Community-Acquired Pneumonia by *Chlamydophila pneumoniae*: A Clinical and Incidence Study in Brazil

Maria Bernadete Chedid[1], Márcio Fernandes Chedid[1], Darcy Oliveira Ilha[3], Mary Clarisse Bozzetti[2], Letícia Chaves[1], Daniela Griza[1] and Paulo Roth Dalcin[1]

[1]Hospital das Clínicas de Porto Alegre, Federal University of Rio Grande do Sul; [2]Epidemiology Department, Federal University of RS; [3]Radiology Service, Santa Casa de Misericórdia de Porto Alegre; Porto Alegre, RS, Brazil

As there was not any data on *Chlamydia pneumoniae* (TWAR) infections in Brazil so far, a prospective cohort study of adult patients hospitalized due to CAP was carried out for one year in a Brazilian university general hospital to detect the incidence of CAP by *Chlamydophila pneumoniae* (TWAR) for one year. During a whole year 645 consecutive patients hospitalized due to an initial presumptive diagnosis of respiratory diseases by ICD-10 (J00-J99), excluding upper respiratory diseases, were screened; 59 consecutive patients with CAP were diagnosed. They had determinations of serum antibodies to *C. pneumoniae* by microimmunofluorescence at the Infectious Diseases Laboratory of University of Louisville (KY, USA); 37 patients (63.8%) had seroreactivity to TWAR antigens, from which 23 (39.6%) had previous infection; 3 patients (5.2%) were diagnosed with CAP by TWAR and got cured. The incidence of TWAR CAP in our hospital by seroconversion was 5.2%. Our incidence of 5.2% is probably underestimated since TWAR culture was not available; we suggest that Real-Time PCR be used along with other diagnostic methods in future studies to detect the actual incidence of TWAR CAP. We propose that the serological criterion of IgM ≥1:16 alone to the diagnosis of acute infection by TWAR are discontinued due a lack of specificity.

Key-Words: CAP, *Chlamydophila pneumoniae*, microimmunofluorescence, TWAR infections.

Chlamydophila pneumoniae is a globally common respiratory pathogen, which causes a variable disease spectrum, being the most common ones pneumonia and bronchitis [1]. Within the last 15 years, it has been worldwide identified as a significant pathogen of community-acquired pneumonia (CAP), being its prevalence in studies of adult patients requiring hospitalization between 6,5% [2] and 17.9% [3], reaching 43% during a *C. pneumoniae* epidemic [4].

An expert meeting [5] recommended the following diagnostic criteria to the serological diagnosis of *C. pneumoniae* infection by MIF: 1st) acute infection, 4-fold increase in IgG or IgM ≥ 16; 2nd) possible acute infection, IgG of ≥1:512; 3rd) presumed past infection, IgG ≥ 1:16. Some researchers [6] using the criterion of "definite"(seroconversion) and "possible" (IgG ≥ 1:512 or IgM ≥1:16) *C. pneumoniae* CAP have demonstrated the effect of the use of different breakpoints in reporting the incidence of TWAR CAP – being, respectively, of 2.8 cases per 100,000 population and 16.5 cases per 100,000 population. Such enormous range of incidence variation suggests that a cutoff of a sole antibody titer of either IgG or IgM antibodies can produce unnecessary mistakes in classifying CAP etiology.

There has not been neither a study on the seroprevalence of *C. pneumoniae* infections nor on the incidence of *C. pneumoniae* CAP in Brazil so far. The aim of this study is to detect the incidence of *C. pneumoniae* CAP in patients requiring hospitalization for one year in our university hospital, reporting their clinical picture and evolution; and to discuss the serological criteria of acute TWAR infection.

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Address for correspondence: Dr. Maria Bernadete Chedid. E-mail: mariab@terra.com.br. Phone/Fax: (55 51) 3328-2472.

