ORIGINAL PAPER
Polish Journal of Microbiology
2023, Vol. 72, No 4, 391–398
https://doi.org/10.33073/pjm-2023-035

Evaluation of the BioFire® FilmArray® Pneumonia *plus* Panel for Detecting Bacterial Etiological Agents of Lower Respiratory Tract Infections in an Oncologic Hospital. Comparison with Conventional Culture Method

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Submitted 26 May 2023, accepted 20 August 2023, published online 10 October 2023

Abstract

Conventional methods used to determine pneumonia pathogens are characterized by low sensitivity and long turnaround times. Introducing new tests with better parameters in patients at higher risk of infections is highly anticipated. The results of the conventional quantitative culture method (CM) in determining the bacterial etiology of pneumonia were compared with the results of the Pneumonia *plus* Panel test (PNP; BioFire® Diagnostics, USA) in 79 samples of bronchoalveolar lavage (BAL). Materials were collected from 79 patients with suspected pneumonia treated in an oncologic hospital due to solid tumors. Only 16/79 BAL samples (20.3%) were true positive (TP) for bacterial etiology in CM vs. 27/79 samples (34.2%) true positive in the PNP test. The total agreement between methods of interpreting the result (positive or negative) was 84.8%. The most prevalent pathogens in both methods were *Staphylococcus aureus*, followed by *Escherichia coli*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. The PNP test identified several respiratory pathogens that were not grown in culture. The semiquantitative value reported by the PNP test was higher than that reported by culture. The PNP test vs. combined test (PNP test and CM methods) demonstrated positive predictive value (PPV) and negative predictive value (NPV) values of 100.0% and 98.1%, and the sensitivity and specificity were 96.4% and 100.0%. The PNP test is a good tool for determining the etiology of bacterial pneumonia and may support the care of an oncologic patient. However, further large-sample studies are needed to research in strictly defined groups of oncologic patients.

K e y w o r d s: BioFire® FilmArray® Pneumonia plus Panel, pneumonia, methods comparison

Introduction

Cancer-related pneumonia is a severe disease with a poor outcome. The course of the disease is usually severe and characterized by high mortality, reaching 35.0% (ICNARC 2017). Many risk factors associated with cancer predispose patients to develop pneumonia. The most common is the disruption of anatomical surfaces, swallowing function, obstruction caused by tumor progression, neutropenia, lymphopenia, and impaired cellular and humoral immunity (Rolston 2001; 2017). In addition, frequent hospitalizations, typical for oncologic patients, increase the exposure of patients to nosocomial pathogens (Wong and Ewans 2017). Diagnosis of pneumonia based on clinical symptoms, which may be poorly expressed in oncologic patients, may be difficult (Wong and Ewans 2017). Therefore, well-documented risk factors for mortality in pneumonia, such as an unidentified etiology (Azoulay et al. 2017), a delay in antibiotic administration in patients with severe disease (Nauclér et al. 2021), and the inappropriate antimicrobial empiric therapy (Fernández-Cruz et al. 2020) are of particular importance among these patients. Pneumonia can be caused by many pathogens, making empiric antibiotic therapy difficult (Leoni et al. 2016; Conway 2018). The type of pathogen depends on the type and stage of the cancer and the type of immunological deficits (Rolston 2001). Typical bacteria, such as Gram-negative bacilli, are a frequent pathogen of these infections (Ren et al. 2021). Infections caused by Gram-positive bacteria (except *Streptococcus pneumoniae* and *Staphylococcus aureus*), *Mycobacterium*

