Variable association between *Chlamydophila psittaci* infection and ocular adnexal lymphomas: methodological biases or true geographical variations?

Didier Decaudin[a], Riccardo Dolcetti[e], Patricia de Cremoux[b], Maurilio Ponzoni[f,g], Anne Vincent-Salomon[b], Claudio Doglioni[f,g], Rémi Dendale[c], Marie-Christine Escande[b], Livia Lumbroso-Le Rouic[d] and Andrés J.M. Ferreri[g,h]

Since the first publication in 2004, a large number of reports have raised the question of an association between ocular adnexal lymphoma and *Chlamydia psittaci*. The results of this scientific debate, however, remain controversial. The primary objective of this paper was therefore to raise important questions concerning the interpretation of the different and heterogeneous data on the association between *Chlamydophila psittaci* and ocular adnexal lymphoma, namely the impact of the methodology used and the epidemiological variability of seroprevalence of *C. psittaci* antibodies. This paper also provides some methodological suggestions for future studies in the field of chlamydia–lymphoma associations. *Anti-Cancer Drugs* 19:761–765 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2008, **19:**761–765

Keywords: *Chlamydia psittaci*, MALT lymphoma, ocular adnexal

[a]Department of Clinical Hematology/Laboratory of Pre-clinical Investigation, Departments of [b]Tumor Biology, [c]Radiation Oncology, [d]Ophthalmology, Institut Curie, France, [e]Cancer Bio-Immunotherapy Unit, Department of Medical Oncology, Centro di Riferimento Oncologico, IRCCS National Cancer Institute, Aviano, [f]Pathology Unit, [g]Unit of Lymphoid Malignancies and [h]Medical Oncology Unit, Department of Oncology, San Raffaele Scientific Institute, Milan, Italy

Correspondence to Dr Didier Decaudin, MD, PhD, Service d'Hématologie, Institut Curie, 26 rue d'Ulm, 75.248 Paris cedex 05, France
Tel: +33 1 44 32 46 90; fax: +33 1 53 10 40 11;
e-mail: didier.decaudin@curie.net

Received 11 May 2008 **Revised form accepted** 4 June 2008

Introduction

Evidence of an association between *Chlamydophila psittaci* infection and ocular adnexal MALT (mucosa-associated lymphoid tissue)-type lymphoma (OAML) has been reported [1]. As suggested for other infection-related lymphomas, a sustained stimulation of the immune system probably constitutes the first necessary step toward transformation in OAML as well. A large number of these patients show an earlier history of chronic conjunctivitis, and some patients with a history of *C. psittaci* infection, follicular conjunctivitis and conjunctival MALT lymphoma have been reported [2]. Moreover, the role of this microorganism is suggested by lymphoma remissions observed after antibiotic therapy with doxycycline [3]. Molecular progression from *C. psittaci*-associated chronic infection to OAML, however, remains to be demonstrated.

Over recent months, several groups from different countries have investigated the prevalence of *C. psittaci* infection in their own series of ocular adnexal lymphomas. Reported studies displayed prevalence rates between 0 and 80%, with wide variability among countries and among different regions within the same country [3–16]. These discrepancies could reflect genuine epidemiological variations or biases related to other intervening factors, including the sensitivity and specificity of detection methods, the common use of wide-spectrum antibiotics before biopsy and the involvement of other doxycycline-responsive infectious agents [17]. Understanding the reasons underlying these discrepancies is of paramount importance in the light of the therapeutic advantages offered by the introduction of doxycycline in the management of OAML patients [3].

Regardless of the intriguing debate on the potential role of *C. psittaci* in lymphomagenesis, this paper critically analyzes methodological differences among published studies and potential interpretation biases that may have affected reported results, and provides some methodological suggestions for future studies in the field of chlamydia–lymphoma associations.

