Medical Journal of Bakırköy

Med J Bakirkoy 2025;21(4):405-413
DOI: 10.4274/BMJ.galenos.2025.2024.4-1

Research

Comparison of PCR with Conventional Methods in the Diagnosis of *Pneumocystis jirovecii* and Atypical *Pneumonia* Agent *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in Intensive Care Patients

Yoğun Bakım Hastalarında *Pneumocystis jirovecii* ve Atipik Pnömoni Etkeni *Chlamydia pneumoniae* ve *Mycoplasma pneumoniae*'nin Tanısında PCR'nin Konvansiyonel Yöntemlerle Karşılaştırılması

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ABSTRACT

Objective: *Pneumocystis jirovecii (P. jirovecii)* can colonize healthy people and other chronic lung patients as well as in immunocompromised or immunosuppressed patients. *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydia pneumoniae* (*C. pneumoniae*) are the most common atypical *pneumonia* agents in the community. This study aimed to investigate the presence of *P. jirovecii*, *M. pneumoniae*, and *C. pneumoniae* colonization/infection by different methods in patients in intensive care units.

Methods: In this study, the presence of *P. jirovecii*, *M. pneumoniae* and *C. pneumoniae* in endotracheal aspirate specimens taken from 50 intensive care patients was investigated by polymerase chain reaction (PCR) method and conventional methods.

Results: Twenty (40%) of 50 patients were diagnosed with *pneumonia*. *P. jirovecii* DNA was detected in six specimens. *P. jirovecii* cysts were detected by Gram-Weigert and Gomori silver staining in one of the patients who were PCR positive. Of 20 patients diagnosed with *pneumonia*, 9 (45%) were *C. pneumoniae* PCR positive, 18 (90%) were *C. pneumoniae* immunoglobulin M (IgM) positive, 8 (40%) were *C. pneumoniae* IgG positive, and 5 (25%) were *M. pneumoniae* IgG positive. Of 30 patients (60%) who were not diagnosed with *pneumonia*, 1 (3.3%) were *C. pneumoniae* IgM positive, 12 (40%) were *C. pneumoniae* IgG positive, and 5 (16.7%) were *M. pneumoniae* IgG positive.

Conclusion: It is important to use the PCR method together with conventional methods for rapid and accurate diagnosis of *P. jirovecii*, *C. pneumoniae*, and *M. pneumoniae* in patients hospitalized in intensive care units, to differentiate colonization/infection, and to prevent the risk of infection development in immune-compromised patients.

Keywords: Atypical *pneumoniae*, *C. pneumoniae*, intensive care unit, *M. pneumoniae*, *P. jirovecii*

ÖZ

Amaç: *Pneumocystis jirovecii (P. jirovecii)* sağlıklı insanlarda ve diğer kronik akciğer hastalarında olduğu gibi immün sistemi baskılanmış hastalarda da kolonize olabilir. *Mycoplasma pneumoniae (M. pneumoniae) ve Chlamydia pneumoniae (C. pneumoniae)* toplumda en sık görülen atipik pnömoni etkenleridir. Bu çalışmada yoğun bakım ünitelerinde yatan hastalarda *P. jirovecii, M. pneumoniae* ve *C. pneumoniae* kolonizasyon/enfeksiyon varlığının farklı yöntemlerle araştırılması amaçlanmıştır.

Gereç ve Yöntem: Bu çalışmada 50 yoğun bakım hastasından alınan endotrakeal aspirat örneklerinde *P. jirovecii, M. pneumoniae* ve *C. pneumoniae* varlığı polimeraz zincir reaksiyonu (PCR) yöntemi ve geleneksel yöntemlerle araştırılmıştır.

Bulgular: Elli hastanın 20'sine (%40) pnömoni tanısı konulmuştur. Altı örnekte *P. jirovecii* DNA'sı tespit edilmiştir. PCR pozitifliği saptanan hastaların sadece birinde Gram-Weigert ve Gomori silver boyama ile *P. jirovecii* kisti tespit edilmiştir. Pnömoni tanısı alan 20 hastanın 9'unda (%45) *C.*

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Cite as: Özel Y, Sarıoğlu N, Vardar Ünlü G, Erel F, Ugün F, Ünlü M. Comparison of PCR with conventional methods in the diagnosis of *Pneumocystis jirovecii* and atypical *pneumonia* agent *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in intensive care patients. Med J Bakirkoy. 2025;21(4):405-413