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tuberculosis, atypical bacteria, viruses, and fungi are diagnosed less frequently (Rolston 2001; Parody et al. 2007; Han et al. 2015; Tabatabai et al. 2022). It is estimated that up to 60% of these infections remain undiagnosed in terms of the pathogen (Jain et al. 2015; Roquilly et al. 2019). In addition, pneumonia, regardless of the pathogen, can cause similar symptoms, but depending on the group of microorganisms causing them, requires different treatment (Watkins 2022). The conventional culture method is among the recommended diagnostic methods for pneumonia caused by typical bacteria (Kumar et al. 2017). However, this method has a number of limitations, caused by, for example, the difficulty in culturing the pathogens and the time to obtain results, leading to unsatisfactory treatment results (Torres et al. 2016). Therefore, introducing new tests with better parameters, such as the ability to detect a wide range of microorganisms and short turnaround times (TAT), is highly anticipated. Molecular tests for microbiological diagnosis of pneumonia may play such a role. In particular, the use of the multiplex PCR (polymerase chain reaction) method, which enables the simultaneous detection and identification of nucleic acids of many bacteria and antibiotic resistance genes in tested samples, can improve diagnosis and enable earlier treatment of these infections, as well as reduce the use of broad-spectrum antibiotics, which are used when the pathogen is unknown. Although many studies have assessed the usefulness of multiplex tests in diagnosing pneumonia, publications on the usefulness of the test in groups of oncologic patients with solid tumors are scarce.

The aim of this study was to compare the results of the conventional quantitative culture method (CM) in determining the bacterial etiology of pneumonia and detecting the underlying mechanisms of microbial resistance with the results of the Pneumonia *plus* Panel test (PNP; BioFire® Diagnostics, USA) among oncologic patients with solid tumors.

Experimental

Materials and Methods

Clinical specimens. Seventy-nine specimens of bronchoalveolar lavage (BAL) collected from patients in whom pneumonia was suspected: outpatients (n = 25) and patients hospitalized in the intensive care unit (n = 25), and other hospital wards (n = 29) of the Prof. F. Łukaszczyk Oncology Centre in Bydgoszcz were collected in the period from June 2020 to February 2023. The samples were tested in parallel using the CM method and the PNP test. The results of the CM method were analyzed independently of the results obtained from the panels. Diagnostic thresholds for the CM method were adopted following the applicable recommendations, 10^4–10^5 CFU/ml for BAL (Hryniewicz et al. 2016). In addition, at the request of the clinician, 69 out of the 79 samples constituted material for testing for yeast-like fungi and mold fungi.

Pneumonia *plus* Panel (PNP; BioFire® Diagnostics, USA). The principle of the method used is a quantitative PCR with a melting curve analysis based on the intercalation properties of the LCGreen®Plus (BioFire Defense, USA) dye into double-stranded and heteroduplex DNA. The PNP test simultaneously detects 26 microorganisms (18 bacteria and eight viruses) and seven antimicrobial resistance genes. The presence of the 15 typical bacteria is reported semi-quantitatively with intervals of 10/4, 10/5, 10/6, or ≥10/7 genomic copies per ml (copies/ml) of the sample. The presence of atypical bacteria, viruses, and antibiotic-resistance genes was determined qualitatively. The microorganisms and resistance genes detected by the PNP test are shown in the Table SI.

Standard microbiological culture, strains identification, antibiotic susceptibility testing, mechanisms of resistance. The test material after prior processing was inoculated quantitatively using calibrated loops with a volume of 0.01 ml (10^{-2}) and 0.001 ml (10^{-3}) on Columbia Agar with Sheep Blood PLUS (Thermo Scientific™, Oxoid™ Deutschland GmbH, Germany), and Haemophilus Selective Agar (Thermo Scientific™, Oxoid Deutschland GmbH, Germany), and reductively on MacConkey Agar No. 3 (Thermo Scientific™, Oxoid™ Deutschland GmbH, Germany), and Cetrimide Agar (Thermo Scientific™, Oxoid™ Deutschland GmbH, Germany). In addition, a direct Gram stain was made. The plates were incubated, respectively, in an atmosphere of 5% CO_2 and aerobic conditions, maintaining the temperature at 35 ± 2°C.