Prevalence of *C. psittaci* infection in ocular adnexal MALT-type lymphoma

The overall prevalence of *C. psittaci* infection in 423 cases of OAML reported in 14 papers is 19%, with a range from 0 to 87% (Table 1). This infection has been detected in 14% (range: 0–69%) of 125 cases of ocular adnexal lymphomas of other histotype and in 8% (range: 0–11%)

0959-4973 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/CAD.0b013e32830b58c4

Table 1 Association between *Chlamydiphila psittaci* and ocular adnexal lymphoma (OAL)

Reference	Countries	OAL of MALT-type (%)	OAL of non-MALT-type (%)	Non-NHL orbital samples	Specimen supports	Technical supports	Notes
[1]	Italy	21/24 (87)	11/16 (69)	0/20 (0)	PEFT	TETR-PCR[a]	/
[4]	UK	4/33 (12)	1/7 (14)	4/40 (10)	PEFT	Single TETR-PCR[b] + direct sequencing	/
	Germany	9/19 (47)	0/9 (0)	1/9 (11)			
	Netherlands	6/21 (33)	2/3 (66)	–			
	Italy	2/15 (13)	0/6 (0)	–			
	China	4/37 (11)	2/20 (10)	0/2 (0)			
	East USA	6/17 (35)	0/8 (0)	–			
[5]	Japan	0/18 (0)	0/3 (0)	0/3 (0)	Frozen and PEFT	TETR-PCR[a] + nested PCR	/
[6]	France	0/8 (0)	1/8 (13)	–	PEFT	TETR-PCR[a]	/
[7]	Japan	0/12 (0)	0/5 (0)	0/4 (0)	PEFT	TETR-PCR + Southern hybridization	Sensitivity data were not given
[8]	Netherlands	0/19 (0)	–	–	PEFT	TETR-PCR[a] + sequencing; omp-A RT-PCR	One additional case with unsuitable DNA
[9]	South USA	0/49 (0)	–	0/2 (0)	Fresh and PEFT	TETR-PCR[a]	/
[10]	USA	0/7 (0)	0/4 (0)	0/6 (0)	PEFT	Single TETR-PCR[b] + 23S specific PCR	Sensitivity data were not given; only 50–200 ng of DNA was analyzed
[12]	Cuba	2/19 (11)	0/7 (0)	0/20 (0)	PEFT	TETR-PCR[a] + direct sequencing	/
[11]	Germany	0/22 (0)	0/18 (0)	–	PEFT	Single TETR-PCR[b]	Only 23 (48%) with amplifiable β-globin; sensitivity: 20 copies of a *C. psittaci* plasmid
[13]	USA	0/30 (0)	–	–	Frozen and PEFT	Omp-1 and omp-2 RT-PCR	/
[14]	Japan	0/23 (0)	–	–	PEFT	16S and 16S-23S spacer PCR protocols	/
[15]	Korea	26/33 (78)	–	5/21 (23)	PEFT	TETR-PCR[a] + direct sequencing	/
[16]	USA	0/17 (0)	0/11 (0)	0/5 (0)	PEFT	16S PCR/gel based assay + 16S RT-PCR	Sensitivity data were not given
Total		80/423 (19)	17/125 (14)	10/132 (8)	/	/	/

LH, lymphoid hyperplasia; MALT, mucosa-associated lymphoid tissue; PEFT, paraffin-embedded fixed tissue.
[a]Madico *et al.* [18].
[b]Monoplex TETR PCR for *C. psittaci* using primers published by Madico *et al.* [18].

of 132 cases of orbit biopsies for non-neoplastic disorders. If only OAML cases are considered, the reported series can be divided into three groups: two series from Italy and Korea reporting a prevalence of *C. psittaci* infection of greater than 75% (median: 83%) [1,15], seven series from Germany, eastern USA, The Netherlands, Italy, UK, China, and Cuba with a prevalence ranging between 10 and 50% (median: 23%) [4,12], and a group of 10 series from Japan, France, The Netherlands, USA, and Germany with a prevalence of 0% [5–11,13,14,16]. Results were homogeneous in the Japanese series [5,7,14] and variable in the series from USA [4,9,13,16], Germany [4,11], Italy [1,4], and The Netherlands [4,8].

Detection methods

Convincing evidence has been provided that *C. psittaci* DNA is present in a limited number of cases or virtually absent in some OAML series; for example, in the French series, where PCR analyses have been performed in duplicate in an independent setting at the Institut Curie and the National Cancer Institute, Aviano [6]. In contrast, it cannot be ruled out that variability in sensitivity and specificity of detection methods could have affected, at least partially, the results obtained so far in the various series analyzed. Most studies used the same multiplex touchdown enzyme time-release PCR described by Madico *et al.* [18] and first applied to ocular adnexal lymphomas by Ferreri *et al.* [1], using CPS 100 and CPS 101 primers for *C. psittaci*. In three studies [4,10,11], these primers were used in 'monoplex' PCR in the same touchdown cycling conditions. Direct sequencing was performed only in four studies to confirm the specificity of the amplified DNA [4,8,12,15], which is a relevant issue considering that the taxonomic classification of chlamydaceae is evolving and that the target fragment amplified by PCR may contain sequences largely overlapping, although belonging to unrelated clamydiaceae. In one study [7], Southern hybridization was performed with specific probes to increase the sensitivity and confirm the specificity of the analysis; however, the probes used were commercially available to be used on fresh samples, whereas tissue samples for analysis were formalin-fixed paraffin-embedded specimens. More recently, real-time PCR specific for *omp1*, *omp2*, or 16S were used as a single approach or in combination with other protocols [10,13,14,16]. Although in most of the studies formalin-fixed paraffin-embedded

