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Value of Rapid Aetiological Diagnosis in Optimization of Antimicrobial Treatment in Bacterial Community Acquired Pneumonia

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A B S T R A C T

In 80 adult patients with community acquired pneumonia (CAP) conventional microbiological methods, polymerase chain reaction (PCR) and serum C-reactive protein (CRP) levels were performed and the appropriateness of the empirical antimicrobial treatment was evaluated according to bacterial pathogen detected. The aetiology was determined in 42 (52.5%) patients, with Streptococcus pneumoniae as the most common pathogen. PCR applied to bronchoalveolar lavage (BAL) provided 2 and PCR on sputum samples 1 additional aetiological diagnosis of CAP. The mean CRP values in the S. pneumoniae group were not significantly higher than in the group with other aetiological diagnoses (166.89 mg/L vs.160.11 mg/L, p=0.457). In 23.8% (10/42) of patients with determined aetiology, the empirical antimicrobial treatment was inappropriate. PCR tests need further investigation, particularly those for the atypical pathogens, as they are predominant in inappropriately treated patients. Our results do not support the use of CRP as a rapid test to guide the antimicrobial treatment in patients with CAP.

Key words: *community acquired pneumonia, PCR, antimicrobial treatment*

Introduction

Community acquired pneumonia (CAP) is a common disease and a frequent cause of morbidity and mortality worldwide. In most studies, *Streptococcus pneumoniae* remains the most frequent bacterial cause of CAP in adult patients. The incidence of other bacterial pathogens varies widely and depends on the geographical area, factors related to the population being studied and microbiological tests used. In 50–70% of patients with CAP the aetiologic agent is not identified, even in carefully conducted prospective studies of pneumonia aetiology[1–6]. Fast and sensitive tests are therefore needed to guide antimicrobial treatment in patients with CAP.

Due to the imperfection of traditional microbiological methods, polymerase chain reaction (PCR) is increasingly being used in order to rapidly determine the aetiological diagnosis of CAP[7–11]. Also, usefulness of the serum C-reactive protein (CRP) levels as an early indicator of aetiology has been previously analyzed but data were discordant[12–14]. During recent years, its usefulness in diagnosis of CAP is considered again[15–19].

Ideally, antimicrobial treatment should be directed against the pathogen that is causing the pneumonia. However, as the aetiology is often not known at presentation, patients are initially receiving empirical antimicrobial treatment. Ewig et al. showed that without microbiological diagnostic results, 21% of cases would have been treated with an inappropriate empirical regimen. However, their findings were based solely on blood culture, bronchoscopy and serology, and molecular methods were not used[20]. In a recent study by van der Eerden et al. initial antimicrobial treatment guided by the clinical and epidemiological presentation was at least effective as treatment guided by the results of rapid microbiological investigation. In that study molecular methods were not used either[21].

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Therefore, the objective of this study was to determine the diagnostic yield of microbiological tests, both conventional and molecular, in patients with bacterial CAP. CRP serum levels as an early marker of the aetiology of CAP was also evaluated. Finally, the value of conventional and molecular microbiological tests in directing antimicrobial treatment was determined by evaluation of the empirical antimicrobial treatment according to the bacterial pathogen detected.

Patients and Methods

Patients

Between February 2007 and January 2008, we prospectively studied 80 patients (aged >18 yr) admitted for CAP to the Department of Pulmonology, Zagreb University Hospital Center, Zagreb, Croatia.

The diagnostic criteria for CAP included a new infiltrate on chest radiograph or auscultatory findings consistent with pneumonia with symptoms of acute lower respiratory tract infection including several (at least 2) of the following: fever or hypothermia, rigors, sweats, new cough with or without sputum production or change in color of respiratory secretions in a patient with chronic cough, chest discomfort, or the onset of dyspnea. Patients hospitalized or residing in a long-term-care facility within the previous 14 days were excluded[22].

The assessment of the severity of CAP was performed using the Pneumonia Severity Index (PSI). Patients meeting a PSI score of IV and V were considered as having a severe pneumonia[22,23].