Received: 01.04.2024
Accepted: 11.02.2025
Publication Date: 18.12.2025

ÖZ

pneumoniae PCR pozitif, 18'inde (%90) *C. pneumoniae* immünoglobulin M (IgM) pozitif, 8'inde (%40) *C. pneumoniae* IgG pozitif ve 5'inde ise (%25) *M. pneumoniae* IgG pozitif olarak saptanmıştır. Pnömoni tanısı konamayan 30 (%60) hastanın 1'inde (%3,3) *C. pneumoniae* IgM pozitif, 12'sinde (%40) *C. pneumoniae* IgG pozitif ve 5'inde (%16,7) *M. pneumoniae* IgG pozitif olarak bulunmuştur.

Sonuç: Yoğun bakım ünitelerinde yatan hastalarda *P. jirovecii, C. pneumoniae* ve *M. pneumoniae*'nin hızlı ve doğru tanısı, kolonizasyon/enfeksiyon ayrımı ve bağışıklık sistemi baskılanmış hastalarda enfeksiyon riskinin önlenmesi için PCR yönteminin konvansiyonel yöntemlerle birlikte kullanılması önemlidir.

Anahtar Kelimeler: Atipik pnömoni, *C. pneumoniae,* yoğun bakım birimi, *M. pneumoniae, P. jirovecii*

INTRODUCTION

Pneumocystis spp. are opportunistic organisms that are unicellular, eukaryotic, and can be found in the lungs of many mammals. Five species of *Pneumocystis* have been identified, namely *Pneumocystis carinii* and *Pneumocystis wakefieldiae* in rats, *Pneumocystis murina* in mice, *Pneumocystis oryctolagi* in rabbits, and *Pneumocystis jirovecii* (*P. jirovecii*) in humans (1). *P. jirovecii* causes *Pneumocystis pneumonia* (PCP), which has a dangerous clinical status, especially in people with compromised immune systems. Initially considered a protozoan, *Pneumocystis* was recognized as a fungus in the late 1980s with the development of gene sequencing techniques (2). Microscopic identification of organisms in lower respiratory tract specimens is considered the gold standard for PCP. Cyst forms of *Pneumocystis* can be stained with Grocott-Gomori metamine silver and trophozoite forms with Giemsa and Diff-Quick, but experience with microscopy is important in the diagnostic examination. Polymerase chain reaction (PCR) is increasingly used in the molecular diagnosis of PCP (3).

Chlamydia pneumoniae (*C. pneumoniae*) is an obligate intracellular bacterium that infects humans and is one of the major causes of atypical pneumonia. *C. pneumoniae* was first isolated from the eye of a child during a trachoma vaccine study in Taiwan in 1965. *C. pneumoniae* pneumonia causes upper and lower respiratory tract symptoms within 1-3 weeks and can occur as an acute or subacute infection (4). Serological diagnosis of *C. pneumoniae* infection is based on a fourfold or greater increase in immunoglobulin M (IgM) or IgG antibodies. Micro-immunofluorescence (IFA) testing is the most preferred method and is species-specific (5).

Mycoplasma pneumoniae (*M. pneumoniae*) is a bacterium that is slow-growing, pleomorphic, non-motile, and without a cell wall. *M. pneumoniae* is easily transmitted, and the infection is most likely to be spread by droplets (6). The most common symptoms include sore throat, hoarseness, fever, cough; initially unproductive but later moderate bloodless sputum, headache, chills, myalgia, earache, and general malaise. A four-fold or greater increase in both IgM and IgG antibody titers in specimens collected at 2-3 week intervals suggests an existing or recent infection (7). Many commercial kits are available based on IFA testing and enzyme immunoassay methods. PCR is another preferred method for the detection of *M. pneumoniae* (8).

Pneumonia is responsible for a significant portion of morbidity and mortality in intensive care units (ICUs). Since atypical pneumonia agents are difficult to grow in culture, data on the frequency of these infectious agents are limited (9). In recent years, advances in molecular microbiology techniques have led to an increased recognition of the importance of atypical pneumonias. However, there are few studies on the etiology of atypical pneumonia in Türkiye (10).

This study aimed to compare the sensitivity of conventional staining methods with PCR for the diagnosis of *P. jirovecii* in ICUs, and to investigate the relationship between the presence of *M. pneumoniae/C. pneumoniae* DNA and serum IgM/IgG antibodies.

