

Clinical Utility of Multiplex PCR for Managing Community-Acquired Pneumonia in Diabetic and Non-Diabetic Patients

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ABSTRACT

Objective: To compare the positivity rates of pathogens detected in diabetic and non-diabetic pneumonia cases, to assess the role of multiplex respiratory polymerase chain reaction (PCR) testing in diagnosis, and to compare inflammatory parameters between the two groups.

Study Design: Observational study.

Place and Duration of the Study: Department of Pulmonology, Hisar Intercontinental Hospital, Istanbul, Turkiye, from September 2021 to June 2023.

Methodology: The study included 151 adults diagnosed with community-acquired pneumonia (CAP). Group 1 comprised patients with diabetes (n = 42), and Group 2 consisted of patients without diabetes (n = 109). Patients' demographic and clinical data were collected and statistically compared using the Mann-Whitney U test, the median test, and the Chi-square Independence test.

Results: The positivity rate of the multiplex respiratory PCR test was significantly greater in Group 1 compared with Group 2 (p = 0.005). In Group 1, the most frequently identified nasopharyngeal pathogen was SARS-CoV-2. In Group 2, *Streptococcus pneumoniae* was the predominant pathogen. Procalcitonin (PCT), C-reactive protein (CRP), and neutrophil levels, as well as the CRP-to-lymphocyte ratio (CLR), were markedly higher in Group 1 compared with Group 2 (p = 0.016, p = 0.002, p = 0.016, and p = 0.016, respectively).

Conclusion: A significantly higher PCR test positivity rate was found in diabetic patients compared with non-diabetic. Differences in the distribution of specific pathogens between the groups were observed. In addition, this study confirmed that diabetic pneumonia cases exhibit elevated levels of inflammatory biomarkers.

Key Words: Pneumonia, Diabetes, Multiplex respiratory polymerase chain reaction test.

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INTRODUCTION

Globally, community-acquired pneumonia (CAP) is the leading cause of infectious hospitalisation and mortality,¹ with its incidence being higher among the very young (under 5 years) and the very old (over 70 years).² Indeed, pneumonia is one of the main causes of death among the elderly. Moreover, in the adult population, the presence of accompanying chronic diseases –such as diabetes mellitus (DM), chronic heart failure, dementia, and ischaemic cardiomyopathy– increases the risk of CAP.³

The most frequent aetiological agent, appearing in approximately two-thirds of cases, is *Streptococcus pneumoniae*. However, because numerous microbial agents can cause CAP, empiric antimicrobial therapy is often initiated while awaiting diagnostic results. Ultimately, identifying the causative agent remains essential for targeted treatment.⁴

DM is a chronic metabolic disorder that contributes to high morbidity and mortality in multiple populations, especially in low- and middle-income countries.⁵ Diabetic patients have weakened defences, stressed islet B cells, and impaired immune function, which collectively contribute to their poor prognosis in various diseases.⁶

Prompt CAP diagnosis and its treatment are vital for reducing morbidity and mortality rate; delayed antibiotics worsen a patient's prognosis.⁷ In cases where a viral pathogen is identified, specific antiviral therapy can be initiated to improve the prognosis. Conventional culture methods for CAP are slow; however, advanced molecular diagnostics offer improved sensitivity and specificity, especially for hard-to-culture

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organisms such as *Mycoplasma pneumoniae*. Multiplex respiratory polymerase chain reaction (PCR) tests offer several benefits. Specifically, they provide a rapid and accurate method to identify multiple agents, including viral and bacterial pathogens, in a single test, and the results are unaffected by prior antibiotic use.^{8,9} This study aimed to investigate the efficacy of multiplex respiratory PCR testing for diagnosis by comparing the positivity rates of pathogens detected in pneumonia cases among diabetic and non-diabetic patients, as well as comparing inflammatory parameters between the two groups.

METHODOLOGY

This single-centre, comparative study was conducted using data obtained from the files of patients treated in the Department of Pulmonology, Hisar Intercontinental Hospital, Istanbul, Turkiye, from September 2021 to June 2023, under the supervision of the archive manager. Ethical committee approval was obtained, and patient confidentiality was ensured by securing all the information as per regulations.