tissue specimens were used, in some instances fresh [9] or frozen [5,13] material was also used. Nevertheless, the number of samples treated under these various conditions is not always mentioned. In most fixed samples, DNA was extracted by commercial silica gel columns, whereas DNA from frozen samples was purified using a classical phenol–chloroform procedure. Notably, the amount of template DNA investigated in the different studies was variably reported; data on the sensitivity of the protocol used were not always given and the quality of the DNA analyzed was not always checked. In the study by Goebel *et al.* [11], in particular, β-globin was amplifiable in only 23 of the 48 samples analyzed, raising some concern about the conclusions drawn in this study. Considering that only scattered cells carry *C. psittaci* infection in OAML samples [1], the load of target DNA is probably very low. On these grounds, the quality and the amount of template DNA investigated, as well as the sensitivity of the PCR protocols used, are crucial for the reliable assessment of the prevalence of this microorganism in OAML biopsies. These issues should be carefully taken into account to obtain reliable prevalence data that may be of value for having a clear picture of the geographical distribution of the association between chlamydial infection and lymphomas.

Interpretation biases

Other than the above-mentioned methodological pitfalls, it is important to underline the role of the use of wide-spectrum antibiotics before biopsy, which is a common practice in OAML patients [17]. In fact, most patients with conjunctival or orbital lesions are first considered as affected by inflammatory or infectious processes instead of lymphomas. This is due to the fact that clinical features at presentation are variable and not pathognomonic for OAML. Thus, these patients usually are treated with more lines of topic or systemic wide-spectrum antibiotics, associated or not with steroids, which could further reduce the local bacterial population resulting in the presence of chlamydial DNA loads below the threshold of PCR detection. Another interfering aspect is related to the potential involvement of other microbial agents in the development and maintenance of OAML. Available evidence seems to rule out the possible involvement of other infectious agents commonly associated with chronic eye diseases, such as *C. trachomatis*, herpes simplex virus 1 and 2 and adenovirus 8 and 19 [1,4], whereas *C. pneumoniae* DNA was detected in a few cases of OAML ([4,19] and Dolcetti, unpublished results). As for other B-cell lymphomas, controversial data on the association between hepatitis C virus infection and OAML exist [20–22]. Intriguingly, lymphoma remission observed in 38% of patients with *C. psittaci*-negative OAML, that is, cases where *C. psittaci* infection was not demonstrated by touchdown enzyme time-release-PCR [3], seems to suggest the involvement of other doxycycline-sensitive agents, perhaps chlamydia strains undetectable with current diagnostic methods. This hypothesis remains a matter of investigation, but it is interesting to think that *C. psittaci* could be replaced by other chlamydiae in series from certain geographical regions. Importantly, regardless of the infectious agents found in lymphoma samples, host immune factors could have a preponderant role in maintaining chronic immune system stimulation, as demonstrated for T-cell regulatory *CTLA4* gene polymorphisms and *Helicobacter pylori* infection [23], leading to a nonepidemiological modulation of the real impact of microbial agents in lymphomagenesis.

Epidemiological aspects

If the above-discussed prevalence discrepancies could not be ascribed to methodological caveats or interpretation biases, it can be assumed that a true epidemiological variability of chlamydial infection in lymphoma patients exists. Unfortunately, no suitable epidemiological data on the actual distribution of this infection in the general population exist. *C. psittaci* infections, usually named 'chlamydiosis', are zoonoses. Chlamydial diffusion in the general population is related to the prevalence of *C. psittaci* infection in wild animals. Prevalence among wild animals in a certain geographic area is difficult to investigate and it is further affected by the transport of this microorganism in migrant birds. Strains of *C. psittaci* have been detected worldwide in more than 460 bird species, mostly in clinically unapparent, latent forms [24]. Although *C. psittaci* strains from birds show some differences in virulence, all should be considered as potentially transmissible to humans. *C. psittaci* infection is commonly asymptomatic both in birds and humans and prolonged contact with an apparently healthy household animals shedding infectious chlamydia can result in repeated infection cycles in humans [25]. On these grounds, it is not surprising that this particular epidemiological issue must be better studied and that the prevalence of *C. psittaci* infection in wild and household animals, in the general population, and in selected risk groups is far from being well known.