METHODS

Ethical Approval

The study was conducted with the permission of Balıkesir University Clinical Research Ethics Committee with the approval number 2018/94, date 09.05.2018. Patients and their first-degree relatives were informed about the content of the study and their consent was obtained.

Collection of Specimens

Fifty patients who were monitored in the ICU of Balıkesir University Research Hospital were included in the study. Patients admitted to the ICU with and without symptoms of pneumonia were included in the study. Endotracheal aspirate (ETA) was collected for the detection of *P. jirovecii, C. pneumoniae,* and *M. pneumoniae,* and serum samples were collected for the serological diagnosis of *C. pneumoniae* and *M. pneumoniae.*

Preparation of Specimens

ETA specimens were centrifuged at 13000 rpm for 10 min. The supernatant was discarded and the pellet was stored as aliquots for staining methods and PCR tests. The pellet was spread on slides by the cytocentrifugation method for Giemsa, Gram-Weigert, and Gomori-Metamine silver staining. For PCR, at least 200 µL of each sample was taken, put into sterile eppendorf tubes, and stored at -20 °C. Blood specimens were centrifuged at 4000 rpm for 10 min. The serum specimens obtained were transferred to sterile tubes and stored at -20 °C.

Giemsa Staining Method

Giemsa solution was prepared by mixing the stock Giemsa (Merck, Darmstadt, Germany) with PBS adjusted to pH 7.2 at a ratio of 1/11. Specimen slides were fixed with methanol for 3 min and stained for 25 min with Giemsa solution. The slides were then rinsed with low-flow tap water from the edges and left upright to dry. The preparations were examined for the presence of *P. jirovecii* trophozoite using immersion oil at 100x magnification under a light microscope.

Gram-Weigert Staining Method

Gram-Weigert dye solution was prepared according to the protocol (11) by preparing crystal violet (Merck, Darmstadt, Germany), Gram iodine (Merck, Darmstadt, Germany), and aniline-xylene solutions (Merck, Darmstadt, Germany) separately. After cytocentrifugation, the slides were dried at room temperature and immersed in 1% eosin Y stain (Merck, Darmstadt, Germany) for 5 minutes. It was then washed in distilled water for 2 min. They were kept in crystal violet and Gram iodine solution for five minutes and then in distilled water for two minutes. The slides were gently dipped in the aniline-xylene solution to remove excess dye and dried at room temperature. They were examined with immersion oil at 100x magnification for the presence of *P. jirovecii* trophozoites and cysts.

Gomori-Methamine Silver Staining Method

A commercial dye (GBL, İstanbul, Türkiye) was used for silver staining. Staining was performed according to the protocols prepared by the manufacturer. The slides were then closed with entellan (Merck, Darmstadt, Germany) and examined for *P. jirovecii* cysts using immersion oil at 100x magnification.

Positive control slides were used to validate the staining methods applied in the study. Additionally, each stained slide that was deemed suspicious was reviewed and verified by at least three researchers who are experts in the field.

DNA Isolation

Specimens stored at -20 °C for PCR were thawed at room temperature and vortexed. The thawed specimens were centrifuged at 13,000 rpm for 10 min, and the supernatant was discarded. 200 µL of animal tissue lysis buffer and 20 µL of proteinase K solution (Qiagen, Hilden, Germany) were added to the pellet and vortexed. The specimens incubated at 56 °C for 60 min and then at 95 °C for 10 min in the heat block were allowed to reach room temperature. DNA isolation was performed using the Qiagen-EZ1 Mini kit and the EZ1 Advanced XL (Qiagen, Hilden, Germany) device in accordance with the manufacturer's instructions.

Real Time PCR

Presence of *P. jirovecii, C. pneumoniae* and *M. pneumoniae* in ETA specimens was examined using a commercial kit (Fast-Track, Esch-sur-Alzette, Luxembourg) and a real-time PCR device (Qiagen Rotor-Gene Q, Hilden, Germany). The amplification protocol was performed according to the manufacturer's instructions. The commercially available *P. jirovecii* and *M. pneumoniae/C. pneumoniae* PCR kit contains a primer/probe mixture, a positive control, a negative control, an internal control (IC), an enzyme mixture, and a PCR buffer solution. Ribonucleic acid was transcribed into cDNA using a specific primer-mediated reverse transcription step, which was immediately followed, in the same tube, by PCR. An increase in fluorescence observed from the relevant dual-labeled probe indicated the presence of specific pathogen sequences in the reaction, which was reported as a cycle threshold value by the real-time thermocycler. The assay used equine arteritis virus (EAV) as both an extraction control and IC. EAV was introduced by the laboratory into each sample and the negative control at the lysis buffer stage of the extraction process. The kit contents were prepared in volumes suitable for the number of samples provided in Table 1.