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Materials and Mathods

This prospective study was approved by the institutional Committee of Ethics in Research. All consenting individuals with age between 18 and 80 admitted to our university general hospital between July 19, 2000 and July 18, 2001 due to an initial presumptive diagnosis of respiratory disease by ICD-10 (J00-J99), excluding upper respiratory diseases, were screened to detect the patients with community-acquired pneumonia. They were enrolled in the study if their chest radiograph taken within 48 hours of admission was consistent with pneumonia and had either one of the major criteria (axillary temperature ≤35.5ºC or ≥37.8ºC, cough or sputum) or two of the minor criteria (dyspnea, abnormal mental status, signs of consolidation by examination, pleuritic chest pain, White blood count > 12,000 cells per cm^3 or band forms > 4%). Underlying chronic disease and immunossupression were defined as others [7-9]. Exclusion criteria were: residents in institutions, those disabled to walk; those discharged from hospital within the last 15 days; pregnant women; HIV-positive patients; patients with cystic fibrosis, bronchiectasis or tracheostomy. Seronegativity to *C. pneumoniae* antigens was defined as IgG ≤1:8 or IgM ≤10. CAP by *C. pneumoniae* was defined by 4-fold rise in IgG or IgM antibodies to *C. pneumoniae* in paired sera and presumed past infection was defined as IgG antibodies ≥ 1:16. Serum samples were collected during the acute phase at the hospital and after 4-12 weeks in the ambulatory. All the survivors were followed for a whole year after their inclusion in the study, being enrolled only once. After the confirmation of the diagnosis of community-acquired pneumonia, sera stored at –70ºC were sent on dry ice in a batch to the Infectious Diseases Laboratory of the

University of Louisville (KY, USA) to be tested to *Chlamydophila pneumoniae* antigens by microimmunofluorescence (MIF) by a kit of MRL Diagnostic (Cypress, CA, USA). Sera tested for IgM antibodies were pretreated to remove possible free and complexed IgG antibodies.

Results

During a whole year, 645 consecutive patients who were hospitalized due to an initial presumptive diagnosis of respiratory disease by ICD-10 (J00-J99, excluding upper respiratory diseases) were examined at hospital admission to exclude or confirm CAP. Most were not included due to reasons given in Table 1.

Only 82 patients were initially eligible to study with the diagnosis of CAP; 23 patients were further excluded either because a new pulmonary infiltrate was not confirmed at chest radiograph (5 patients) or alternative diagnoses were made (COPD, 5 patients; heart failure, 3; tuberculosis, 2; collagen vascular diseases, 1; idiopathic pulmonary fibrosis, 1). Other six patients had exclusion criteria by being HIV positive (1), by presenting bronchiectasis (4) or previous pneumatocele (1). Thus, 59 patients constituted the final study group, being 20 females and 38 males, with a median age of 57.6 years (SD = 10.5). From this group, 36 (61%) patients had underlying chronic diseases; 61% were smokers and 19% were imunossupressed; only 58 patients had enough amount of serum to be tested to the presence of *C. pneumoniae* antibodies.

From a total of 58 patients, 21(36.2%) were seronegatives (IgG≤1:8, IgM ≤1:10) to *C. pneumoniae* antigens, while 37 (63.8%) had seropositivity (Table 2). Thus, the seroprevalence of *C. pneumoniae* infection in Brazilian adults is 63.8%, as it has been reported in many countries [10,11].

From a total of 37 seropositive patients, 28 patients (48.2%) had IgG antibody ≥1:32 and 7 patients (12.1%) had IgG antibodies at 1:16. The remaining 2 patients had negative IgG but positive IgM antibodies (IgM>1:10).

