SHORT COMMUNICATION

Antibodies Anti-*Chlamydia pneumoniae* and Anti-*Mycoplasma pneumoniae* in Patients with Negative Serology for Hantavirus. Retrospective Study

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The seroprevalence of Chlamydia pneumoniae *and* Mycoplasma pneumoniae *in hantavirus seronegative patients, who had symptoms and signs compatible with pneumonia was established. For this purpose we used the indirect fluorescent antibody test. Titers ≥ 1:16 for* C. pneumoniae *and* M. pneumoniae *were found in 8.6% and 17.1% of the serum, respectively, showing evidence of recent or current infection.*

Key words: antibodies - *Chlamydia pneumoniae* - *Mycoplasma pneumoniae* - indirect fluorescent antibody test

Hantaviruses have been recognized as the etiologic agents of two acute diseases: haemorrhagic fever with renal syndrome in Asia and Europe, and hantavirus pulmonary syndrome (HPS) in the Americas (Mills et al. 1999). Serologic studies showed that hantaviruses are spread all over the world, and their reservoirs are different species of rodents. Transmission to human beings usually occurs by aerosols (Mills et al. 1999).

The symptoms and signs of HPS are very similar to other atypical pulmonary infections (MINSAL 1997, Barrera 1998); for this reason differential diagnosis must be carried out. The most common atypical pathogens are *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* considered as important causes of community-acquired pneumonia (Tan 1999). Infection rates for these agents are not well established because many clinicians and investigators do not routinely test for them and therefore their infections are empirically treated (Tan 1999). Laboratory diagnosis is difficult because most of these agents cannot be easily cultured. Diagnosis relies on either high acute antibody titers or enhanced titers in serum serology samples (Tan 1999).

HPS was first recognized in Chile in 1993 (Barrera 1998), and up to date 124 cases have been notified, with a mortality rate of 49.2% (MINSAL 2000). A great number of blood samples from patients with clinical diagnosis of HPS have been sent to our laboratory for serologic diagnosis. However, many of them were negative for hantavirus.

Considering that in Chile *M. pneumoniae* and *C. pneumoniae* are not routinely investigated, the aim of this study was to establish the retrospective seroprevalence of *C. pneumoniae* and *M. pneumoniae* in hantavirus seronegative patients.

With this purpose, 35 out of 107 hantavirus negative sera were stored in the Instituto de Microbiología Clínica, Universidad Austral de Chile. They were selected from patients showing signs and symptoms (myalgias, fever, chest discomfort and chest radiograph with infiltrate) compatible with pneumonia. The patients' age varied from 2 to 88 years.

We used two indirect fluorescent antibody tests (IFA). The IFA test for *Chlamydia* (Fuller Laboratories) uses slides with elementary bodies as substrate antigen. To performe the test, patients' sera were initially diluted (1:8) in IgM sample diluent, where IgG antibody is selectively immunoprecipitated. The diluted serum was then transferred to a separate substrate slide well to allow reaction of IgM antibody with the antigens. After appropriate incubation (37ºC in wet chamber for 90 min) the slide was washed to remove unreacted serum proteins and a fluorescence-labelled anti-human IgM (conjugate) was added. This conjugate was allowed 30 min to bind the antigen-antibody complexes before washing again to remove untreated conjugate. The resulting reaction was observed under fluorescence microscopy, where a positive reaction is seen as sharply defined applegreen fluorescent elementary body in the cytoplasm of

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10-20% of the infected cells. A IgM titer of 1:16 or higher was considered to support recent or current infection (Kuo et al. 1995, Hammerschlag 1996).

To detect the presence of circulating IgM antibodies to *M. pneumoniae* in human sera, we used the IFA (Zeus Scientific Inc.). *M. pneumoniae* antigenic substrate is fixed onto a multiwell microscope slide. Serum samples were incubated with this substrate and antibody, if present, could be observed after staining with a fluorescein-labeled anti-human IgM conjugate. A fluorescence microscope demonstrate characteristic positive, bright, applegreen fluorescence of the reaction. According to the manufacturer's instructions, an IgM titer of 1:16 or higher was considered as a positive result indicating active or recent infection with *M. pneumoniae*.