Real-time PCR procedures were performed according to the following protocol: hold at 42 °C for 15 minutes, hold at 94 °C for 3 minutes, followed by 40 cycles of 94 °C for 8 seconds and 60 °C for 34 seconds. The analysis results were interpreted in cases where the negative control was negative, and both the positive and ICs were positive. For detailed validation data such as sensitivity, specificity, clinical studies and external quality panel results, please refer to the related validation file at: www.fast-trackdiagnostics.com

Immunofluorescent Antibody Method

C. pneumoniae and *M. pneumoniae* IgG and IgM antibodies in serum specimens were investigated by the IFA method using a commercial kit (Euroimmun, Lübeck,

Table 1. Required reagent volumes for PCR reaction based on the number of samples

Number of reactions	1	15	32	64
PPmix	1.5 µL	22.5 µL	48 µL	96 µL
Buffer	12.5 µL	187.5 µL	400 µL	800 µL
Enzyme	1 µL	15 µL	32 µL	64 µL
Total	15 µL	225 µL	480 µL	960 µL

PPmix: Primer/prop mixture, PCR: Polymerase chain reaction

Germany). IgG-type antibodies in the patient's serum were removed with Eurosorb (Euroimmun, Lübeck, Germany) reagent before the detection of IgM-type antibodies. The presence of IgG and IgM type antibodies was examined under a fluorescence microscope at 40x magnification by comparison with positive and negative controls.

The evaluation of IFA images was performed by comparing the positive and negative control images provided in the kit with the clinical sample images. The results were reviewed and validated by at least three researchers who are experts in the field.

Statistical Analysis

Data were analyzed using IBM SPSS 25.0 statistical software (IBM SPSS Inc., Chicago, IL, USA). Continuous variables are expressed with the average±standard deviation, and minimum and maximum values. The differences between categorical variables are examined by chi-square analysis and Fisher's exact test.

RESULTS

Distribution of Patient Specimens by Units

Specimens included in the study and their distribution according to ICUs are shown in Table 2.

Diagnosis and Clinical Findings of the Patients

Twenty of 50 patients were diagnosed with pneumonia. The most common findings in all patients were cough and shortness of breath.

Table 2. Distribution of ETA samples by intensive care units

Intensive care	Specimen
Anesthesia intensive care	26
General intensive care	14
Coroner intensive care	5
CVS intensive care	5
Total	50

ETA: Endotracheal aspirate, CVS: Cardiovasculer surgery

P. Jirovecii PCR Results in ETA Specimen

P. jirovecii DNA was detected in six specimens; the DNA load was high in one patient, while it was found to be low in the other five.

P. Jirovecii Staining Results of ETA Specimen

P. jirovecii trophozoite forms were not detected in any of the specimens using Giemsa staining. *P. jirovecii* cysts were detected by Gram-Weigert and Gomori Silver staining in only one of the patients who were positive by PCR. In the other five PCR-positive patients, *P. jirovecii* cyst or trophozoite form were not observed by staining methods. PCR-negative patients, were also negative by staining methods. Five of the PCR-positive patients were followed up in the anesthesia ICU, and one was in the cardiovascular ICU. While two of the six patients with a positive PCR test for PCP had symptoms of pneumonia, the patient with the highest parasite load had no symptoms of pneumonia. The distribution of *P. jirovecii* positive patients is given in Table 3 according to the diagnostic methods and pneumoniae findings. PCR and Gram Weigert and Gomori silver staining images of the patient with the highest parasite load are shown in Figure 1.