Out of 37 seropositive patients (Table 2), 23 (39.6%) presented positive IgG antibodies ≥ 1:16 in the first or second serum sample, thus being cases of previous infection by *C. pneumoniae* [5]. The other 11 seropositive patients had only one serum sample of serum: 5 patients did not have second serum sample because they did not return to the ambulatory at time, while the remaining 6 patients died (3 deaths by CAP and another 3 deaths by end-stage cancer). In all these 11 patients we can not exclude TWAR CAP; neither can we classify them as "previous infection" by TWAR. The remaining three seropositive patients are the ones with *C. pneumoniae* CAP: two female patients without detectable IgG antibodies but with seroconversion of IgM (patients number 13 and 29, Table 2); and a male patient (number 36, Table 2) who presented seroconversion of IgG antibodies.

In our series of 59 patients, there were 7 deaths (11.9%) by pneumonia and other 4 patients whose deaths were attributed to end-stage neoplasia. The three patients with *C. pneumoniae* CAP got cured.

Case Report 1

A 33-year-old female was hospitalized due to pleuritic chest pain, chills and dry cough. She reported headache, nausea and fever of 38ºC some days before. She had had an otitis episode with right tympanic membrane perforation one year ago. At the admission, she complained shortness of breath, coughing, chills and generalized muscle pain. She had purulent nasal secretions and rales were heard in the left lung, but no sputum sample was obtained. No axillary temperature greater than 37.2ºC was noticed during her hospital stay. Chest radiograph showed patchy consolidation in part of the lingular segments with small pleural effusion (Figure 1). Paranasal sinus X-Ray showed polypoid opacity within the right maxillary sinus. ESR was 59 mM per hour. WBC count was 7,500/mm^3 with 20.6% lymphocytes, 70.3% neutrophils and no band forms. Serum MIF showed IgM antibodies to *C. pneumoniae* in the dilution of 1:640, which fell to 1:80 after 5 weeks. IgG serum antibodies were negative. She took PO levofloxacin 500 mg q12h, with subsequent normalization of the ERS. She was discharged after 3 days. Chest radiograph after one month (Figure 2) showed regression of lung lesions; there was no longer lesion in the right maxillary sinus.

Case Report 2

A 60-year-old female was admitted for coughing with hemoptysis of 10 mL in the last 24 hours; she was taking propranolol 40 mg q8 h for systemic hypertension. She mentioned that 3 weeks before she had been consulted in an emergency room due to a cold with small amount of sputum and had taken PO trimethoprim-sulfamethoxazole 800 mg q12h for seven days. At admission, rales were heard in the left hemithorax. Chest radiograph showed patchy consolidation of lingular segments with atelectasis (Figure 3). High resolution computed tomography (HRCT) showed sub segmental consolidation of the lingula with air bronchograms (Figure 4) and a few small calcified nodules within the lesions, properly seen in some CT slices. Paranasal sinus X-Ray showed opacification of ethmoidal cells and mucosal thickening of left maxillary sinus. During her hospital stay she had no axillary temperature greater than 37.3ºC. She had neither elevated WBC count nor elevated band forms. She started on levofloxacin PO 500 mg q12h. After four days, a fiberbronchoscopy with bronchoalveolar lavage (BAL) detected blood clots inside the lingular bronchi. Exam of the BAL showed a few Gram-positive isolated cocci and many leucocytes. Ziehl-Neelsen staining of the BAL was negative as well as the culture; no malignant cells were seen either. Differential count showed predominant neutrophils (59%), with 35% macrophages, 1% eosinophils and 5% lymphocytes. Chest radiograph after two months showed almost complete regression of the pulmonary lesions. Serum sample drawn on the second day after hospital admission showed IgM antibodies to *C. pneumoniae* of 1:640,

Table 1. Exclusion criteria in 563 patients presenting presumed respiratory disease at hospital admission by ICD-10 (J00-J99), excluding upper respiratory diseases

