Anti-chlamydial IgG Neutralizing Ability in Nonzoonotic Atypical Community Acquired Respiratory Tract Infections

Salvatore Pignanelli¹ · Giovanna Pulcrano² · Vita Dora Iula² · Alisa Shurdhi³

Received: 15 December 2014 / Accepted: 6 April 2015 / Published online: 5 May 2015
© Association of Microbiologists of India 2015

Abstract *Chlamydophila pneumoniae* is a pathogenic agent, involved in various types of infection. This study has evaluated the ability of IgG antibodies in outpatient, with acute respiratory tract infections from *C. pneumoniae*, to neutralize in vitro purified elementary bodies of this bacterium, revealing a good neutralizing performance of IgG antibodies.

Keywords *Chlamydophila pneumoniae* · EBs · IgG · Neutralizing activity

Abbreviations

CAP	Community-acquired pneumonia
EBs	Elementary bodies
HBSS	Hanks balanced salt solution
MIF	Microimmunofluorescence
RT-PCR	Real time-polymerase chain reaction
SPG	Sucrose–potassium phosphate–glutamic acid

Chlamydophila pneumoniae is an atypical bacterium: first isolation dates back to 1965 [1], established as a human respiratory pathogen in 1986 [2], and potential zoonotic transmission described in 2010 [1]. *C. pneumoniae* is an obligate intracellular bacterium, which is detected from 5 to 10 % of community-acquired pneumonia (CAP) [3]. Moreover, *C. pneumoniae* is involved in various types of infection (sub-acute, acute, and chronic). Acute disease from this bacterium is most common [4]. Seroprevalence increases with age, e.g. in young it is about 50 %, while in elderly about 75 % [4]. Despite some pathological conditions have been attributed to *C. pneumoniae* [5–7], this bacterium frequently causes mild or asymptomatic infections, which often remain unrecognized and consequently untreated [4]. For these reasons this study has evaluated the ability of IgG antibodies from outpatient, with acute *C. pneumoniae* infection, to neutralize in vitro purified elementary bodies (EBs) of this pathogenic agent. We have studied 50 non consecutive outpatients in 2012–2013 with acute infection by *C. pneumoniae*, with following clinical, imaging and laboratory data: nonproductive cough, low-grade fever, and radiological images in suspicion of interstitial pneumonia, and positivity of real time-polymerase chain reaction (RT-PCR) and/or serological techniques, as described previously [8]. From these patients we have collected 200 sera samples. The sera were selected as follows: 50 at the same time of acute infection diagnosis (1st set), 50 after 2 weeks (2nd set), 50 after 12 weeks (3th set) and 50 after 20 weeks (4th set). In addition, to evaluate specificity of this assay, 25 sera are studied as control: 10 sera positive for antibodies anti-*Mycoplasma pneumoniae*, 5 with antibodies anti-*Legionella pneumophila*, and 10 negative sera for antibodies against *C. pneumoniae*, *M. pneumoniae* and *L. pneumophila*. The sera positive against *C. pneumoniae* are tested also against purified EBs of

✉ Salvatore Pignanelli
dott.pignanelli@libero.it

¹ Department of Diagnostic Services, O.U. Laboratory Analysis of Clinical Chemistry and Microbiology, S. Maria della Scaletta Hospital, Via Montericco 4, 40026 Imola, BO, Italy

² Department of Molecular and Cellular Biology and Pathology “Luigi Califano”, Medicine School, University of Napoli Federico II, Naples, Italy

³ Department of Pathology and Laboratory Medicine, Section of Microbiology, University Hospital of Parma, 14 Viale Gramsci, 43126 Parma, Italy