Empirical antimicrobial regimens were classified as beta-lactam monotherapy, beta-lactam plus macrolide, macrolide monotherapy, quinolone monotherapy and other regimens.

The study was approved by the ethics committee of School of Medicine, University of Zagreb (date of issue 6 February 2007, registration number 04-76/2007-32). The patients gave their informed consent to participate in this study.

Microbiological and laboratory investigations

Clinical specimens obtained in the study included sputum samples for bacterial culture and PCR, urine samples for bacterial antigen detection and serum samples for PCR and serological testing. Serum samples for serological testing were drawn during the acute stage of illness and 3–4 weeks later. Serum samples were also used for determining CRP levels by an automated analyzer (Boehringer Mannheim; Mannheim, Germany) within 24 hours of admission. Blood samples for bacterial culture and bronchoalveolar lavage (BAL) samples for bacterial culture and PCR were obtained according to the clinical judgment of the physician in charge.

Standard methods were used for bacterial culture. Sputum data were evaluated only in samples with >25 polymorphonuclear leucocytes and <10 epithelial cells per low-power microscopy field.

Urine samples were tested for pneumococcal antigen (NOW *Streptococcus pneumoniae* test; Binax Inc., Maine, US) and, in case of severe pneumonia, for *Legionella pneumophila* serogroup 1 antigen (NOW *Legionella* Urinary Antigen Test; Binax Inc., Maine, US).

Chlamydophila pneumophila and *L. pneumophila* serogroup 1–7 antibodies IgG and IgM, as well as *Mycoplasma pneumoniae* IgA, IgM and IgG antibodies in paired sera from the acute and convalescent phase were determined using commercial enzyme immunoassay kits (Novagnost™, Dade Behring Marburg GmbH, Marburg, Germany; SERION ELISA, Institut Virion/Serion GmbH, Würzburg, Germany).

DNA for PCR reactions was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany).

PCR for *S. pneumoniae* and *C. pneumoniae* was performed as previously described[7,10]. For *M. pneumoniae* and *Legionella* species commercial PCR kits were used (Venor®Mp and Onar®Ls, Minerva Biolabs, Berlin, Germany). PCR for *S. pneumoniae* was performed on serum and BAL samples with the same PCR method. The only difference was that BAL samples (because of their viscosity) were treated with mucolytic agent 6% N-acetyl-L-cysteine prior to DNA isolation. PCR for for *C. pneumoniae*, *M. pneumoniae* and *Legionella* species was performed on sputum and BAL samples.

Empirical antimicrobial treatment was that one prescribed within the first 24 hours of admission.

Criteria for aetiological diagnosis

Categorisation of positive microbiological test results into definite, probable, and possible bacterial aetiologies of community-acquired pneumonia was done as previously described[24,25].

Criteria for definite aetiology were the following: 1) blood cultures or pleural fluid cultures yielding a bacterial pathogen; or 2) positive urinary *L. pneumophila* serogroup 1 antigen; or 3) detection of *Legionella* species by PCR from sputum or BAL sample; or 4) seroconversion or antibody titre increase (according to manufacturer's interpretation) of *M. pneumoniae* IgG, *C. pneumoniae* IgG, or *L. pneumophila* serogroup 1–7 IgG.

Criteria for probable aetiology were the following: 1) positive urinary pneumococcal antigen; or 2) positive sputum culture when a predominant microorganism was isolated from purulent sample and the finding of Gram stain was compatible; or 3) positive BAL culture when the organism was isolated in numbers $\geq 10^4$ colony-forming units/ml; or 4) detection of *M. pneumoniae* or *C. pneumoniae* by PCR from sputum or BAL sample; or 5) positive IgA or IgM antibodies for *M. pneumoniae*, or positive IgM antibodies for *C. pneumoniae*.

Criterion for possible aetiology was detection of *S. pneumoniae* by PCR from serum or BAL sample.

Statistical analysis

Means of serum CRP levels among different aetiological groups were compared by using two-tailed unpaired t-tests. Values of $p<0.05$ were considered significant.