Criteria	N	%
Patients immediately excluded		
<18 year-old	42	7.5
≥80 year-old	47	8.3
Hospital discharge in the preceding 15 days	27	4.8
Institutionalized patients, nursing home	2	0.3
HIV-positives at admission	18	3.2
Using tracheostomy	2	0.3
Cistic fibrosis	3	0.5
Hemiparesis	57	10.1
Paralysis of leg and foot	7	1.2
Bronquiectasis	7	1.2
Patients completely disabled to walk	14	2.5
Total	226	40.1
Patients further examined who didn´t have any infiltrate in Chest X-Ray in the first 48 h		
Hypertensive crisis	1	0.2
Cirrosis (acute)	1	0.2
Renal failure	3	0.5
Stroke (acute)	1	0.2
Meningitis	4	0.7
Cancer	9	1.6
Febrile neutropenia	2	0.3
Alcohol withdrawal syndrome	1	0.2
Ketoacidosis	2	0.3
Shock or septicemia (lung not the primary source)	6	1.1
Diagnosis not known yet	158	28.1
Total	188	33.4
Presumed pneumonia patients whose clinical or radiological criteria of pneumonia were not confirmed in the first week of hospitalization		
Pneumotórax	4	0.7
COPD only	23	4.1
Asthma exacerbation	7	1.2
TB (active)	26	4.6
TB (not active) with hemoptysis and/ or respiratory bacterial infection	2	0.3
Pleural effusion only	9	1.6
Pulmonary fibrosis	7	1.2
Lung tumour only	12	2.1
Sinus infection and bronchitis (acute)	2	0.3
Silicosis	1	0.2
Mitral stenosis	2	0.3
Acute pulmonary edema	2	0.3
MI	1	0.2
Congestive heart failure	10	1.8
Mediastinal mass	1	0.2
Total	109	19.4
Patients not examined		
Death in the first 24-48 h	12	2.1
Discharged in the first 24-48 h	16	2.9
Patients denying participation	3	0.5
Patients not available to be examined more than twice	9	1.6
Total	40	7.1
Total	**563**	**100**

Table 2. Patients with CAP who had seropositivity in the MIF test to *Chlamydophila pneumoniae* at the Infectious Diseases Laboratory of University of Louisville

Patient initials	IgG1st sample	IgG 2nd sample	IgM 1st sample	IgM 2nd sample
AB	32	16	NR	NR
NJ	16	-	NR	-
AL	32	-	NR	-
AZ	32	32	NR	NR
HS	256	256	NR	NR
ES	NR	16	NR	NR
GO	128	-	NR	-
FS	64	-	NR	-
PN	128	128	NR	NR
NF	256	256	NR	NR
VS	256	256	NR	NR
NO	64	16	NR	NR
JC	NR	NR	640	80
CR	128	32	NR	NR
CC	32	32	NR	NR
NR	128	-	NR	-
SM	32	-	NR	-
MS	32	-	NR	-
OJ	128	64	NR	NR
CL	128	-	NR	-
LP	16	NR	NR	NR
NR	16	16	NR	NR
HS	64	128	NR	NR
LS	128	128	NR	NR
NT	NR	64	NR	NR
FD	16	16	NR	NR
VM	64	-	NR	-
AF	64	-	NR	-
OC	NR	NR	640	NR
OA	16	-	NR	-
JS	NR	16	NR	NR
MB	128	32	NR	NR
JÁ	64	128	NR	NR
WP	32	64	NR	NR
AC	128	128	NR	NR
JS	128	1024	NR	NR
PS	16	16	NR	NR

NR – Not Reagent at the dilution 1:8; serum sample not available.

while serum sample 6 weeks later had no IgM antibodies. Both samples were IgG negative.

