SERODIAGNOSTICS OF CHLAMYDIAL INFECTIONS – SIGNIFICANCE OF POSITIVITY IN IGA AND/OR IGM ANTIBODY CLASSES ONLY*

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Received: September 15, 2002

Key words: *Chlamydia* / Infection / Antibodies / Significance

There was followed the development of serological findings in patients with proved positivity only in classes IgA and/or IgM of chlamydial antibodies (without IgG), which can be suspected of showing “false” positivity. 184 patients were repeatedly examined for chlamydial antibodies in their sera (interval between collections up to three months) using a genus specific rELISA. Sera were also tested for the evidence of IgM antibodies against capsid antigen of *Epstein-Barr* virus (EBV) and against cytomegalovirus (CMV) using ELISA methods. In 75 (40.8%) of patients, IgA/IgM individual positivities were demonstrated even during the following sample test(s). In 28 (15.2%) of them, IgG evidence preceded and in 29 (15.7%) other patients positive seroconversion followed in this class. In 13 (7.1%) patients, IgG antibodies disappeared and subsequently reappeared. Only in 39 (21.2%) of these probands, antibodies IgA/IgM were not demonstrated at another examination. Active EBV, resp. CMV infection was proved in 24 (13.0%), resp. in 18 (9.8%) of patients. It is concluded that the evidence of positivities only in classes IgA and/or IgM mostly signal the onset of a primary infection (reinfection) or an active infection in patients with IgG production failures respectively. In these cases, a “false” positivity can be supposed to occur only in a minor extent.

INTRODUCTION

Serological examination methods play an important role in the diagnostics of chlamydial infections, above all at diseases caused by species *Chlamydia* (*Chlamydia pneumoniae* and *C. psittaci*)¹. Mostly, micro-immunofluorescent (MIF) and immunoenzymatic (ELISA) tests are being used. As the marker of infection activity, namely positivity in A and M immunoglobulin classes is considered^{1,2}. IgM antibodies are rather typical for primary infections, while IgA for reinfections and chronic persisting infections. It is important that IgA and/or IgM antibodies are usually detected together with immunoglobulins G². The positivity found only in IgA and/or IgM (without IgG evidence) can be considered as suspicious (“false” or “non-specific” positivity) and clinicians take such findings with reserve. It happens rather often that the next test does not prove any presence of chlamydial antibodies and the patient is seronegative.

The aim of this study is to contribute to the elucidation of this problem by monitoring of repeatedly tested patients with above described serological findings. In these patients, an eventual presence of an active infec-

tion by *Epstein-Barr* virus (EBV) and/or cytomegalovirus (CMV) was also checked.

PATIENTS AND METHODS

In 1997–2000, the patients of the Teaching Hospital in Olomouc whose sera contained chlamydial antibodies class IgA and/or IgM (without IgG) were monitored in the frame of routine diagnostic procedures. The majority of patients were tested only once, so the course of serological findings could not be registered, event. the second test was performed in an interval longer than three months.

Therefore, the study involved only those patients who underwent a control examination within an interval of three months. Their sera were also tested for the evidence of IgM antibodies against viral capsid antigen (VCA) of EBV and against CMV (markers of active EBV, resp. CMV infection)³.

The detection of IgA, IgG and IgM antibodies against the recombinant fragment of lipopolysaccharide (LPS) antigen of chlamydias was performed using sets “Chlamydia-rELISA” (medac GmbH, Wedel, Germany). An-

* This study was presented at the 53. Tagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie, Aachen, Germany, 30. 9. – 4. 10. 2001.

tibodies against VCA EBV class IgM were tested with the commercial set "ETI-EBV-M reverse" (DiaSorin s.r.l., Saluggia, Italy), anti-CMV IgM were assessed with the set "EIA Cytomegalo IgM" (Test-Line Ltd., Brno, Czechia). The principles for use given by the producers were observed at all testing and evaluation of results. To reach the maximum objectivity, all borderline values were considered as negative ones.

RESULTS

IgA and/or IgM antibodies (without IgG) were proved in 692 serum samples of 544 patients (356 women, 188 men), at whom a test of chlamydial antibodies evidence was demanded. Most frequently, only IgA class antibodies (379 cases, 54.8%) were demonstrated, further only IgM (206, 29.8%) and IgA + IgM (107, 15.4%). Among the most frequent clinical diagnoses, pneumonia (11.6%), chlamydial infection (11.4%), arthritis and arthropathy (8.1%) were present. Other diseases were rather sporadic. The set was very heterogeneous, more than 100 clinical diagnoses were registered.