Results

Patients' characteristics

The demographic and clinical characteristics of the 80 patients with CAP included in the study are described in Table 1. Sixty-one patients (76.3%) had at least one underlying disease. In 24 (30.0%) patients antimicrobial treatment was taken prior to hospital admission for pneumonia. According to the PSI score, 23 (28.8%) patients were in class I, 9 (11.3%) in class II, 16 (20.0%) in class III, and 32 (40.0%) in classes IV and V combined.

Aetiology of pneumonia and yield of different microbiological methods

The microbial aetiology of CAP was established in 42 (52.5%) patients with a total of 49 bacterial pathogens detected. A single pathogen was detected in 35 (43.8%) and 2 pathogens in 7 (8.8%) patients. Bacterial pathogens according to criteria for aetiological diagnosis are described in Table 2. Of these, 2 fulfilled the criteria for a definite, 45 for probable and 2 for possible aetiology. *S. pneumoniae* was the most common pathogen and was identified in 25 (31.3%) patients followed by *M. pneumoniae* in 7 (8.8%) and *Haemophilus influenzae* in 5 (6.3%) patients. *S. pneumoniae* with *H. influenzae* was the most common combination and was detected in 3 patients.

Diagnostic yield of various microbiological methods is described in Table 3. Urine samples for the pneumococcal urinary antigen detection was obtained from 76 (95.0%) patients. The test result was positive in 16 (21.1%) patients. In 14 (18.4%) of the 76 patients tested, the pneumococcal urinary antigen test was the only test positive and provided an additional diagnosis that other diagnostic tests did not provide. *L. pneumophila* serogroup 1 antigen detection was performed in urine samples from 32 patients and 2 (6.3%) were positive.

TABLE 1
DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF 80 PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA

Characteristics	Number (%) of patients*
Age ($\overline{X}$±SD)	62.6±15.5
Male gender	52 (65.0)
Smokers	23 (28.8)
Underlying disease	
Cardiovascular disease	27 (33.8)
Chronic obstructive pulmonary disease	21 (26.3)
Diabetes mellitus	8 (10.0)
Neoplasia	7 (8.8)
Cerebrovascular disease	5 (6.3)
Asthma	3 (3.8)
Chronic renal failure	2 (2.5)
Distribution according to PSI score	
PSI I-III	48 (60.0)
PSI IV-V	32 (40.0)

*Dara are presented as number of subjects (%) unless otherwise indicated, PSI – pneumonia severity index

Blood cultures were obtained in 11 (13.8%) patients and they were all negative.

Sputum and BAL samples were obtained from 64 (80.0%) and 19 (23.8%) patients, respectively. Sputum samples were adequate for culture in 36 (56.3%) patients. Bacterial pathogens were isolated in 17 (26.6%) of these patients including 8 cases of *S. pneumoniae*, 3 cases of *H. influenzae*, 4 cases of *Pseudomonas aeruginosa,* and 2 cases of *K. pneumoniae*. BAL culture was positive in 5 (26.3%) patients including 1 case of *S. pneumoniae*, 1 case of *H. influenzae*, 1 case of *P. aeruginosa*, and 2 cases of *K. pneumoniae*.

S. pneumoniae PCR was positive in 15.8% (3/19) BAL samples. All patients who underwent fibreoptic bronchoscopy (FOB) had taken antimicrobial treatment prior to bronchoscopic examination. Serum samples for *S. pneumoniae* PCR was obtained from 79 patients and all were PCR-negative. *M. pneumoniae* PCR was positive in

TABLE 2
BACTERIAL PATHOGENS IN 80 PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA ACCORDING TO CRITERIA FOR AETIOLOGICAL DIAGNOSIS

Bacterial pathogen	Definite diagnosis N	Probable diagnosis N	Possible diagnosis N
Streptococcus pneumoniae	0	23	2
Mycoplasma pneumoniae	0	7	0
Haemophilus influenzae	0	5	0
Chlamydophila pneumoniae	0	4	0
Pseudomonas aeruginosa	0	4	0
Legionella species	2	0	0
Klebsiella pneumoniae	0	2	0