Case Report 3

A 59-year-old male was admitted to the hospital, complaining of dyspnea worsening, coughing and chills during the last 5 days. He was a former heavy smoker with chronic obstructive pulmonary disease (COPD) and had coronary chronic disease. He complained of chills and pleuritic chest pain in his right thorax and right shoulder that had started the day before. He was taking angiotensin-converting enzyme inhibitors, β-blockers and PO aspirin 100 mg/day. He presented axillary temperature of 38.8ºC, cough with purulent sputum, and diminished breath sounds were heard in both lungs; at inferior right lung, rales could be heard. Chest radiograph at admission showed patchy consolidation of the right anterior basal segment of the lung (Figure 5). WBC count was 10,900 cells per mm^3, with 64% neutrophils, 2% band forms, 1% eosinophils and 23% lymphocytes. Gram stain showed many isolated Gram-positive cocci, a few extracellular Gram-negative diplococci, a few Gram-negative bacilli and rare leucocytes; Ziehl-Neelsen stain showed no acid-fast bacilli and sputum culture grew only saprophytic colonies. Serum sample drawn on the third day after hospitalization showed IgG antibodies

Figure 1. Chest radiograph of patient 1 at admission showing incomplete patchy consolidation of lingular segments with small amount of pleural fluid.

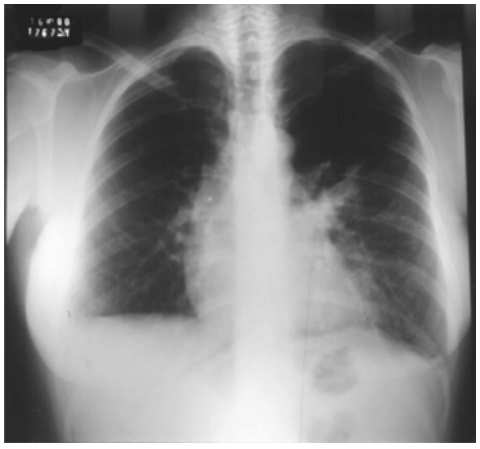


Figure 2. Chest X-Ray of patient 1 after 1 month shows regression of the pulmonary lesions.

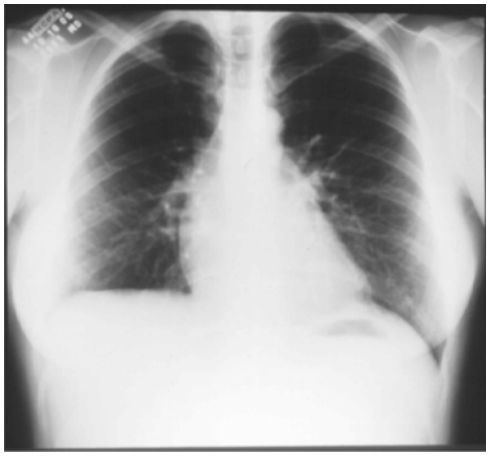


Figure 3. Chest radiograph of patient 2 at admission showing patchy consolidation of lingular segments with atelectasis.

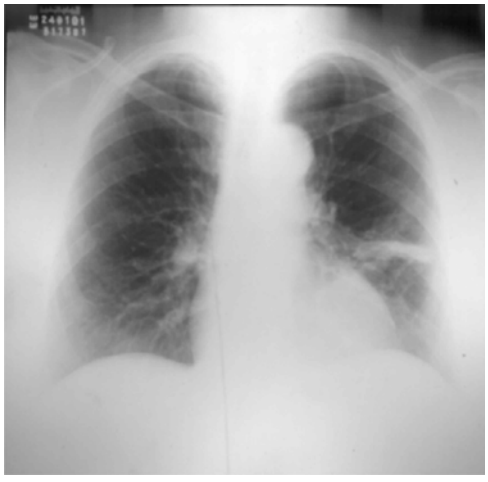


Figure 4. Thorax HRCT of patient 2 taken after 1 week, showing patchy lingular consolidation with air bronchograms.