Seroprevalence is often used to recognize endemic areas for a certain microorganism potentially associated with cancer. The most extensively studied example is hepatitis C virus seroprevalence and its association with different lymphoma entities [26,27]. The seroprevalence of anti-*C. psittaci* antibodies, determined by various methodologies, varies between 0 and 49% (median: 5–10%) in selected groups [28–38]; this prevalence increases to 49% in individuals professionally exposed to chlamydial infections or to cohabitation with infected animals [28,30,34]. Although seroprevalence has been investigated in these highly selected individuals, large screening studies on the general population are rare. Moreover, the sensitivity and specificity of serologic tests are extremely

heterogeneous with high cross-reactivity for other chlamydial species such as *C. pneumoniae*. As a consequence, serologic tests are unreliable methods to predict an association with *C. psittaci* in lymphoma patients, and the comparison of seroprevalence of *C. psittaci* antibodies between the general population and OAML patients would exhibit another relevant limitation related to the difficulties to test, in the same period, subgroups of individuals matched for age, sex, and epidemiological characteristics.

Conclusion and suggestions

In conclusion, available literature is not focused on the potential role for *C. psittaci* infection in lymphomagenesis. Reported studies only consider a bacteria–lymphoma association, which exhibits an apparent prevalence variability among different geographical regions. In this respect, the possible role of methodological pitfalls and other above-mentioned confounding factors should, however, be carefully considered. Investigators analyzing the prevalence of chlamydial infections in their own series should take into account some simple suggestions such as (i) performing direct sequencing to confirm the amplicon specificity, which should be parallel to the continuous update of molecular taxonomic classification of chlamydaceae; (ii) using more primers to exclude overlapping with unrelated clamydiaceae; (iii) specifying in reports most details on experimental conditions, such as number of investigated samples, specimens characteristics, DNA extraction procedures, amount of DNA template, sensitivity data of the protocol used, and quality of the DNA analyzed. Any potential interfering factor, as well as geographical area and years of diagnosis of the studied series, should be considered and reported. This strategy should be applied, especially to international prospective trials with clinical, biological, and translational elements, which should receive higher priority to improve our knowledge on the fascinating topic of chlamydia-related lymphomagenesis.

References

1 Ferreri AJ, Guidoboni M, Ponzoni M, De Conciliis C, Dell'Oro S, Fleischhauer K, *et al*. Evidence for an association between Chlamydia psittaci and ocular adnexal lymphoma. *J Natl Cancer Inst* 2004; **96**:586–594.

2 Yeung L, Tsao YP, Chen PY, Kuo TT, Lin KK, Lai LJ. Combination of adult inclusion conjunctivitis and mucosa-associated lymphoid tissue (MALT) lymphoma in a young adult. *Cornea* 2004; **231**:71–75.

3 Ferreri AJ, Ponzoni M, Guidoboni M, Resti AG, Politi LS, Cortelazzo S, *et al*. Bacteria-eradicating therapy with doxycycline in ocular adnexal MALT lymphoma: a multicenter prospective trial. *J Natl Cancer Inst* 2006; **98**:1375–1382.

4 Chanudet E, Zhou Y, Bacon CM, Wotherspoon AC, Müller-Hermelink HK, Adam P, *et al*. Chlamydia psittaci is variably associated with ocular adnexal MALT lymphoma in different geographical regions. *J Pathol* 2006; **209**:344–351.

5 Daibata M, Nemoto Y, Togitani K, Fukushima A, Ueno H, Ouchi K, *et al*. Absence of Chlamydia psittaci in ocular adnexal lymphoma from Japanese patients. *Br J Haematol* 2006; **132**:651–661.

6 De Cremoux P, Subtil A, Ferreri AJM, Vincent-Salomon A, Ponzoni M, Chaoui D, *et al*. Re: evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. *J Natl Cancer Inst* 2006; **98**:365–366.