*Numbers include 7 patients with dual bacterial pathogens (*S. pneumoniae* with *H. influenzae*, 3 patients; *S. pneumoniae* with *K. pneumoniae*, 1 patient; *S. pneumoniae* with *M. pneumoniae*, 1 patient; *P. aeruginosa* with *M. pneumoniae,* 2 patients)

TABLE 3
DIAGNOSTIC YIELD OF MICROBIOLOGICAL METHODS IN 80 PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA

Microbiological method	Number of positive/tested (%)
Streptococus pneumoniae antigen detection in urine sample	16/76 (21.1)
Legionella pneumophila serogroup 1 antigen detection in urine sample	2/32 (6.3)
Blood cultures	0/11
Sputum culture	17/64 (26.6)
BAL culture	5/19 (26.3)
Streptococcus pneumoniae PCR in serum	0/70
Streptococcus pneumoniae PCR in BAL	3/19 (15.8)
Mycoplasma pneumoniae PCR in sputum	1/64 (1.6)
Mycoplasma pneumoniae PCR in BAL	0/19
Chlamydophila pneumoniae PCR in sputum	0/64
Chlamydophila pneumoniae PCR in BAL	0/19
Legionella species PCR in sputum	0/64
Legionella species PCR in BAL	0/19
Serology*	10/79 (12.7)

*Including serology for *M. pneumoniae*, *C. pneumoniae* and *Legionella pneumophila* serogroup 1-7
BAL – bronchoalveolar lavage, PCR – polymerase chain reaction

1.6% (1/64) of sputum samples and BAL samples were negative. PCR tests for *C. pneumoniae* and *Legionella* species were negative in sputum and BAL samples.

A total of 6 cases of *M. pneumoniae* and 4 cases of *C. pneumoniae* pneumonia were identified by serology. IgM antibodies for *L. pneumophila* serogroup 1–7 in all serum samples were negative. IgG antibodies were positive in 2 serum samples, but there was no 4-fold increase in antibody levels in convalescent phase serum.

CRP values compared with causative pathogens

Out of 80 patients with CAP, 51 had a CRP measurement within the first 24 hours of admission. Mean CRP levels according to the respective bacterial pathogens are shown in Table 4. When grouping the patients in those with *S. pneumoniae* (N=30) and those with all other pathogens detected (N=11), the mean CRP values in the *S. pneumoniae* group were not significantly higher than in the group with other aetiological diagnoses (166.89 mg/L *vs.* 160.11 mg/L, p=0.457). Also, the mean CRP values in patients with *S. pneumoniae* were not significantly higher than in patients with CAP of unknown aetiology (166.89 mg/L *vs.* 148.72 mg/L, p=0.519).

TABLE 4
SERUM CRP VALUES ACCORDING TO CAUSATIVE PATHOGEN IN 51 PATIENTS WITH COMMUNITY ACQUIRED PNEUMONIA

Causative pathogens	Patients N	CRP $\overline{X}$ mg/L
Streptococcus pneumoniae	11	177.11
*Legionella pneumophila**	2	380.4
*Mycoplasma pneumoniae**	3	106.97
Chlamydophila pneumoniae*	4	185.63
Other	2	118.4
Patients with >1 pathogen identified*	3	234.27
CAP of unkonwn aetiology	26	148.72
Total	51	

*Not included in statistycal analyses due to the small number of cases
CRP – C-reactive protein

Evaluation of empirical antimicrobial treatment

The relation between the empirically selected antimicrobial agents for 80 patients and the respective bacterial pathogens is shown in Table 5. In 10 (23.8%) out of 42 patients with determined aetiology, the initially selected antimicrobial agents were retrospectively considered inappropriate. Antimicrobials were inappropriately used in 4 patients with *C. pneumoniae*, 2 patients with *P. aeruginosa,* 2 patients with *P. aeruginosa* plus *M. pneumoniae*, 1 patient with *M. pneumoniae*, and 1 patient with *L. pneumophila* serogroup 1 infection. In 8 (80%) out of 10 inappropriately treated patients, established aetiological agents were atypical pathogens *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila* serogroup 1.