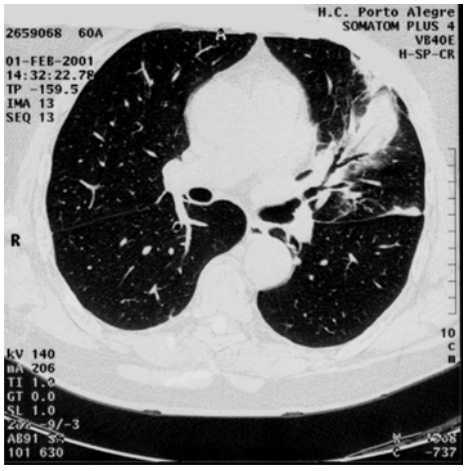


Figure 5. Chest X-Ray of patient 3 at admission showing patchy consolidation of the right basal segment of the lung.

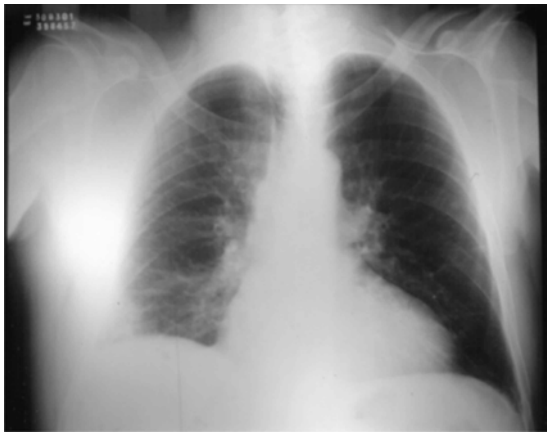
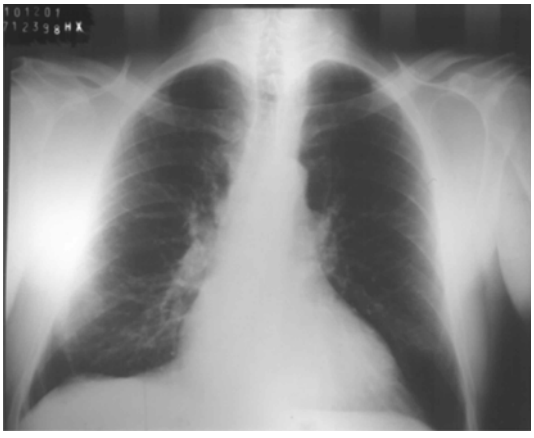


Figure 6. Chest X-Ray of patient 3 after 5 weeks shows almost complete regression of pulmonary lesions.



to *C. pneumoniae* of 1:128.The patient took IV ampicillin-sulbactam 1g qh8 and was discharged after 3 days. Serum IgG antibodies to *C. pneumoniae* after 4 ½ weeks were positive at 1: 1024. IgM antibodies were negative in both serum samples. Chest radiograph after 5 weeks showed regression of the bronchopneumonic lesions (Figure 6).

Discussion

Our two female patients with TWAR CAP presented mild clinical diseases, similar to reported cases [12,13]. Both diseases had a subacute course with symptoms for more than one week before hospitalization. One case presented initially a biphasic pattern with symptoms of upper respiratory infection and after 2 ½ weeks had pneumonia, as reported [10]. Both had acute sinus infection at the presentation and no detectable fever in the hospital, neither high WBC count nor elevated band forms; at least in one case the ESR was abnormal. Although there is not a distinct radiological pattern to *C. pneumoniae* CAP, chest radiographs demonstrated subsegmental pattern as reported [14].

The first patient was admitted due to nausea and fever with signs of pneumonia. Concomitant otitis was excluded. Getting improvement on levofloxacin, she could be discharged after 3 days. The second patient used trimethoprim-sulfamethoxazole for 7 days, but persistent cough and the rising of hemoptysis after 20 days led to hospital admission. A fiberbronchoscopy was done, whose BAL showed predominance of neutrophils, tuberculosis was ruled out. She recovered on PO levofloxacin. The third patient had an acute exacerbation of COPD and presented pneumonia at hospital admission. Sputum samples before antibiotic therapy revealed no predominant organism at Gram stain, and the culture grew saprophytic organisms. This patient had a more severe clinical course maybe due to his previous diseases. He improved on amoxicillin, which is a common finding in TWAR pneumonia [3].