In this study, a population of 190 patients over the age of 18 years were assessed for suitability; they had been diagnosed with CAP via a multiplex respiratory PCR test. Patients with an infection other than pneumonia, as well as those diagnosed with neoplasm, those transferred to the critical care unit, and pregnant patients were excluded. Following the application of the exclusion criteria, 151 patients were included in the study. The inclusion criteria were as follows: adult patients with pneumonia confirmed by clinical symptoms and chest radiography, and Type II DM diagnosed using standard criteria, including fasting glucose ≥ 126 mg/dL, HbA1c $\geq 6.5\%$, or glucose ≥ 200 mg/dL in the presence of symptoms. Only patients with confirmed Type II DM were included in the study. Type I DM cases were excluded based on clinical history (early onset, insulin dependence), C-peptide levels, and other autoimmune laboratory markers. Forty-two patients (27.8%) had Type II DM (Group 1), and 109 (72.2%) did not (Group 2). Given the study's cross-sectional design, the difference in group sizes can be explained by a naturally lower number of diabetic cases. The inclusion of more than 30 cases in Group 1 ($n = 42$) enabled controlled statistical testing. The clinical and demographic data collected included gender, age, PCR results, C-reactive protein (CRP), procalcitonin (PCT), white blood cell (WBCs), neutrophil, lymphocyte, neutrophil-to-lymphocyte ratio (NLR), and CRP-to-lymphocyte ratio (CLR) levels. It was determined that the two patient groups were similar in terms of age, comorbidity status, and the use of corticosteroids and antibiotics. Pneumonia was defined as acute fever, purulent sputum, elevated leucocytes, a decline in oxygenation, and new lung infiltrate of infectious origin seen on chest radiography.^{8,10}

Venous blood was collected for complete blood count (CBC) and biochemical analyses. Test measurements were performed using a Sysmex XN-1000 (blood count / flow cytometry) and Architect 4100 analysers (CRP/turbidimetry, PCT/immunoassay).

Reverse transcription PCR (RT-PCR; Thermo Fisher/Quant Studio-5) was performed using nasopharyngeal swabs. The multiplex PCR panel included *Influenza A/B*, *Respiratory syncytial virus (RSV)*, *Parainfluenza 1-4*, *SARS-CoV-2*, *Rhinovirus*, *Enterovirus*, *Adenovirus*, *Metapneumovirus*, *Bocavirus*, *Streptococcus pneumoniae*, *Bordetella pertussis*, *Legionella pneumophila*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*.

The statistical analysis was conducted using SPSS version 25.0 (Chicago, IL, USA). Normality was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Differences in laboratory data between patient groups were analysed using the Mann-Whitney U and the median tests. Data were expressed as median and inter-quartile range (IQR). Confidence intervals and p-values were calculated.

The Chi-square asymptotic significance test and Fisher's exact significance test were used to analyse the differences in PCR positivity rates between the groups. In this study, pathogens with frequencies less than five that did not fit the asymptotic distribution were evaluated using Fisher's exact test. The differences were regarded as statistically significant if the p-value was below 0.05.

RESULTS

A total of 151 patients diagnosed with CAP through PCR tests on admission were included. Table I presents the characteristics of the study groups.

Table II shows a statistically significant difference in PCR test positivity between the two groups. Twenty-five out of 42 patients with diabetes tested positive, and 39 out of 109 patients without diabetes tested positive. The difference was statistically significant ($p = 0.005$, Figure 1).

In Group 1, most identified pathogens were SARS-CoV-2 and *Streptococcus pneumoniae*. SARS-CoV-2 was detected significantly more in Group 1 than in Group 2. In Group 2, *Streptococcus pneumoniae* was the most dominant agent. None of the samples tested were positive for *Haemophilus influenzae*, *Metapneumovirus*, or *Parainfluenza virus* in Group 1. In contrast, in Group 2, *Metapneumovirus*, *Haemophilus influenzae*, and *Parainfluenza virus* were found in two cases, one case, and one case, respectively (Figure 2).

Table I: Demographic profiles of the groups.

Variables	Group 1 (n = 42)	Group 2 (n = 109)	p-values
Gender: female, n (%)	11 (26.82%)	41 (37.61%)	0.186*
Age (years), median (IQR)	62 (51-73)	51 (43-66)	0.002**

Group 1: Diabetic pneumonia; Group 2: Non-diabetic pneumonia. *Chi-square test; **Median test.

Table II: Positive rates of respiratory pathogens in multiplex PCR groups.