Discussion

In most cases, the initial approach in patients with CAP involves empirical antimicrobial treatment. The main problem for clinicians is to estimate the likely bacterial pathogen and to select the most appropriate antimicrobial agents. Fast microbiological methods and early markers for guiding the clinician in the initial selection of antimicrobial treatment are therefore needed.

In this study PCR was used simultaneously with conventional microbiological methods to determine the bacterial pathogens in patients with CAP.

TABLE 5
DESCRIPTION OF EMPIRICAL ANTIMICROBIAL TREATMENT ACCORDING TO BACTERIAL PATHOGENS IN 80 PATIENTS WITH CAP

Causative pathogen	Antimicrobial agents used					
	Number of patients	Beta-lactam monotherapy	Beta-lactam plus macrolide	Quinolone monotherapy	Macrolide monotherapy	Other regimen
Streptococcus pneumoniae	20	10	5	3	1	1
Streptococcus pneumoniae + *Mycoplasma pneumoniae*	1	0	1	1	0	0
Streptococcus pneumoniae + *Haemophilus influenzae*		2	0	0	0	0
Streptococcus pneumoniae + *Klebsiella pneumoniae*	1	1	0	0	0	0
Klebsiella pneumoniae	1	1	0	0	0	0
Haemophilus influenzae	2	2	0	0	0	0
Pseudomonas aeruginosa	2	2*	0	0	0	0
Pseudomonas aeruginosa + *Mycoplasma pneumoniae*	2	2*	0	0	0	0
Mycoplasma pneumoniae	4	1*	0	1	0	1
Chlamydophila pneumoniae	4	4*	0	0	0	0
Legionella species	2	1*	1	0	0	0
Unknown	38	21	5	7	5	1
Total	80	47	12	12	6	3

*Antimicrobial agents were used inappropriately

CAP – community acquired pneumonia

The most useful method for identifying *S. pneumoniae* in our study was the pneumococcal urinary antigen, as was the case in some previous studies[25]. There is no reason to doubt in a cross-reactions with other micro-organisms and a reliability of positive pneumococcal urinary antigen in patients in which this was the only test positive for *S. pneumoniae*. Beside manufacturer's performance data which claims the possibility of false-positive results only in healthy children with nasopharyngeal carriage of *S. pneumoniae* and in the 48 hours following pneumococcal vaccination, there is also a plenty of scientific data supporting a high specifity of this test. In our study only the adult patients were included, and none of the patients received pneumococcal vaccine 48 hours before testing. In the study by Genne et al., 67 adults with CAP were compared with 81 patients with suspected urinary tract infection to determine sensitivity and specificity of pneumococal urinary antigen test. An aetiology could be found for 22 (33%) using conventional methods and increased to 33 patients (49%) with the addition of the urinary antigen test. Nine out of 14 patients diagnosed with *S. pneumoniae* as the aetiologic agent for their CAP had detectable urinary antigen levels (sensitivity 64%; 1/81 positive control urine samples, specificity 99%). Pneumococcal infection was diagnosed by pneumococcal urinary antigen in 24% without an aetiologic identification by conventional methods[26]. High specificity of pneumococcal urinary antigen test was also determined in a study by Diederen et al[27]. Our results may be somewhat diminished by the fact that a positive result of pneumococcal urinary antigen test may persist in patients with chronic obstructive pulmonary disease or recent pneumococcal infection[28]. However, *S. pneumoniae* remains the most frequent bacterial cause of CAP in most studies including adult patients[1–6]. Therefore, we agree with authors who claim that it might be justified to use pneumoccocal urinary antigen test as a basic test for diagnosis of pneumococcal pneumonia[25].