The BAL culture of the second patient was negative probably due to the use of antibiotics. In our third case both Gram stain and culture of sputum samples obtained before antibioticotherapy don´t suggest concomitant pneumococcal infection. The mild severity of our clinical cases suggests TWAR etiology only, according to former reports showing that *C. pneumoniae* CAP is milder than pneumococcal pneumonia [12,13]; and that pneumonia due to concomitant infection with *C. pneumoniae* and *S. pneumoniae* results in a more severe illness than did TWAR infections alone [15]. As we did not exclude other etiological agents, we cannot attribute the etiology of our CAP cases to TWAR only. Our point, tough, was to diagnose *C. pneumoniae* as a causative agent, no matter if combined to other pathogen or no.

MIF test is still recommended as the only one species specific test to TWAR infections diagnosis [5], despite problems in the performance of test and interpretation of its results [16]. Some validated commercial MIF kits [17,18] have been used by many clinical laboratories and researchers [11,17,19-21]. Laboratorial diagnosis of *C. pneumoniae* infection in our study was established on the basis of a significant and clear-cut antibody response by MIF test as reported [11] and recommended [5]; we use seroconversion as criterium of definite acute infection as others [6].

Carrier state differs from asymptomatic infection because in the former there is no concomitant serological evidence of acute infection (seroconversion). Carrier state has been considerably described by culture and more recently by PCR. In one report 4.7% of 234 healthy adults [22] and 2 of 41 healthy children [23] had TWAR isolated from throat cultures. Others identified TWAR by PCR or nasopharingeal culture in 2 of 104 healthy adults [24].

Recently Miyashita et al. [25] found 14 positive nasopharyngeal specimens by either culture or PCR in 1,018 healthy adults, getting follow-up cultures, PCR and serology in 10 out of these 14 patients. They had no serological evidence of acute infection, despite being PCR positives in 3 cases for 4-12 weeks. Their conclusion was that the prevalence of "asymptomatic infection" was 1.4%. Subclinical infection of these very patients was neither documented nor ruled out by clinical or radiological exam. We can't tell if they had an asymptomatic infection in which their immunological system was not able to produce antibodies IgG or IgM, [16]. Thus, they should be classified as carriers only.

It has been already pointed out that isolations and positive PCR findings without seroconversion in paired samples may be a sign of a mere carrier stage of TWAR [24,26,27]; and also that the sole identification of the organism in the throat could not mean TWAR is the etiological agent of the infection [24,28].

We think that the definition of asymptomatic infection should be reserved to the culture or PCR- positive patients without symptoms who have either seroconversion of antibodies or any lung infiltrate at thorax HRCT consistent with pneumonia- since it has been reported that HRCT has greater sensitivity than Chest X-Ray in diagnosing pneumonia [29,30].

As the same researchers [25] detected other 4 asymptomatic individuals with an IgM titer of ≥1:16 (who had no positive PCR or culture) we can not presume again either they had "asymptomatic infection" because their IgM antibody might be slowing down after a recent infection by TWAR. Others [24] had already found 12% of 103 healthy individuals with an isolate titer of IgM ≥1:16, challenging the diagnostic utility of the MIF test. There is not an error of MIF technique but of the interpretation of its results due to the use of cut-off point of a sole antibody titer (IgG ≥1:512 or IgM ≥1:16) as criteria of acute infection. We can not diagnose acute infection in asymptomatic persons by means of an only antibody titer without clinical evaluation and follow up. And finally, a complete clinical evaluation is needed to exclude current infection in culture or PCR- positive patients before presume they are carriers only.