After an incubation period of 16–24 hours (or incubation extended to 48 hours, in the case of a negative result after the first day of incubation), identification was carried out using mass spectrometry (matrix-assisted laser desorption/ionization-time of flight mass spectrometry) (IVD MALDI Biotyper Smart System, microflex® LT/SH smart, Bruker Daltonics GmbH & Co., Germany). At the request of the clinician, quantitative inoculation for fungi was also performed on Sabouraud Glucose Selective Agar with Gentamicin and Chloramphenicol (Thermo Scientific™, Oxoid™ Deutschland GmbH, Germany), and on Brilliance™ Candida Agar (Thermo Scientific™, Oxoid™ Deutschland GmbH, Germany). Incubation was carried out, respectively: up to 7 days, on the first day at a temperature of 35 ± 2°C, on days 2–7 at a temperature of 25 ± 2°C, and up to 3 days at a temperature of 30 ± 2°C. Colonies that grew were identified by mass spectrometry. The results obtained were presented as a number of CFU/ml (CFU – colony-forming units).

Antibiotic susceptibility was assessed using the Kirby-Bauer disc diffusion method on Mueller-Hinton E agar and Mueller-Hinton agar with 5% horse blood + 20 mg/l β-NAD (bioMérieux, France), as well as the automatic nephelometric method (BD Phoenix (BD, Sparks, MD), or additionally using antibiotic strips in a concentration gradient – ETEST® (bioMérieux, France), following the recommendations of European Committee on Antimicrobial Susceptibility Testing (EUCAST 2023a; 2023b).

Data analysis. Consistency in the interpretation of the results of the detection of bacterial etiology of pneumonia by the PNP test (reference method) and the CM method was assessed. If only viral pathogens were detected by the PNP assay, the samples were categorized as "bacteriologically negative" for the analysis, and failure to culture bacterial pathogens by the culture method was considered consistent with the PNP result. Results were considered positive in the PNP assay when at least one target microorganism was detected (at $\geq 10^4$ copies/ml for semi-quantitative bacteria). In the comparative analysis of the results of the PNP test with the results of the CM in determining the etiology of pneumonia and detecting the basic mechanisms of microbial resistance, use was made of the parameters assessed in the verification and validation processes of the research method and laboratory tests: accuracy (%), sensitivity (%), specificity (%), positive predictive value (PPV) (%) and negative predictive value (NPV) (%) (PDA 2000; Clark et al. 2009; Murphy et al. 2015). The numbers of True Positive and True Negative results (TP; TN), and the numbers of False Positive and False Negative results (FP; FN) were used to determine the indicated parameters of the PNP test.

Statistical analysis was performed using Statistica software version 13.3 (TIBCO® Software Inc., USA). Specificity, Sensitivity, Accuracy, PPV, and NPV were evaluated using the supplement "Zestaw Plus" ver. 5.0.80 (StatSoft Polska Sp. z o.o., Poland). Relationships between diagnostic methods were assessed using R-Spearman correlation analysis. In all statistical analyses, the cut-off value for the probability ratio was set at $p \leq 0.05$.

Results

Analysis of detected typical bacteria and resistance mechanism to antibiotics. In the case of 32 out of 79 samples (40.5%) of the BAL, the result of the PNP test was "positive", including three samples with both bacterial and viral pathogens detected. In five out of the 32 samples (15.6%), only viral pathogens were detected by the PNP test, meaning that these materials were negative for bacterial agents. Thus, 27 bacteriological positive and 51 negative results were considered in the reference method. Bacteriological results specified by the pathogen, including 27 positive BAL according to the criteria, are presented in Table I. The PNP test detected 42 bacteria vs. 36 bacteria isolated in the CM method, although only 21 out of 36 bacteria (58.3%) were cultured equal to or above the diagnostic threshold. The most frequently detected bacteria in both methods, if the quantitative criterion for individual bacteria in the CM method was excluded from the analysis, was *S. aureus*, followed by *Escherichia coli*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. Most pathogens that did not meet the quantitative criterion (11 out of 15) were quantified at 10^3–10^4 CFU/ml. In two samples considered TP in the PNP test, no growth of *Serratia marcescens* and *Enterobacter cloacae complex* was obtained. These samples contained two or more pathogens. Moreover, in five samples, with the PNP test confirming the presence of genetic material (in genomic copies/ml), respectively: *E. coli* 10^6, *Moraxella (Branhamella) catarrhalis* 10^5, *S. aureus* 10^4, *P. aeruginosa* 10^4, and *S. pneumoniae* 10^5, the culture results were negative. Overall, using quantitative criteria in the CM method showed a concordance between methods in 16 of 27 positive results. These data demonstrated a 69.0% increase positive BAL samples. Furthermore, the culture analysis in one case showed the growth of *P. aeruginosa* on the border of the diagnostic threshold, but the genetic material of *P. aeruginosa* was not detected in the PNP assay.