Blood cultures were obtained in 13.8% patients. In our study we considered it unnecessary to perform the blood cultures in every patient. Several studies have provided evidence that their sensitivity in CAP is low (particularly for patients with non-severe CAP, no co-morbid disease and for those who have received antimicrobial treatment before admission), and, when management decisions are analyzed, the impact of positive blood cultures is minor[29–32]. Therefore, in our study we left the decision for obtaining the blood cultures to the clinical judgment of the physician in charge. The explanation for all negative blood cultures (N=11) in our study could be prior antimicrobial treatment in 1 patient and pneumonia severity of PSI class I-II in 6 patients. The PCR tests for *S. pneumoniae* applied to serum samples were not useful as a diagnostic test for pneumococcal pneumonia. Because of inhibitors that could be present in serum samples, in our study we did: 1. dilution of serum samples by pooling; in this way the amount of inhibitors possibly present in serum samples was decreased; and 2. repeating of PCR reaction after spiking of PCR-negative serum samples prior to amplification with pneumococcal DNA previously isolated from bacterial suspension of *S. pneumoniae*; in this way the presence of PCR-inhibitors and false-negative PCR results were excluded. This pro-

cedure was also done on PCR-negative BAL samples. The inhibitors were detected only in one serum sample in which PCR remained negative even after spiking with pneumococcal DNA. Inhibitors were not detected in BAL samples. Also, after dilution of serum samples with pooling all PCR results in serum samples remained negative. BAL PCR provided 2 additional diagnoses of *S. pneumoniae*, despite the fact that all patients who underwent bronchoscopy have received antimicrobial treatment before procedure. These results are in agreement with previous reports[33,34]. Despite 2 additional diagnoses of pneumococcal pneumonia with BAL PCR, BAL culture and BAL PCR have shown overall a low diagnostic yield with positive results in only 26.3% (5/19) and 15.8% (3/19) of patients who underwent bronchoscopy, respectively. These results are not surprising knowing that in our study BAL was not performed immediately on admission, but when it was considered clinically indicated due to diagnostic or therapeutic difficulties. In studies in which BAL was performed within 24 hours of admission and before administration of antimicrobial treatment, yield of BAL culture and PCR was higher[34,35]. However, our and similar studies are showing much better the value of BAL in patients with CAP in routine clinical situations[36].

Although PCR added 1 additional case diagnosed as *M. pneumoniae*, majority of cases were diagnosed by serology. In the present study, the results by the 2 approaches – PCR on samples from respiratory tract and serology were discordant, which was also the case in previous studies[9]. Positive PCR alone without an accompanying serological response can indicate carrier status, but can also be due to a deficient immune response, a condition that is common in elderly people. Dorigo-Zetsma et al. reported that patients with positive PCR and negative serology were significantly older than the patients with positive *M. pneumoniae* serology[37]. Indeed, in our study the patient with positive PCR for *M. pneumoniae* had negative serology and was 80 years old. The other way round, negative PCR results in patients with otherwise serologically confirmed mycoplasma respiratory tract disease could be explained by early disappearance of *M. pneumoniae* due to antimicrobial therapy or immune response of the host[9]. Although PCR added only 1 additional case of *M. pneumoniae* pneumonia, it could be useful in patients with deficient immune response that is common in elderly people and immunocompromised patients. All 4 cases of *C. pneumoniae* were diagnosed by serology. The low yield of PCR for *M. pneumoniae* and *C. pneumoniae* is also reported by other investigators and is suggesting that more reliable rapid tests for these atypical pathogens are necessary[23]. Both cases of *L. pneumophila* serogroup 1 infection were detected by urinary antigen test. In these 2 patients PCR on sputum samples was not performed because they could not produce sputum sample. It is a well-known fact that less than one-half of patients with *Legionella* infection can produce sputum[11]. In our study PCR on sputum and BAL samples did not provide an additional diagnosis of *Legionella* infection. It might be useful to evaluate it on different type of clinical samples, as it has recently been suggested for serum samples[38].