We think that whereas four-fold rises in antibody titer in the IgM or IgG serum fraction are clearly related to current TWAR infection, a high titer of antibody in a single specimen may be due to persistence after a recent or not-too-recent infection [10,27]. Patients with high IgM antibody titer either due to previous TWAR infection 4-6 months before or to a more recent asymptomatic infection may present current pneumonia due to another bacteria (e.g. pneumococcal pneumonia, whose sputum and blood culture are often negatives) - thus the finding of a sole titer of IgM 1:16 in this very patient might take to the erroneous classification of pneumonia by TWAR. Then, we propose that even in a patient with pneumonia, the diagnosis of TWAR etiology should not be made by an only IgM titer of ≥1:16. We wonder if the incidence of *C. pneumoniae* CAP reported in most studies was higher than should actually should had not been used a sole titer of IgM ≥1:16 to diagnose acute infection [6].

All our patients with TWAR CAP had seroconversion. Our two first cases of TWAR CAP had seroconversion of IgM antibodies, being cases of primary TWAR infection, in which pattern IgM antibodies appear three weeks after the symptoms without detectable IgG antibodies. Our third patient had a pattern of reinfection, by presenting seroconversion of IgG antibodies with negative IgM [31].

According to many studies, it is now evident that *C. pneumoniae* can be isolated by culture or PCR from asymptomatic persons who may present a sole IgM antibody titer of ≥1:16, meaning either recent infection or carrier state; or at the utmost possible current infection. The conclusion is that a cutoff point of a single titer of IgM antibody ≥1:16 has no specificity to diagnose acute *C. pneumoniae* infection.

We agree we should compare patients with CAP by TWAR with definitive criterion (only the ones with seroconversion) with patients with possible TWAR CAP (who had a sole antibody titer of IgM ≥1:16 or IgG ≥1:512) as proposed [32]; this study should include the results of culture and PCR techniques as well. We also should use other tests (immunological, molecular tests) to typical agents to improve the diagnosis of mixed infections. A comprehensive study like that can make us understand the role of *C. pneumoniae* as a sole pathogen and as co-pathogen in CAP; and also the true meaning of the detection of *C. pneumoniae* in respiratory specimen by culture and PCR.

The weakness of the present study is the small sample size due to limited financial resources; and as many researchers, we haven´t made culture or PCR in respiratory samples. Culture is made only at reference laboratories and PCR techniques are not available in clinical routine. Then, we think we might have underdiagnosed the incidence of pneumonias by *C. pneumoniae* in our study. It was recently reported (by a study that also did not make cultures to *C. pneumoniae*) that sera samples of 2 patients whose BAL samples were twice positives by Real-Time PCR to *Chlamydia pneumoniae* were negatives by MIF serologies. All samples whose tested positive by the molecular assay were diagnosed as pneumonia by Chest X-Ray. The discrepancy of the results of serologies and Real-Time PCR to *C. pneumoniae* was attributed to possibly early stage of the disease, where antibody detection is an insensitive diagnostic tool [33].

Real-Time PCR was the most sensitive test to the diagnosis of pneumonia by *C. pneumoniae* [33,34]. Blood base polymerase chain reaction using nested polymerase chain reaction did not seem to be a marker for acute respiratory infection by *C. pneumoniae* [35]. We wonder if Real-Time PCR in blood monocytes might be a marker for acute respiratory *C. pneumoniae* infection and if it could monitor antichlamydial therapy as suggested [36].

We think we have underestimated the real incidence of *C. pneumoniae* pneumonias in our hospital by using only serologies, since at the time of this study (2000-2001) we did not do PCR techniques; we agree that serologies itself are not enough to the diagnosis of *C. pneumoniae* acute infections; if used, an only IgM titer of ≥1:16 has no specificity to the diagnosis of TWAR acute infection; and finally it is urgent to improve molecular diagnosis rather by Real-Time PCR tests along with other methods to get the true incidence of the disease.

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