A false positive culture result was obtained in one sample containing *S. aureus* and *Klebsiella pneumoniae*. Other pathogens detected in the CM were excluded from further analysis because they were not included in the panel. They were α-hemolytic streptococci (n = 15), *Haemophilus parainfluenzae* (n = 5), *Haemophilus haemolyticus* (n = 3), *Aggregatibacter segnis* (n = 2), *Neisseria subflava* (n = 1), *Rothia mucilaginosa* (n = 1), *Staphylococcus epidermidis* (n = 1), *Streptococcus pseudopneumoniae* (n = 1), and *Hafnia alvei* (n = 1). However, most of them are not considered respiratory pathogens.

In the phenotypic method, resistance to carbapenems was detected in one of the four *P. aeruginosa* strains, not due to the production of carbapenemases. In one *E. coli* strain, the production of extended-spectrum β-lactamases (ESBLs) was confirmed. The PNP test detects six genes associated with the production of carbapenemases and ESBLs in selected Gram-negative bacilli. The panel detected the presence of the gene responsible to produce CTX-M-type ESBLs in *E. coli*. However, but the production of KPC, VIM, IMP, and NDM carbapenemases was not confirmed in *P. aeruginosa*, which was consistent with the phenotypic tests. No methicillin resistance was found among the strains of *S. aureus* tested by the phenotypic method. Similarly,

Table I
Comparison of bacteria identified by the BioFire® FilmArray® Pneumonia *plus* Panel (PNP) and the conventional quantitative culture methods (CM) in 27 bacteriologically positive BAL samples.

Bacterium	Conventional quantitative culture methods (CM)	BioFire® FilmArray® Pneumonia *plus* Panel (PNP)
	Total number of bacteria detected*	Total number of bacteria detected
*Enterobacter cloacae complex***	1 (0)	2
*Escherichia coli***	7 (4)	8
Haemophilus influenzae	4 (3)	4
Klebsiella oxytoca	1 (1)	1
Klebsiella pneumoniae group	3 (2)	3
*Moraxella (Branhamella) catarrhalis***	0 (0)	1
Proteus spp.	3 (1)	3
*Pseudomonas aeruginosa***/****	5 (3)	5
*Serratia marcescens***	0 (0)	1
*Staphylococcus aureus***	9 (4)	10
Streptococcus agalactiae	1 (1)	1
*Streptococcus pneumoniae***	2 (2)	3
Total number	36 (21)	42
Resistance genes		
CTX-M	1#	1
IMP	0	0
KPC	0	0
NDM	0	0
VIM	0	0
OXA-48-like	0	0
mecA/*C* and MREJ	0	0
Total number	1	1

Seventy-nine samples of BAL (bronchoalveolar lavage) were analyzed by CM and PNP. Twenty-seven samples were bacteriologically positive in the PNP test (reference method) at the detection level at $\geq 10^4$ genomic copies/ml.
* – bacteria identified in CM, regardless of the result of the diagnostic titer; in the brackets number of cultures equal to or above the diagnostic threshold ($\geq 10^4$–10^5 CFU/ml (CFU – colony-forming units))
** – microorganisms not detected in the culture method
*** – microorganism not detected in the PNP test
# – ESBLs positive (extended-spectrum β-lactamases)

the presence of the mecA/mecC and MREJ genes was not detected in the PNP test.