In recent studies CRP is again evaluated as a diagnostic tool in patients with CAP. In these studies authors are occupied with a thought that CRP, as an early marker, could be useful in predicting the aetiology of CAP and the initial selection of an adequate empiric antimicrobial treatment[15–19]. Some of these studies are actually indicating that serum CRP values could be useful in selecting empiric antimicrobial treatment. Almirall et al. concluded that high plasma levels of CRP are more common when the pathogens are *S. pneumoniae* and *L. pneumophila* or when the illness is more severe[39]. Peters and al. found positive correlation between *S. pneumoniae* DNA load in blood with CRP levels in patients with CAP[40]. On the other hand, von Baum et al. found that patients with *Mycoplasma pneumonae* pneumonia show a lower inflammatory response in terms of CRP values[41]. In our study CRP values in patients with pneumonia caused by *S. pneumoniae* were not significantly higher than in patients with other proven aetiological diagnoses. This observation was also reported by other authors[13]. In our study, the highest CRP values were detected in 2 patients with *Legionella* infection. Although this number of cases was too small to be included in statistical analysis, this data are in agreement with previous studies in which the mean CRP values in the *L. pneumophila* group were significantly higher than those in the group with other diagnoses, and the authors raised the question of whether *L. pneumophila* triggers more (or different) inflammatory pathways than other atypical microrganisms[39,42]. In our study the lowest CRP values were found in patients with *M. pneumoniae* infection. This finding is also in agreement with other similar studies, but our number of cases was too small to be included in statistical analysis[41]. Cals et al. found differences in CRP values between bacteriological and virological causative agents of CAP and also that CRP assisted in prescribing decisions. As we focused on bacterial CAP, there is no information about the presence of virological agents in the respiratory samples of our patients and their relation to CRP values[43].

Although there are studies showing that CRP could be a useful tool in predicting the aetiology of CAP and the initial selection of an adequate empirical antimicrobial treatment, the systematic review by van der Meer et al. is showing quite opposite. They concluded that testing for CRP is neither sufficiently sensitive to rule out nor sufficiently specific to rule in bacterial from viral aetiology of lower respiratory tract infection[44]. Although we are aware of limitations of our study with regard to small number of patients with CRP measurements, our results are in agreement with van der Meer et al. and their statement that the current evidence does not support a wide introduction of CRP as a rapid test to guide antimicrobial treatment[44].

Thirty percent of the patients included in the study received antimicrobial treatment before admission to the hospital. Although we are aware that previous antimicrobial treatment changes reliability of microbiological methods and CRP measurements, this fact is not di-

minishing the results of our study. Even more, it allows us to show the yield of these methods not in a research-oriented setting, but in routine clinical setting where patients are often treated prior to hospitalization.

Although there was heterogeneity in antimicrobial regimens, the most widely used antibimicrobial regimen was beta-lactam monotherapy. In this study, the antimicrobial agents that were empirically selected were inappropriately administered to 23.8% out of 42 patients with determined aetiology. In a similar study, Ewig et al. reported that among 19 adult patients with CAP with established aetiological agent, 21.0% (4/19) cases would have been treated with an inappropriate regimen without diagnostic results[20]. Higher percent of inappropriately treated patients in our study could be explained by extensive microbiological work-up, including PCR tests, which helped to establish the aetiological diagnosis in a larger number of patients than in a previous study.

To our knowledge little is known about consequences of both appropriate and inappropriate antimicrobial treatment on clinical outcome. There are only 2 existing studies referring to this problem so far. The first is the one by Nakayama et al. in which the relation between the empirically selected antimicrobial agent and causative pathogen for 117 cases of CAP was examined. Antimicrobials were inappropriately used in 11 (9.4%) cases, but no patient experienced a relapse or was refractory to the treatment[45]. The second study is one by van der Eerden et al. already mentioned in our paper. In that study there was no significant difference in length of stay (LOS), 30 day mortality, clinical failure, or resolution of fever between patients who received pathogen directed treatment and those who received empirical broad spectrum antimicrobial treatment[21].