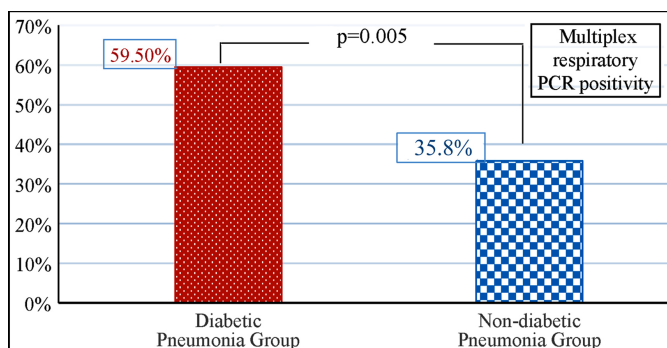
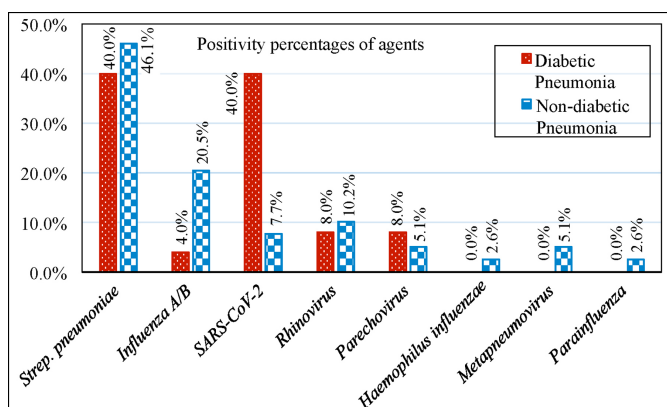
Pathogens	Group 1 (n = 42)	Group 2 (n = 109)	p-values
Test positivity n (%)	25 (59.5)	39 (35.8)	0.005
<i>Streptococcus pneumoniae</i> , n (%)	10 (40)	18 (46.1)	0.599
SARS-CoV-2, n (%)	10 (40)	3 (7.7)	0.002
<i>Influenzae A/B</i> , n (%)	1 (4)	8 (20.5)	0.173
<i>Rhinovirus</i> , n (%)	2 (8)	4 (10.2)	0.750
<i>Parachovirus</i> , n (%)	2 (8)	2 (5.1)	0.654
<i>Metapneumovirus</i> , n (%)	-	2 (5.1)	
<i>Haemophilus influenzae</i> , n (%)	-	1 (2.6)	
<i>Parainfluenza virus</i> , n (%)	-	1 (2.6)	

Group 1: Diabetic pneumonia; Group 2: Non-diabetic pneumonia. Chi-square test; Significance of the difference between the PCR positivity percentages of the groups.

Table III: The results of the laboratory findings of the groups.

Variables	Group 1		Group 2		95% CI [LL- UL]	p-values
	Median	(IQR)	Median	(IQR)		
PCT (ng/mL)	0.06	(0.03-0.18)	0.04	(0.03-0.08)	-0.15-1.42	0.016
CRP (mg/L)	64.5	(11.6-120.7)	13.9	(5.89-57.77)	15.3-59.1	0.002
WBC ($10^3/\mu\text{L}$)	8.20	(6.18-11.47)	7.51	(5.79-9.78)	-0.30-2.12	0.180
Neutrophil ($10^3/\mu\text{L}$)	7.07	(5.08-9.96)	4.86	(3.44-6.87)	0.97-3.51	0.016
Lymphocyte ($10^3/\mu\text{L}$)	1.14	(0.70-1.68)	1.43	(0.82-1.87)	-0.49-0.03	0.138
CLR	49.8	(11.17-134.2)	15.2	(3.35-42.25)	22.8-95.2	0.016
NLR	6.13	(3.21-13.19)	3.62	(2.17-7.07)	2.19-8.71	0.186

Data are expressed as median (IQR). The median test was used in statistical analysis. CI [LL- UL]: Confidence interval [Lower limit - Upper limit]; PCT: Procalcitonin; CRP: C-reactive protein; WBC: White blood cell; CLR: CRP/lymphocyte ratio; NLR: Neutrophil lymphocyte ratio.

**Figure 1: Positive multiplex PCR test rates in study groups.****Figure 2: Positivity percentages of viral and bacterial agents in PCR tests.**

As demonstrated in Table III, PCT and CRP at entry level were higher in Group 1, and the result was statistically significant ($p = 0.016$, $p = 0.002$, respectively).

In addition, Group 1 had higher neutrophil and CLR levels compared with Group 2 ($p = 0.016$ and $p = 0.016$, respectively). However, although the median values of NLR were higher in Group 1 than that of in Group 2, there was no statistically significant difference between the results ($p = 0.186$).