Table 3. Distribution of *P. jirovecii* positive patients by specimen type, method and diagnosis

No	Giemsa staining	GW staining	Silver staining	PCR	Pneumonia
1	-	+	+	+	-
2	-	-	-	+	+
3	-	-	-	+	-
4	-	-	-	+	-
5	-	-	-	+	+
6	-	-	-	+	-

GW: Gram-Weigert staining, PCR: Polymerase chain reaction

C. Pneumoniae/M. Pneumoniae PCR and Antibody Results of the Patients With and Without Pneumonia

Of the 50 patients included in the study, 20 (40%) were diagnosed with pneumonia. Of 20 patients diagnosed with pneumonia, 9 (45%) were *C. pneumoniae* PCR positive, 18 (90%) *C. pneumoniae* IgM positive, 8 (40%) *C. pneumoniae* IgG positive, and 5 (25%) *M. pneumoniae* IgG positive. Of 30 patients (%60) who were not diagnosed with *pneumonia*, 1 (3.3%) *C. pneumoniae* IgM positive, 12 (40%) *C. pneumoniae* IgG positive, and 5 (16.7%) *M. pneumoniae* IgG positive. While *C. pneumonia* PCR and IgM results were found to be statistically significant in the group diagnosed with pneumonia compared to the other group ($p<0.05$), for *C. pneumoniae* IgG there was no significant correlation between the groups ($p>0.05$). The data for *M. pneumonia* are not sufficient for statistical evaluation in terms of PCR, IgM, and IgG positivity in both groups. PCR and immunofluorescent antibody results of patients with and without pneumonia are shown in Table 4. *C. pneumoniae/M. pneumoniae* amplification curve and IgG/IgM type antibodies images are shown in Figures 2 and 3.

C. Pneumoniae Antibody and PCR Results of the Patients With/Without *Pneumonia*

Clinical findings such as respiratory distress, dyspnea, cough, and sputum were observed in the majority of patients with *C. pneumoniae* detected by DNA and IgM antibody presence. The comparisons of *C. pneumoniae* antibody and PCR results are shown in Table 5.

M. Pneumoniae Antibody and PCR Results of the Patients With/Without *Pneumonia*

There was only one patient who tested positive for *M. pneumoniae* PCR and had chronic obstructive pulmonary disease (COPD), cerebrovascular disease, and signs of unconsciousness. IgG antibody was positive in 10 patients, while *M. pneumoniae* IgM antibody was negative in all patients. The comparisons of *M. pneumoniae* antibody and PCR results is shown in in Table 6.

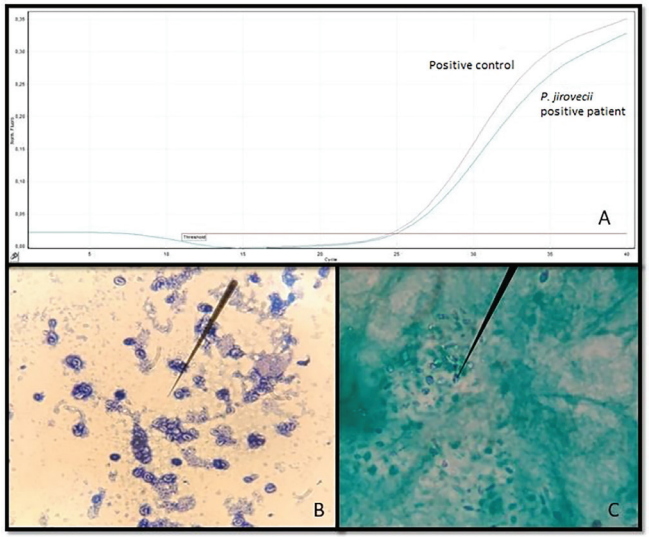

Figure 1. *P. jirovecii* amplification curve and staining images. **A)** *P. jirovecii* amplification curve in RT-PCR method, **B)** *P. jirovecii* cysts in Gram-Weigert staining, **C)** *P. jirovecii* cysts in Gomori-metamine silver staining
RT-PCR: Reverse transcription-polymerase chain reaction

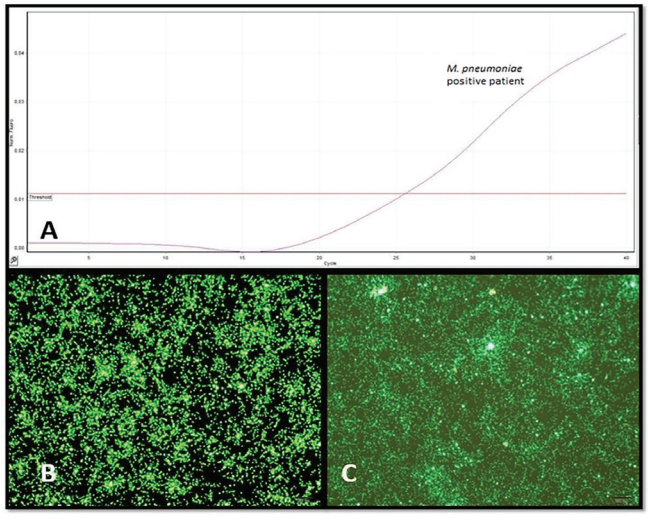

Figure 2. *M. pneumoniae* amplification curve and IFA images **A.** RT-PCR amplification curve, **B.** IgM type antibodies detected by IFA method (positive control), **C.** IgG type antibodies detected by IFA method
IFA: Immunofluorescence, RT-PCR: Reverse transcription-polymerase chain reaction, Ig: Immunoglobulin