Interesting similarity between our and study by Nakayama et al. is that predominant pathogens in inappropriately treated patient were atypical ones: in our study *M. pneumoniae, C. pneumoniae and L. pneumophila* serogoup 1, and in the study by Nakayama et al. viruses and *M. pneumoniae*. If in this inappropriately treated patients outcome is not different, as Nakayama et al. showed, an ancient question is raised again: what is, if any, a role of atypical pathogens in CAP and what is their position in pathogenesis in relation to *S. pneumoniae*[45]?

In our study the most original result displayed is the percent of patients that receive inappropriate empirical antimicrobial treatment based on aetiological tests which also included molecular methods. However, the real value of molecular methods and their influence on treatment outcome is yet to be established. Clinically relevant outcome parameters such as mortality, LOS or readmission rate should be evaluated to measure cost-benefit of these methods and their effect on the treatment outcome[22,46,47].

Predominant pathogens in inappropriately treated patients were atypical pathogens *M. pneumoniae, C. pneumoniae* and *L. pneumophila* serogroup 1. This finding further emphasize two existing problems: first, a dilemma about necessity of empirical antimicrobial coverage of atypical pathogens in patients with CAP, and the second, a need for more reliable rapid tests for the detection of atypical pathogens[25,48–50].

In conclusion, our study indicates that *S. pneumoniae* is the most common cause of CAP in adult patients. Pneumococcal urinary antigen is the most useful test, but PCR on BAL samples can also provide an additional diagnosis of pneumococcal pneumonia, even in patients with previously administered antimicrobial treatment. As 23.8% of patients were inappropriately treated according to aetiological diagnosis, microbiological test in patients with CAP are still necessary. Particularly PCR tests for the diagnostics of *M. pneumoniae, C. pneumoniae* and *Legionella* species need further investigation and improvement because these causative pathogens are predominant in inappropriately treated patients. Our results do not support the use of CRP as a rapid test to guide antimicrobial treatment.

Until rapid methods are improved, initial decisions about empirical antimicrobial treatment will still be based on the knowledge of predominant microbial patterns in CAP on particular geographical area. It is therefore important that teaching hospitals conduct periodic surveys and determine their microbial patterns prevalent in their hospitalized patients with CAP.

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ZNAČENJE BRZE ETIOLOŠKE DIJAGNOZE U OPTIMIZACIJI ANTIMIKROBNOG LIJEČENJA BOLESNIKA S IZVANBOLNIČKOM PNEUMONIJOM UZROKOVANOM BAKTERIJAMA

S A Ž E T A K

Kod 80 bolesnika s izvanbolničkom pneumonijom napravljene su konvencionalne mikrobiološke pretrage i lančana reakcija polimeraze (PCR), te određen C-reaktivni protein (CRP) u serumu. Adekvatnost empirijskog antimikrobnog liječenja evaluirana je prema detektiranom bakterijskom patogenu. Etiologija je utvrđena kod 42 (52,5%) bolesnika, a najčešći patogen bio je *Streptococcus pneumoniae.* PCR-om na uzorcima bronhoalveolarnih lavata i sputuma utvrđena je etiološka dijagnoza kod 2 odnosno 1 bolesnika kod kojih to nije bilo moguće s konvencionalnim mikrobiološkim metodama. Prosječne CRP vrijednosti kod bolesnika sa *S. pneumoniae* nisu bile statistički značajno veće u usporedbi s onima kod bolesnika s drugom etiologijom pneumonije (166,89 mg/L *vs.*160,11 mg/L, p=0,457). Kod 23.8% (10/42) bolesnika s utvrđenom etiologijom empirijsko antimikrobno liječenje bilo je nedekvatno. PCR metode u dijagnostici izvanbolničkih pneumonija zahtijevaju daljnje istraživanje. To se osobito odnosi na PCR metode za detekciju atipičnih patogena budući da oni prevladavaju kod neadekvatno liječenih bolesnika. Rezultati ovog istraživanja ne pokazuju da bi CRP bio koristan kao brzi test prema kojem bi se usmjeravalo antimikrobno liječenje kod bolesnika s izvanbolničkom pneumonijom.