Chlamydophila psittaci and *Chlamydia trachomatis* to evaluate the specificity of neutralising antibodies against *C. pneumoniae*. The software and criteria used to perform statistical analysis were described previously [9]. The microimmunofluorescence (MIF) assay was used to detect and measure the titre of IgG antibodies anti-*C. pneumoniae* (strain IOL-207) in the sera (Fig. 1). This assay and the diagnostic criteria of acute/past infection used in this work are described earlier [8]. A sucrose gradient was used to obtain purified EBs (titre 5×10^5 inclusion forming units/mL) of *C. pneumoniae* (strain IOL-207) and to perform neutralizing test [10]. Rabbit serum was used as a source of complement (5 % final concentration). The sera anti-*C. pneumoniae* were inactivated at 56 °C for 30 min and diluted 1:5 in Hanks balanced salt solution (HBSS) and then 100 μ L were distributed in triplicate over 96-well microtitre plates. EBs were diluted in 0.25 M sucrose–10 mM potassium phosphate–5 mM glutamic acid (SPG), to obtain 2×10^4 inclusion forming units/mL, and 90 μ L was added to prediluted sera and to 100 μ L of HBSS to control. The mixtures (serum and EBs) and control (HBSS and EBs), incubated for 30 min at 37 °C, were then inoculated in triplicate onto monolayers of a continuous LLC-MK2 cell line derived from Rhesus monkey kidney tissue grown in 24-well plates containing a glass coverslip at the bottom [10]. Subsequently, 800 μ L of chlamydial growth medium (Eagle's minimum essential medium with 10 % heat-inactivated fetal calf serum, 5 μ g/mL of glucose, 1 μ g/mL of cycloheximide) was added to obtain a final serum dilution of 1:10. After centrifugation at 1000 *g* for 1 h, the monolayers were incubated at 37 °C for 48 h, fixed in methanol and then stained with a fluorescein-conjugated monoclonal antibody specific for the chlamydial LPS (Meridian, Cincinnati, USA). An average was taken and the results were expressed as reduction percentage of inclusion forming units/mL from control monolayers. A

Fig. 1 Bright apple-green staining due to reaction between immunocomplexes (IgG and *C. pneumoniae* EBs) and a fluorescein-conjugated monoclonal antibody anti-human IgG

≤ 50 % reduction of inclusion forming units/mL from control was defined as neutralizing. Moreover, in order to obtain a neutralizing titre, two dilutions (1:20 and 1:40) were performed. In addition, neutralisation reactions using purified EBs of genovar D of *Chlamydia trachomatis* and 6BC of *Chlamydophila psittaci* were performed to control the specificity of neutralising antibodies anti-*C. pneumoniae*. The targets of immune response have been highlighted using an immunoblot analysis. Briefly, proteins were separated by SDS-PAGE using 12 % (w/v) precast gel (Invitrogen, Paisley, UK) and transferred to nitrocellulose membranes (Sigma, St. Louis, USA). These membranes were blotted with the 50 positive sera of 4th set, diluted 1/100. Chlamydia-specific bands were detected with peroxidase-labelled goat anti-human IgG (Dako, Glostrup, Denmark) diluted 1:500, as the secondary antibody and with 4-chloro-naphthol (Bio-Rad, Milan, Italy) for colorimetric visualization. Table 1 shows the distribution of IgG titre in sera from patients with acute infection by *C. pneumoniae*. The 40 % of the 1st and 2nd sera set did not show IgG antibodies, which appear more late (12 weeks). High titres of IgG are reached in 4th set (20 weeks), although 40 % of the sera of the 3th set has a titre of IgG from 256 to 512. Table 1 exhibit the neutralizing ability of IgG, with average neutralizing activity (percentage) and modal titre value. The neutralizing activity increases in direct proportion to increase antibody titer, but also increasing IgG age (Table 1). Only 24 % of 1st sera set shows neutralizing activity, with low average percentage (58 %) and low modal titre (1:10) (Table 1). On the contrary, the 3th and 4th set of sera exhibit excellent neutralizing activity with very high values (average percentage 89.4 % and titre modal value 1:40) (Table 1). All sera from patients with acute *C. pneumoniae* infection, in the absence of IgG show no neutralizing activity (IgG titre < 32 in 1st and 2nd sera sets) (Table 1). The immunoblot has showed a reactivity of sera against the 40, 100 and 120 kDa proteins (Fig. 2).

