoniae and multiple sclerosis: the latest etiologic candidate. Epidemiology 2003;14:133–134.
19. Hao Q, Miyashita N, Matsui M, Wang HY, Matsushima T, Saida T. Chlamydia pneumoniae infection associated with enhanced MRI spinal lesions in multiple sclerosis. Mult Scler 2002;8:436–440.
20. Derfuss T, Gurkov R, Bergh FT, et al. Intrathecal antibody production against Chlamydia pneumoniae in multiple sclerosis is part of a polyspecific immune response. Brain 2001;124(Pt 7):1325–1335.
21. Grimaldi LM, Pincherle A, Martinelli-Boneschi F, et al. An MRI study of Chlamydia pneumoniae infection in Italian multiple sclerosis patients. Mult Scler 2003;9:467–471.
22. Hintzen RQ, Verkooyen RP, Hop W, van der Zwaan E, van Doorn PA, Buljevac D. Chlamydia pneumoniae infections are associated with increased disease activity in multiple sclerosis. Neurology 2003;60(5 S1):A60–A61.
23. Du C, Yao SY, Ljunggren-Rose A, Sriram S. Chlamydia pneumoniae infection of the central nervous system worsens experimental allergic encephalitis. J Exp Med 2002;196:1639–1644.