7 Liu YC, Ohyashiki JH, Ito Y, Iwaya K, Serizawa H, Mukai K, *et al*. Chlamydia psittaci in ocular adnexal lymphoma: Japanese experience. *Leuk Res* 2006; **30**:1587–1589.

8 Mulder MM, Heddema ER, Pannekoek Y, Faridpooya K, Oud ME, Schilder-Tol E, *et al*. No evidence for an association of ocular adnexal lymphoma with Chlamydia psittaci in a cohort of patients from the Netherlands. *Leuk Res* 2006; **30**:1305–1307.

9 Rosado MF, Byrne GE Jr, Ding F, Fields KA, Ruiz P, Dubovy SR, *et al*. Ocular adnexal lymphoma: a clinicopathologic study of a large cohort of patients with no evidence for an association with Chlamydia psittaci. *Blood* 2006; **107**:467–472.

10 Vargas RL, Fallone E, Felgar RE, Friedberg JW, Arbini AA, Andersen AA, *et al*. Is there an association between ocular adnexal lymphoma and infection with Chlamydia psittaci? The University of Rochester experience. *Leuk Res* 2006; **30**:547–551.

11 Goebel N, Serr A, Mittelviefhaus H, Reinhard T, Bogdan C, Auw-Haedrich C. Chlamydia psittaci, Helicobacter pylori and ocular adnexal lymphoma-is there an association? The German experience. *Leuk Res* 2007; **31**: 1450–1452.

12 Gracia E, Frösch P, Mazzucchelli L, Martin V, Rodríguez-Abreu D, Jiménez J, *et al*. Low prevalence of Chlamydia psittaci in ocular adnexal lymphomas from Cuban patients. *Leuk Lymphoma* 2007; **48**:104–108.

13 Ruiz A, Reischl U, Swerdlow SH, Hartke M, Streubel B, Procop G, *et al*. Extranodal marginal zone B-cell lymphomas of the ocular adnexa: multiparameter analysis of 34 cases including interphase molecular cytogenetics and PCR for Chlamydia psittaci. *Am J Surg Pathol* 2007; **31**:792–802.

14 Yakushijin Y, Kodama T, Takaora I, Tanimoto K, Bessho H, Sakai I, *et al*. Absence of chlamydial infection in Japanese patients with ocular adnexal lymphoma of mucosa-associated lymphoid tissue. *Int J Hematol* 2007; **85**:223–230.

15 Yoo C, Ryu M-H, Huh J, Park JH, Kang HJ, Ahn HS, *et al*. Chlamydia psittaci infection and clinicopathologic analysis of ocular adnexal lymphomas in Korea. *Am J Hematol* 2007; **82**:821–823.

16 Zhang GS, Winter JN, Variakojis D, Reich S, Lissner GS, Bryar P, *et al*. Lack of an association between Chlamydia psittaci and ocular adnexal lymphoma. *Leuk Lymphoma* 2007; **48**:577–583.

17 Ferreri AJ, Ponzoni M, Dognini GP, Du MQ, Doglioni C, Radford J, *et al*. Bacteria-eradicating therapy for ocular adnexal MALT lymphoma: questions for an open international prospective trial. *Ann Oncol* 2006; **17**:1721–1722.

18 Madico G, Quinn TC, Boman J, Gaydos CA. Touchdown enzyme time release-PCR for detection and identification of Chlamydia trachomatis, C. pneumoniae, and C. psittaci using the 16S and 16S–23S spacer rRNA genes. *J Clin Microbiol* 2000; **38**:1085–1093.

19 Chan CC, Shen D, Mochizuki M, Gonzales JA, Yuen HK, Guex-Crosier Y, *et al*. Detection of Helicobacter pylori and Chlamydia pneumoniae genes in primary orbital lymphoma. *Trans Am Ophthalmol Soc* 2006; **104**:62–70.

20 Ferreri AJM, Viale E, Guidoboni M, Resti AG, De Conciliis C, Politi L, *et al*. Clinical implications of hepatitis C virus infection in MALT-type lymphoma of the ocular adnexa. *Ann Oncol* 2006; **17**:769–772.

21 Arcaini L, Burcheri S, Rossi A, Paulli M, Bruno R, Passamonti F, *et al*. Prevalence of HCV infection in nongastric marginal zone B-cell lymphoma of MALT. *Ann Oncol* 2007; **18**:346–350.