DISCUSSION

This research involved a comparative analysis of CAP patients with and without diabetes. It highlighted the value of multiplex respiratory PCR testing in detecting causative agents and compared inflammatory parameters in the two groups. Consistent with the previous literature, PCR testing in diabetic patients proved to be beneficial and time-saving; SARS-CoV-2 was identified as the most frequent pathogen.

Despite advances in diabetes prevention and treatment, diabetes remains a global health burden, increasing mortality rates. Hyperglycaemia, a condition that commonly affects people with diabetes, weakens immunity and increases their infection risk by impairing neutrophils, cellular immunity, and complement.^{5,6}

Hellenthal *et al.* reported that pneumonia triggers systemic inflammation, affecting vascular permeability and diabetes.¹¹ Timely diagnosis, pathogen identification, and treatment are key to reducing CAP morbidity and mortality. CAP can be caused by various pathogens, including bacteria, viruses, fungi, and atypical organisms, making diagnosis challenging for clinicians. Indeed, the causative microorganism cannot be determined in almost half of the cases of pneumonia.³

In recent years, multiplex respiratory PCR testing has gained wide acceptance in patients with CAP as a more sensitive and rapid diagnostic method compared with traditional cultures.¹² Therefore, this method was employed in the present research. The PCR results revealed higher positivity rates for *Streptococcus pneumoniae*, followed by SARS-CoV-2, Influenza A/B, Rhinovirus, and Parvovirus in pneumoniae cases. Although *Streptococcus pneumoniae* was detected first among non-diabetic *pneumoniae* patients, *Streptococcus pneumoniae* and SARS-CoV-2 were most prevalent in diabetic patients.

Thomsen *et al.* showed that DM is a leading cause of *Streptococcus pneumoniae* infection and *pneumococcal bacteraemia*. In addition, they found that *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and influenza virus are more prevalent in diabetic patients.¹³

The SARS-CoV-2 pandemic in 2019-2020 changed lives, with preventive measures such as masking and handwashing affecting respiratory pathogens. Previous research has shown that individuals with diabetes are more susceptible to COVID-19.¹⁴ Abdi *et al.* reviewed 18 trials and reported that diabetic patients are more likely to develop COVID-19.¹⁵ The present study showed *Streptococcus pneumoniae* as the main bacterial agent, while SARS-CoV-2 as the most frequent viral pathogen in pneumonia cases.

CRP, PCT, and NLR are key biomarkers of pulmonary infections. NLR also predicts CAP severity and prognosis more accurately than standard markers do.¹⁶ Huang *et al.* studied

80 CAP patients and found elevated NLR levels, showing the superior diagnostic value and reliability of this biomarker for CAP.¹⁷ Zhang *et al.* found that PCT, CLR, CRP, and NLR were elevated in intensive care unit patients with bacteraemia, concluding that these markers could predict bacteraemia.¹⁸ In the present study, patients with CAP and diabetes exhibited increased CRP, PCT, neutrophil, and CLR levels, consistent with the previous findings. As mentioned above, CRP and PCT may also be affected by non-infectious diseases, including diabetes, and it has been speculated that CAP patients with diabetes exhibit higher levels of inflammatory biomarkers compared to those without diabetes.

This study had several limitations. First, its retrospective design, relied on medical records, limited the ability to compare findings with bacterial culture results. Second, the COVID-19 pandemic may have led to the underdetection of other viruses. Third, additional risk factors, such as chronic lung disease or asthma, were not assessed. Moreover, variables such as sample collection, sample suitability, disease stage, and pre-analytical conditions should be carefully considered to ensure the accuracy of PCR test results, as they can significantly affect the reliability of the test results, and, consequently, clinical decision-making.

CONCLUSION

The findings demonstrated a markedly higher PCR test positivity rate in diabetic patients compared to non-diabetic patients. Moreover, the type of pathogens detected are different between the two groups, and diabetic patients exhibited higher inflammatory biomarker levels compared to non-diabetic patients.

ETHICAL APPROVAL:

Ethics approval was obtained from the Hospital's Ethics Committee of Hisar Intercontinental Hospital, Istanbul, Türkiye (No: 23-7/42, July 21, 2023).

PATIENTS' CONSENT:

Informed consent was obtained from all the patients before the procedure.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

OD, BSU: Conception and design of the study, interpretation of the data, and drafting of the manuscript.

SA, SG: Interpretation of the data and drafting of the manuscript.

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