In addition, IgM anti-*C. pneumoniae* were detected in 90 % of 1st set and in 100 % of 2nd set of samples. The 25 sera, used as controls, are all negative for detection of antibodies anti-*C. pneumoniae* and show no neutralizing activity against *C. pneumoniae* EBs ($p < 0.001$). Furthermore, the sera show no neutralizing activity against EBs of *C. trachomatis* and *C. psittaci* ($p < 0.001$). Therefore the assay specificity has been excellent (Fig. 1). These data display that this assay is not limited by cross-reactivity among *Chlamydiae*, strict human pathogens. This work has shown in vitro an excellent activity of IgG antibodies, titre and time-dependent, to neutralize *C. pneumoniae* EBs. These results may explain why this bacterium frequently causes infections often unrecognized and untreated, despite some pathological conditions attributed to that bacterium

4. Kuo CC, Jackson LA, Campbell LA, Grayston JT (1995) *Chlamydia pneumoniae* (TWAR). Clin Microbiol Rev 8:451–461
5. Boman J, Hammerschlag MR (2002) *Chlamydia pneumoniae* and atherosclerosis: critical assessment of diagnostic methods and relevance to treatment studies. Clin Microbiol Rev 15:1–20. doi:[10.1128/CMR.15.1.1-20.2002](https://doi.org/10.1128/CMR.15.1.1-20.2002)
6. Busatto P, Blasi F, Casanova F, Selmi C, Centanni S, Zuin M (2005) Lack of PBC-specific antimitochondrial antibodies in patients with *Chlamydia pneumoniae* infection. J Gastroenterol Hepatol 20:1626–1627. doi:[10.1111/j.1440-1746.2005.03941.x](https://doi.org/10.1111/j.1440-1746.2005.03941.x)
7. Fainardi E, Castellazzi M, Seraceni S, Granieri E, Contini C (2008) Under the microscope: focus on *Chlamydia pneumoniae* infection and multiple sclerosis. Curr Neurovasc Res 5:60–70. doi:[10.2174/156720208783565609](https://doi.org/10.2174/156720208783565609)
8. Pignanelli S, Shurdhi A, Delucca F, Donati M (2009) Simultaneous use of direct and indirect diagnostic techniques in atypical respiratory infections from *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. J Clin Lab Anal 23:206–209. doi:[10.1002/jcla.20332](https://doi.org/10.1002/jcla.20332)
9. Pignanelli S, Pulcrano G, Iula VD, Zaccherini P, Testa A, Catania MR (2014) In vitro antimicrobial profile of *Ureaplasma urealyticum* from genital tract of childbearing-aged women in northern and southern Italy. APMIS 122:552–555. doi:[10.1111/apm.12184](https://doi.org/10.1111/apm.12184)
10. Donati M, Di Francesco A, Baldelli R, Magnino S, Pignanelli S, Shurdhi A, Delucca F, Cevenini R (2009) In vitro detection of neutralising antibodies to *Chlamydia suis* in pig sera. Vet Rec 164:173–174. doi:[10.1136/vr.164.6.173](https://doi.org/10.1136/vr.164.6.173)
11. Pignanelli S, Shurdhi A (2011) In vitro activity of rifaximin against *Chlamydia suis*. Vet Rec 169:584. doi:[10.1136/vr.100027](https://doi.org/10.1136/vr.100027)
12. Bartolini E, Ianni E, Frigimelica E, Petracca R, Galli G, Berlanda Scorza F, Norais N, Laera D, Giusti F, Pierleoni A, Donati M, Cevenini R, Finco O, Grandi G, Grifantini R (2013) Recombinant outer membrane vesicles carrying *Chlamydia muridarum* HtrA induce antibodies that neutralize chlamydial infection in vitro. J Extracell Vesicles. doi:[10.3402/jev.v2i0.20181](https://doi.org/10.3402/jev.v2i0.20181)
13. Phoon MC, Yee GW, Koh WP, Chow VT (2011) Comparative seroepidemiologic analysis of *Chlamydia pneumoniae* infection using microimmunofluorescence, enzyme immunoassay and neutralization test: implications for serodiagnosis. Indian J Microbiol 51:223–229. doi:[10.1007/s12088-011-0168-4](https://doi.org/10.1007/s12088-011-0168-4)
14. Baud D, Regan L, Greub G (2010) Comparison of five commercial serological tests for the detection of anti-*Chlamydia trachomatis* antibodies. Eur J Clin Microbiol Infect Dis 29:669–675. doi:[10.1007/s10096-010-0912-4](https://doi.org/10.1007/s10096-010-0912-4)
15. Pignanelli S (2011) Laboratory diagnosis of *Toxoplasma gondii* infection with direct and indirect diagnostic techniques. Indian J Pathol Microbiol 54:786–789. doi:[10.4103/0377-4929.91503](https://doi.org/10.4103/0377-4929.91503)