22 Arnaud P, Escande M-C, Lecuit M, Validire P, Levy C, Plancher C, *et al*. Hepatitis C virus infection and MALT-type ocular adnexal lymphoma. *Ann Oncol* 2007; **18**:400–401.

23 Cheng T-Y, Lin J-T, Chen L-T, Shun CT, Wang HP, Lin MT, *et al*. Association of T-cell regulatory gene polymorphisms with susceptibility to gastric mucosa-associated lymphoid tissue lymphoma. *J Clin Oncol* 2006; **24**:3483–3489.

24 Kaleta EF, Taday EM. Avian host range of Chlamydophila spp. based on isolation, antigen detection and serology. *Avian Pathol* 2003; **32**:435–461.

25 Ferreri AJ, Dolcetti R, Magnino S, Doglioni C, Cangi MG, Pecciarini L, *et al*. A woman and her canary: a tale of chlamydiae and lymphomas. *J Natl Cancer Inst* 2008 (in press).

26 Gisbert JP, Garcia-Buey L, Arranz R, Blas C, Pinilla I, Khorrami S, *et al*. The prevalence of hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Eur J Gastroenterol Hepatol* 2004; **16**:135–138.

27 Seve P, Renaudier P, Sasco AJ, Dumontet C, Salles G, Coiffier B, *et al*. Hepatitis C virus infection and B-cell non-Hodgkin's lymphoma: a cross-sectional study in Lyon, France. *Eur J Gastroenterol Hepatol* 2004; **16**:1361–1365.

28 Orfila J, Pinault JF, Loisier E, Thomas D. Epidemiologic study of Chlamydia psittaci infections in miming environment of the North of France. *Virologie* 1982; **33**:23–33.

29 Maffei C, Marracino A, Di Stanislao F, Pauri P, Clementi M, Varaldo PE. Psittacosis in a highly endemic area in Italy. *Epidemiol Infect* 1987; **99**:413–419.
30 Musso A, Riva C, Balbo L, Valpreda A, Garbaccio P, Migliore G, *et al*. Anti-Chlamydia psittaci antibodies in a healthy pediatric population. *Minerva Pediatr* 1991; **43**:305–309.
31 Bourke SJ, Carrington D, Frew CE, McSharry CP, Boyd G. A comparison of the seroepidemiology of chlamydial infection in pigeon fanciers and farmers in the UK. *J Infect* 1992; **25**:91–98.
32 Moss TR, Darougar S, Woodland RM, Nathan M, Dines RJ, Cathrine V. Antibodies to Chlamydia species in patients attending a genitourinary clinic and the impact of antibodies to C. pneumoniae and C. psittaci on the sensitivity and the specificity of C. trachomatis serology tests. *Sex Transm Dis* 1993; **20**:61–65.
33 Mahmoud E, Elshibly S, Mardh PA. Seroepidemiologic study of Chlamydia pneumoniae and other Chlamydia species in a hyperendemic area for trachoma in the Sudan. *Am J Trop Med Hyg* 1994; **51**:489–494.
34 Bergstrom K, Domeika M, Vaitkiene D, Persson K, Mardh PA. Prevalence of Chlamydia trachomatis, Chlamydia psittaci and Chlamydia pneumoniae antibodies in blood donors and attendees of STD clinics. *Clin Microbiol Infect* 1996; **1**:253–260.
35 Ni AP, Lin GY, Yang L, He HY, Huang CW, Liu ZJ, *et al*. A seroepidemiologic study of Chlamydia pneumoniae, Chlamydia trachomatis and Chlamydia psittaci in different populations on the mainland of China. *Scand J Infect Dis* 1996; **28**:553–557.
36 Pospisil L, Veznik Z, Diblikova I, Pejcoch M. Occurrence of antibodies to chlamydial group antigens in the population of the Czech Republic. *Epidemiol Mikrobiol Imunol* 1997; **46**:13–17.
37 Koivisto AL, Isoaho R, Von Hertzen L, Töyrylä M, Laippala P, Kivelä SL, *et al*. Chlamydial antibodies in an elderly Finnish population. *Scand J Infect Dis* 1999; **31**:135–139.
38 Heine RP, Ness RB, Roberts JM. Seroprevalence of antibodies to Chlamydia pneumoniae in women with preeclampsia. *Obstet Gynecol* 2003; **101**:221–226.