Analysis of detected viruses and atypical bacteria. No atypical bacteria were detected in the tested samples in the analyzed period. Nine samples were positive for viruses, including five samples in monoculture. Among the detected viruses were Respiratory Syncytial Virus (RSV) (n = 3), influenza A virus (n = 2), parainfluenza virus (n = 1), adenovirus (n = 1), and human rhinovirus/enterovirus (n = 4).

Characteristics of the test. The overall agreement of the interpretation of the result between methods (positive or negative) was 84.8% (n = 67/79). The culture method showed a concordance of 16/27 positive results (TP) according to the criteria and 51/51 negative results (TN), which accounted for 59.3% and 100.0%, respectively, in the given result groups. Of the TN, 19 materials (n = 19/51; 37.3%) grew one to three potentially pathogenic bacterial species, but the growth titer (CFU/ml) was lower than the established diagnostic threshold. In the remaining clinical samples (n = 12/79; 15.2%), the CM method yielded incorrect results: 1/12 FP (8.3%) and 11/12 FN (91.7%). Among the materials for which a false-negative result was obtained, 6/11 (54.5%) of the materials were negative. In comparison, in 5/11 (45.5%) samples the pathogen was cultured at a titer below the cut-off value. Summarizing the individual categories of results concerning the total number of BAL tested (n = 79), the data are as follows: TP – 16/79 (20.3%); TN – 51/79 (64.6%); FP – 1/79 (1.3%) and FN – 11/79 (13.9%). Based on the obtained results, the sensitivity, specificity, PPV and NPV were

Table II
Comparison of conventional quantitative culture method (CM) and the BioFire® FilmArray® Pneumonia *plus* Panel (PNP). Overall sensitivity, specificity, accuracy, and positive and negative predictive values.

Methods	Samples	TP	FP	FN	TN	SENS	SP	ACC	PPV	NPV	FPR	FNR	AUC	SE	*p*
PNP vs. comb	BAL	27	0	1	51	0.964	1.000	0.987	1.000	0.981	0	0.036	0.982	0.02	**0.0000**
CM vs. comb	BAL	17	0	11	51	0.607	1.000	0.861	1.000	0.823	0.000	0.393	0.804	0.06	**0.0000**
CM vs. PNP	BAL	16	1	11	51	0.593	0.981	0.848	0.941	0.823	0.019	0.407	0.787	0.062	**0.0000**

BAL – bronchoalveolar lavage, Comb = CM + PNP, TP – true positives, FP – false positives, FN – false negatives, TN – true negatives, SENS – sensitivity, SP – specificity, ACC – accuracy, PPV – positive predictive value, NPV – negative predictive value, FPR – false positive ratio, FNR – false negative ratio, AUC – area under the curve, SE – standard error of AUC, *p* – statistical significance level
The Spearman correlation coefficient R = 0.7466, $p < 0.001$

calculated. The cumulative results are presented in Table II. Moreover, in the case of 28/69 (40,6%) samples of clinical materials sent by clinicians for mycological tests, fungi growth was observed. However, only in 1/28 (3.6%) samples the growth titer was higher than the established diagnostic threshold (Table III). The Spearman correlation coefficient, determining the correlation between diagnostic methods, was R = 0.7466, which confirms a strong relationship between the results of the compared diagnostic methods. This relationship was statistically significant ($p < 0.0001$).

Table III
Performance of quantitative growth of fungi in conventional quantitative culture method (CM).