There were 184 patients (124 women, 60 men) who were examined repeatedly. The age range was seven months – 86 years, with the median of 39 years. According to the development of serological findings, five groups of patients could be assessed:

1. Only IgA and/or IgM antibodies were proved throughout the follow-up. This group was the largest one (75 patients, 40.8%).
2. No chlamydial antibodies were evidenced in the following test(s), the patients were seronegative (39, 21.2%). In 18 (46.2%) out of these 39 patients, antichlamydial antibiotic agents were applied in the intervals between tests (in 4 cases clarithromycin, in 3 cases azithromycin and doxycycline, in 2 cases ofloxacin and clarithromycin + ofloxacin, once erythromycin, roxithromycin, azithromycin + ofloxacin and clarithromycin + ciprofloxacin).
3. In 29 (15.7%) patients, a seroconversion in the class IgG appeared.
4. In 28 (15.2%) patients, IgG antibodies were present in preceding test(s). Thus, they disappeared in the course of monitoring.
5. In 13 (7.1%) patients, IgG antibodies disappeared and consequently reappeared, event. the disappearance was preceded by seroconversion in the class IgG.

An active EBV infection (positive anti-VCA EBV IgM) was evidenced in 24 (13.0%) out of 184 probands, an active CMV infection (positive anti-CMV IgM) in 18 persons (9.8%). No simultaneous active infection of EBV and CMV was registered. The comparison of occurrence of anti-VCA EBV IgM and anti-CMV IgM in individual groups was not realized due to small numbers of patients.

DISCUSSION

Serological diagnostics of active chlamydial infection is mostly based on the evidence of antibodies of classes IgA and/or IgM and on significant changes of their titres. These immunoglobulins are usually detected together with IgG antibodies^{1,2}. However, the positivity limited to the classes IgA and/or IgM is rather frequent. In a previous study of the authors⁴, these findings represented approximately 12% of all seropositive samples and covered almost 20% of serological representations of active infections. Other authors also mention isolated IgA and/or IgM positivity, both at infections caused by *C. pneumoniae* and *C. trachomatis*^{5–10}. Sporadically, it can be seen at healthy blood donors, too⁴. Contrarily, in seminal plasma IgA antibodies quite often appear isolated, as dominant locally produced immunoglobulins¹⁰.

However, such findings are always suspect of "false" or "nonspecific" positivity and clinicians as well as microbiologists tend to verify the diagnosis by evidencing the seroconversion in the class IgG. It happens sometimes that the next test of serum sample does not reveal any antibodies, the patient is seronegative and the diagnosis of an active chlamydial infection is not confirmed. In our set, it happened in 21% of the monitored patients. It is to be noted that almost half of them were treated with antichlamydial antibiotics in the intervals between tests, with consequent early suppression of an eventual onset of chlamydial infection or reinfection.

Seroconversion in the class IgG was registered in almost 16% of patients. That situation could be evaluated as a primary infection or reinfection, and it was described by other authors, too⁶.

Surprisingly, in more than 22% of patients (groups 3 and 5), IgG antibodies were evidenced in previous test(s), or they intermittently appeared and disappeared. This fact may reflect disorders in antibodies formation, though it may be possible that in some patients during a certain stage of disease there occurs a shift of immune response from chlamydial LPS antigen to another one – major outer membrane protein (MOMP), heat shock protein 60 etc. – and back. *Bas* and *Vischer*¹¹ describe a wide variety of antibody response to eight different recombinant antigens in patients with *C. trachomatis* infection. Discrepancies between the results of rELISA (LPS antigen) and MIF (MOMP antigen) tests are known to occur^{1,6}, usually explained by an earlier onset and shorter time of presence of anti-LPS antibodies¹. In our opinion, a part of these differences may be due by the mentioned irregularities in the antibody response of some patients.

In the major group of patients, IgG positivity was not evidenced throughout the observation period (group 1). It is probable that some of them would be transferred into groups 3, 4 and 5, if a greater number of samples were available. Nevertheless, it is evident that some patients do not form anti-LPS IgG antibodies even in the course of an infection. It is then difficult to

define which stage of the disease the patient undergoes (primary infection, reinfection, chronic persisting infection?).

In spite of a certain possibility of “false” positivity, isolated IgA and/or IgM positivity is of high diagnostic importance. Sometimes it is the only serological marker of an active chlamydial infection, and it can be confirmed by the evidence of antigen or nucleic acid by polymerase chain reaction^{5,8}.