Table 4. *C. pneumoniae/M. pneumoniae* PCR and antibody results of the patients with and without pneumonia

Atypical *pneumonia* agents	Pneumonia (n=20)		Non-pneumonia (n=30)		p-value
	n	%	n	%	
C. pneumoniae PCR	9	45	0	0	p<0.05
C. pneumoniae IgM	18	90	1	3.3	p<0.05
C. pneumoniae IgG	8	40	12	40	p>0.05
M. pneumoniae PCR	0	0	1	3.3	-
M. pneumoniae IgM	0	0	0	0	-
M. pneumoniae IgG	5	25	5	16.7	-

PCR: Polymerase chain reaction, Ig: Immunoglobulin

Table 5. Comparison of the *C. pneumoniae* antibody and PCR results of the patients with pneumonia

No	IgM	IgG	PCR	Pneumonia
1	+	-	+	+
2	-	+	-	-
3	+	-	-	+
4	+	-	+	+
5	-	+	-	-
6	+	-	-	+
7	+	-	-	+
8	-	+	-	-
9	+	-	-	+
10	-	+	-	-
11	-	+	-	-
12	-	+	-	-
13	+	-	-	+
14	+	+	+	+
15	-	+	-	-
16	+	-	-	+
17	-	+	-	-
18	+	-	+	+
19	+	+	+	+
20	+	-	+	+
21	+	+	+	+
22	-	+	-	-
23	-	+	-	-
24	+	+	+	+
25	-	+	-	+
26	+	+	-	-
27	+	-	-	+
28	+	-	-	+
29	-	+	-	-
30	+	+	-	+
31	-	+	-	+
32	+	+	+	+

PCR: Polymerase chain reaction, Ig: Immunoglobulin

Table 6. Comparison of the *M. pneumoniae* antibody and PCR results of the patients with pneumonia

No	IgM	IgG	PCR	Pneumonia
1	-	+	-	+
2	-	+	-	+
3	-	+	-	+
4	-	+	-	-
5	-	+	-	-
6	-	+	-	-
7	-	+	-	-
8	-	+	-	+
9	-	+	-	-
10	-	-	+	-
11	-	+	-	+

PCR: Polymerase chain reaction, Ig: Immunoglobulin

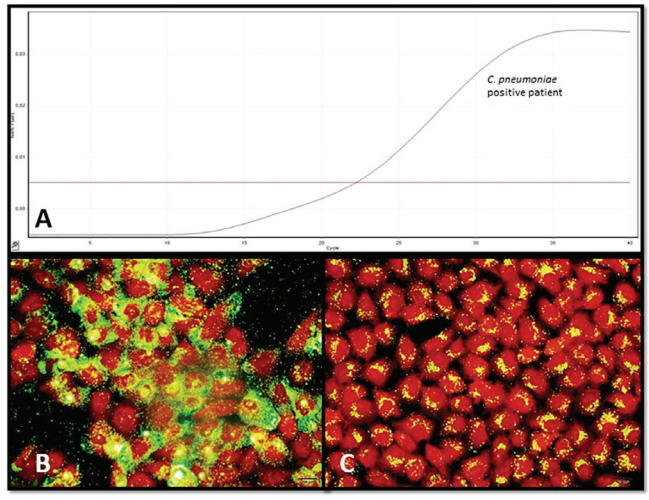

Figure 3. *C. pneumoniae* amplification curve and IFA images. **A.** RT-PCR amplification curve, **B.** IgM type antibodies detected by IFA method, **C.** IgG type antibodies detected by IFA method
IFA: Immunofluorescence, RT-PCR: Reverse transcription-polymerase chain reaction, Ig: Immunoglobulin