In our study, 8.6% of the serum presented IgM antibodies for *C. pneumoniae* with titers ≥ 1:16. This result is similar to that reported by other authors such as Peeling and Brunham (1996) and Kuo et al. (1995) who reported *C. pneumoniae* as responsible for 6 to 10% of the community-acquired pneumonia. Data on the incidence of infections by *C. pneumoniae* are not available in Chile. However, Martinez et al. (1999) reported 60% of seroprevalence in asymptomatic subjects, frequency that is higher than that reported worldwide (Peeling & Brunham 1996), suggesting that *C. pneumoniae* infection is endemic in Chile.

With regard to *M. pneumoniae* antibodies, 17.1% of the sera were positive for IgM with titers ≥ 1:16. Similar results were described (Tan 1999, Dorigo-Zetsma et al. 1999). Unfortunately, due to the inexistence of data on this subject in our country, we are unable to make comparisons. However, due to the high prevalence reported in Chile for other species of *Mycoplasma* in different samples (Wilson et al. 1986, Otth et al. 1990), we can speculate that *M. pneumoniae* should also be present among the Chilean population.

The analysis of a second sera sample obtained in the convalescent phase is necessary to establish diagnosis. Being this a retrospective study, we were unable to obtain the second sample. However, having in mind that all 35 patients showed signs and symptoms compatible with pneumonia and all the complementary cultures (spinal fluid, blood, articular and pleural fluid) were negative, we could infer that *M. pneumoniae* or *C. pneumoniae* could be responsible for the clinical features observed in the patients.

Being our results comparable to those obtained in other countries, we propose the implementation of laboratory techniques to perform the differential diagnosis of these pathogens, since their treatment and prognosis is totally different from that of hantavirus. On the other hand, literature reports that these bacteria are agents of other pathologies such as coronary heart disease, reactivate arthritis, polyarthritis, and skin lesions in Stevens-Johnson-syndrome (Tully 1993, Martínez et al. 1999).

REFERENCES

Barrera ME 1998. *Hanta la Respuesta Chilena*, Ministerio de Salud de Chile, 116 pp.

Dorigo-Zetsma JW, Zaat SAJ, Wertheim-van Dillen PME, Spanjaard L, Rijntjes J, van Waveren G, Jensen JS, Angulo AF, Dankert J 1999. Comparison of PCR, culture and serological test for diagnosis of *Mycoplasma pneumoniae* respiratory tract infection in children. *J Clin Microbiol 37*: 14-17.

Hammerschlag MR 1996. Diagnostic methods for intracellular pathogens. *CMI 1* (S1): 3-8.

Kuo C-C, Jackson LA, Campbell LA, Grayston JT 1995. *Clamydia pneumoniae* (Twar). *Clin Microbiol Rev 8*: 451-461.

Martínez MA, Kogan R, Silva JJ, Pinto ME, Vidal C, Huppo H 1999. Seroprevalence of *Chlamydia pneumoniae* in Chile. *Scand J Infect Dis 31*: 103-104.

Mills JN, Yates TL, Ksiazek TG, Peters CJ, Childs JE 1999. Long-term studies of hantavirus reservoir populations in the southwestern United States: rationale, potencial, and methods. *Emerg Infect Dis 5*: 95-101.

MINSAL - Ministerio de Salud de Chile 1997. Infeccion por virus hanta diagnostico y tratamiento, Santiago, Chile, p.1-5.

MINSAL. *Hantavirus: Situación Epidemiológica*. Informe al 1 de Noviembre 2000. [en línea], Departamento de Epidemiología. Santiago Chile, 15 de Noviembre 2000. http:// epi.minsal.cl.

Otth L, Gutierrez MA, Wilson M, Tejero A, Brousain MT, Moreno MI, Montaña J 1990. Estudio microbiológico de la uretritis en el hombre. *Rev Med Chile 118*: 543-547.

Peeling RW, Brunham RC 1996. Clamydiae as pathogens: new species and new issue. *Emerg Infect Dis 2*: 307-317.

Tan JS 1999. Role of atypical pneumonia pathogens in respiratory tract infections. *Can Respir J 6* (A): 15-19.

Tully JS 1993. Current status of the mollicutes flora of humans. *Clin Infec Dis* 17: 2-9.

Wilson M, Zaror L, Rosas C 1986. Estudio microbiológico de la uretritis en el hombre. *Rev Med Chile 114*: 742-747.