A known cause of “nonspecific” IgM positivity is e.g. the presence of the rheumatoid factor⁹. Modern serological sets should be able to eliminate this risk. Another cause of “false” IgA/IgM positivity could be an active EBV or CMV infection with concomitant effect on activities of the immune system. In a serological survey of the Czech Republic in 1996, anti-VCA EBV IgM antibodies (one of markers of active EBV infection) were evidenced in 0–3% of population, and only in the age group above 60 years they reached 8.5%³. Thus it is evident that the occurrence of anti-VCA EBV IgM was higher in our set of patients. However, it is not to be stated that an active EBV or CMV infection should be a major cause of “nonspecific” positivity in IgA and/or IgM classes of antibodies against chlamydial LPS antigen.

It can be concluded that a finding of chlamydial antibodies only in IgA/IgM classes usually reflects the very onset of a primary infection or reinfection, event. an active infection in patients with disorders or irregularities in IgG formation. Therefore, it should not be underestimated. A “false” transitory positivity in these classes occurs only in a minor part of patients.

ACKNOWLEDGEMENT

This investigation was supported by the Research project (Výzkumný záměr) MSM 151100002.

REFERENCES

1. Verkooyen RP, Van Lent NA, Mousavi Joulandan SA, Snijder RJ, Van den Bosch JM, Van Helden HP, Verbrugh HA (1997) Diagnosis of *Chlamydia pneumoniae* infection in patients with chronic obstructive pulmonary disease by micro-immunofluorescence and ELISA. *J. Med. Microbiol.* 46, 959–964.
2. Mazzoli S, Tofani N, Fantini A, Semplici F, Bandini F, Salvi A, Vergassola R (1998) *Chlamydia pneumoniae* antibody response in patients with acute myocardial infarction and their follow-up. *Am. Heart J.* 135, 15–20.
3. Roubalová K, Seeman J (1998) Sérologický přehled protilátek proti herpetickým virům CMV, EBV, VZV. *Zprávy CEM (SZÚ, Praha) 7 (Suppl 1)*, 29–31.
4. Hejnar P, Krátká J (2000) Prävalenz von IgA-, IgG- und IgM-Antikörpern gegen Chlamydien mit Hilfe von rekombinanten ELISA-Testen von medac bei Patienten der Universitätsklinik Olomouc und Bedeutung des IgM-Nachweises für die Diagnostik aktiver Infektionen. *Mikrobiologie* 10, 209–212.
5. Birkebaek NH, Jensen JS, Seefeldt T, Degn J, Huniche B, Andersen PL, Ostergaard L (2000) *Chlamydia pneumoniae* infection in adults with chronic cough compared with healthy blood donors. *Eur. Respir. J.* 16, 108–111.
6. Dreesbach K, Ridgway G, Carder C, Mumtaz G (1996) *Chlamydia* serology: diagnostic evaluation of a new recombinant ELISA. *Proceedings 3rd Meeting of the European Society for Chlamydia Research, Vienna*, 360.
7. Song YG, Kwon HM, Kim JM, Hong BK, Kim DS, Huh AJ, Chang KH, Kim HY, Kang TS, Lee BK, Choi DH, Jang Y, Kim HS (2000) Serologic and histopathologic study of *Chlamydia pneumoniae* infection in atherosclerosis: a possible pathogenetic mechanism of atherosclerosis induced by *Chlamydia pneumoniae*. *Yonsei Med. J.* 41, 319–327.
8. Srugo I, Gershtein R, Madjar S, Elias I, Tal J, Nativ O (2000) Acute primary *Chlamydia trachomatis* infection in male adolescents after their first sexual contact. *Arch. Pediat. Adol. Med.* 154, 169–172.
9. Wareham D, Templeton K, Aitken C (2000) The use of an IgM recombinant enzyme immunoassay for the diagnosis of atypical pneumonia due to *Chlamydia pneumoniae*. *Proceedings 4th Meeting of the European Society for Chlamydia Research, Helsinki*, 151.
10. Weidner W, Floren E, Zimmermann O, Thiele D, Ludwig M (1996) Chlamydial antibodies in semen: search for “silent” chlamydial infections in asymptomatic andrological patients. *Infection* 24, 309–313.
11. Bas S, Vischer TL (2000) Chlamydial serology: comparative diagnostic evaluation of immunoblotting and immunoassays using different recombinant proteins as antigens. *Proceedings 4th Meeting of the European Society for Chlamydia Research, Helsinki*, 78.