DISCUSSION

Community-acquired pneumonia (CAP) is a lung disease that threatens human health and has a high mortality rate, which is often associated with long hospital stays (12). More than two million children under five die from pneumonia each year (13). Detection of *P. jirovecii* DNA in patients without any symptoms has been defined as "*P. jirovecii* colonization/carrier", despite the risk of PCP (14). However, due to the low organismal load associated with colonization, the frequency of detection of colonization varies greatly from one study to another depending on the methods used, such as PCR or immunohistochemical staining of respiratory samples. *P. jirovecii* DNA has also been detected in individuals who are not immunocompromised, such as those with chronic lung disease, smokers, or pregnant women (15). In a study by Mori et al. (16), it was reported that PCP developed within 2-4 weeks in patients who had *P. jirovecii* DNA detected by PCR, did not have respiratory symptoms, and received immunosuppressive therapy for rheumatoid arthritis. Therefore, it has been reported that the detection of *P. jirovecii* DNA requires prophylaxis in conditions of clinical immune suppression that carry a risk of PCP risk (17).

In a study by Seyhan (18), the presence of *P. jirovecii* was investigated by comparing the PCR with various staining methods (Giemsa, Gram-weigert, and Gomori silver staining) in 100 immunocompromised patients. In that study, *P. jirovecii* DNA was detected by PCR in 4 of 100 patients, although *P. jirovecii* cysts and trophozoites could not be detected by Giemsa, Gram-Weigert, and Gomori silver staining methods in these patients. *P. jirovecii* cysts were observed with Gomori silver staining in a patient whose *P. jirovecii* DNA was not detected by PCR in the same study. In a study by Töz et al. (19), bronchoalveolar lavage (BAL) specimens of 42 patients with a pre-diagnosis of PCP were investigated using Giemsa, Gram-Weigert staining, and PCR. In this study, 13 patients were found to be positive by the PCR method, while three patients were found to be positive by both the staining and PCR methods, and, a total of 16 out of 42 patients were found to be positive for PCP. In a study conducted in İzmir, *P. jirovecii* positivity was found in 21 of 30 patients without pneumonia findings by PCR, six by direct fluorescent antibody (DFA), and one by staining. Four of these patients showed positivity with both PCR and DFA; furthermore, it was stated that PCR is a good method for demonstrating colonization (20).

In this study, two of the six patients with positive *P. jirovecii* DNA, followed up in the ICU, had pneumonia. In the patient who was PCR positive and had a high DNA load, *P. jirovecii* cysts were detected only in Gram-Weigert and Gomori methenamine silver staining, and there were no signs of pneumonia in this patient. This patient was thought to have *P. jirovecii* colonization. *P. jirovecii* was not detected in the three staining methods, which were applied to the samples from the other five patients with low DNA load. According to these data, the conclusion was that the probability of detecting *P. jirovecii* by staining methods is low. For this reason, it is thought that PCR will be suitable for investigating patients who may be at risk for PCP contamination, especially in ICUs, and will contribute to the identification of colonized patients.

In addition to cell culture and PCR, serological methods are also used in the diagnosis of *C. pneumoniae* infection. The serological diagnosis of *C. pneumoniae* infection is based on the detection of a 4-fold increase in IgG or IgA levels in serum specimens taken during the acute and convalescent periods. The retrospective nature of the diagnosis in this method means that serological results have little effect on treatment decisions. Specific IgM antibodies begin to form in 2-3 weeks, and IgG antibodies begin to form in 6-8 weeks. Therefore, it seems that serological methods are not effective in the diagnosis of acute infection (21). For this reason, PCR is thought to be the most sensitive and effective method in lower respiratory tract specimens.

Twenty of the 50 patients included in the study, (40%) had signs of pneumonia. *C. pneumoniae* PCR positivity was detected in 9 patients among those diagnosed with pneumonia. Of the 20 patients in the same group, 18 were positive for *C. pneumoniae* IgM and 8 were positive for *C. pneumoniae* IgG. In nine patients, *C. pneumoniae* DNA and IgM antibodies were found to be positive together. Similarly, in the thesis study conducted by Gökçınar (22), the lower respiratory tract and serum specimens, of 50 patients were investigated for *C. pneumoniae* using PCR and serological methods. Although IgM antibodies were detected in two of 50 patients in the related study, *C. pneumoniae* DNA was not detected in any of the patients by PCR. In this study, IgM antibody was positive in 18 of 20 patients (90%) with pneumonia symptoms. In 8 of these patients (40%), IgM was found to be positive alone, and in 9 (45%), it was positive with PCR. In the group without pneumonia symptoms, only IgM was found positive in one patient. IgM antibodies detected by the IFA method were observed to be associated with the presence of pneumonia symptoms. In this context, it is thought that studying IgM and PCR tests together, may be useful in indicating acute infection. In the patient group with pneumonia symptoms, IgM was found positive in 18 patients. However, PCR positivity was detected less frequently than expected. The potential reason for this result may be that ETA samples were used instead of BAL samples in the study. BAL samples were not preferred in this study because they require an invasive process, which is a limitation of our study.

Among the 15 *Mycoplasma* species that can infect humans, *M. pneumoniae* is the best-known atypical pneumonia agent (23). While IgM antibodies were not detected in any of the patient sera included in this study, IgG antibodies were detected in 10 of them. *M. pneumoniae* DNA was detected in one of these patients, and IgM and IgG antibodies were not detected in the same patient. *Mycoplasma* infection is frequently seen in children, but it has been reported that it may rarely be associated with certain diseases in the elderly. Many studies have shown that *M. pneumoniae* infection is associated with myocarditis, pericarditis, cerebral stroke, and vasculitis (24-26). In addition to playing a role in the pathogenesis of atherosclerosis, *M. pneumoniae* also induces cardiovascular disease and chronic inflammation (27). In this study, PCR was positive only in a 95-year-old patient who was followed up in the ICU, with the diagnosis of COPD and cerebrovascular disease.

Zhang et al. (28) extensively compared PCR and serological methods for the diagnosis of *M. pneumoniae* in a meta-analysis. Researchers have reported that commercial PCR tests exhibit high specificity but lower and more variable sensitivity. Despite their advantages, they still cannot replace serology, and PCR is a reliable and accurate method to be used together with serological diagnosis. Copete et al. (29) compared the presence of *M. pneumoniae* in children with and without CAP by serology and PCR; 13.9% of children with CAP were found positive by PCR and/or serology. While 10.3% of them were positive by PCR and 6.7% serologically, 2.8% of the cases were positive with both tests. In the acute phase, 32% of children with CAP and 38.3% of healthy children had positive IgM titers. It has been reported that IgM titer alone cannot be useful and PCR tests alone cannot distinguish between infection/colonization; therefore, it would be more reliable to use both tests together.

Study Limitations

The most significant limitation of the study is the small sample size. We believe that conducting similar studies with much larger sample sizes, within the context of financial resources and laboratory infrastructure, would be highly valuable. The data we have obtained can provide valuable preliminary information for larger-scale studies.

In this study, both PCR and serological methods were utilized. While these methods provide high sensitivity and specificity, they also have certain limitations. PCR, despite being a highly sensitive technique, is heavily reliant on sample quality and the preservation of genetic material. On the other hand, serological tests are valuable for detecting past or current infections but may have reduced sensitivity in the early stages of infection when antibody production is insufficient.

These limitations highlight the necessity of using complementary diagnostic tests, particularly in cases where a definitive diagnosis cannot be achieved with a single method. In this context, the combined use of PCR and serological methods can contribute to achieving more accurate and reliable diagnostic outcomes. Future studies should focus on evaluating the effectiveness of combining different diagnostic methods and further refining diagnostic protocols to enhance their utility.

CONCLUSION

As a result, the use of PCR together with conventional methods for the rapid and accurate diagnosis of *P. jirovecii* (PCP) and atypical pneumonia agents (*C. pneumoniae* and *M. pneumoniae*) in patients hospitalized in ICUs, guides the diagnosis. It is important because people with colonization act as reservoirs for spreading the agent within the community, and they also carry the risk of developing pneumonia if they have immune difficulties.

ETHICS

Ethics Committee Approval: The study was conducted with the permission of Balıkesir University Clinical Research Ethics Committee with the approval number 2018/94, date 09.05.2018.

Informed Consent: Patients and their first-degree relatives were informed about the content of the study and their consent was obtained.

FOOTNOTES

Authorship Contributions

Concept: Y.Ö., N.S., M.Ü., Design: Y.Ö., N.S., M.Ü., Data Collection or Processing: Y.Ö., N.S., F.E., F.U., Analysis or Interpretation: Y.Ö., G.V.Ü., F.E., F.U., M.Ü., Literature Search: Y.Ö., N.S., G.V.Ü., F.E., F.U., M.Ü., Writing: Y.Ö., M.Ü.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that this study received no financial support.

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