Name of fungi	Quantity (CFU/ml)			
	10^2–10^3	10^3–10^4	10^4–10^5	$>10^5$
Aspergillus fumigatus	4	3	0	0
Candida glabrata	5	8	0	0
Candida albicans	6	5	1	0
Candida tropicalis	2	2	0	0
Candida dubliniensis	3	0	0	0
Kluyveromyces marxianus (formerly *Candida kefyr*)	1	0	0	0
Pichia kudriavzevii (formerly *Candida krusei*)	0	1	0	0
Saccharomyces cerevisiae	1	0	0	0
Penicillium spp.	1	0	0	0
Total number	23	19	1	0

Sixty-nine samples were analyzed for fungi by CM.
The results are presented as CFU/ml (CFU – colony-forming units).
The diagnostic threshold was ≥ 10^4 – 10^5 CFU/ml. Fungi growth was obtained in 28 out of 69 samples (40.6%), but only in one sample was the growth of *Candida* in the diagnostic titer.

Discussion

In the current work, we analyzed the results of studies of bacterial pathogens in samples of BAL among patients with solid tumors using the rapid PNP test and CM. Only 20.3% of samples were TP for bacterial etiology in CM compared to 34.2% TP in the PNP test. More than 60.0% of the analyzed samples were TN in both methods. The most prevalent pathogens in both methods were *S. aureus* followed by *E. coli*, *P. aeruginosa*, and *H. influenzae*. The CM method, compared to the PNP test for total bacterial pathogens, achieved a sensitivity and specificity, of 59.3% and 98.1%, indicating that the etiology for only slightly more than half of the cases of bacterial infections could be established using conventional methods. The semiquantitative value reported by the PNP test was higher than reported by culture. The PNP test vs. combined tests (PNP test and CM methods) demonstrated PPV and NPV values of 100.0% and 98.1%, and a sensitivity and specificity of 96.4% and 100.0%.

These results correlate with the results described in other works, in which the authors emphasize the low sensitivity of the CM method compared to the high sensitivity of the PNP test (Kamel et al. 2022; Jitmuang et al. 2022). Ginochio et al. (2021) showed that the sensitivity of the PNP test in detecting the etiology of hospital-acquired and ventilator-associated pneumonia is over 98.0%. The advantage of the PNP test over the CM method is the detection of the presence of microbial genetic material. Since the method is not limited by the pathogen activity, detecting the DNA of damaged or non-viable pathogens can be a disadvantage if it generates FP results. It is also an advantage if the material is collected during antibiotic therapy, which is common among hospitalized patients (Buchan et al. 2020). Some bacteria are more difficult to detect using routine cultures (Ginocchio et al. 2021). In our study, differences in non-growth bacteria were found in *M. catarrhalis* and *S. pneumoniae*, but also in *S. aureus*, *P. aeruginosa*, and *E. coli*. It may be related to the frequent use of empiric broad-spectrum antibiotics among oncologic patients with suspicion of pneumonia rather than to the difficulty in obtaining the growth of bacteria in culture. Interpreting these results is problematic: it must refer to the specific clinical situation and requires close cooperation between the microbiologist and the clinician. In case of no growth of bacteria in culture,

some guidelines recommend discontinuing antibiotic treatment. However, clinical factors should alter the decision (Kalil et al. 2016).

The simultaneous use of the CM method and the PNP test can increase the sensitivity of the diagnostics. Edin et al. (2020), combining the PNP test with CM, determined the etiology in 73.0% of the examined patients, which helped to make quick therapeutic decisions. With the PNP test, we also identified many more positive samples. Using the PNP test allowed us to determine the bacterial etiology in 11 (13.9%) out of the 79 samples tested.

The ability of the PNP test to identify genes responsible for important resistance mechanisms is helpful, especially in the case of the high prevalence of resistance detected by the PNP test in a given area. In the samples tested in the current study, the gene responsible for the production of CTX-M-type ESBLs cephalosporinases in *E. coli* was detected, which was confirmed by the phenotypic method. ESBLs hydrolyze expanded spectrum cephalosporins (ceftriaxone, cefepime). A positive result suggests that anti-Gram-negative therapy should usually be escalated to a carbapenem as ESBLs confer resistance to cephalosporins (Rodríguez-Baño et al. 2018). However, we did not detect other resistance mechanisms; it is worth emphasizing that the lack of resistance gene does not rule out antibiotic resistance in mechanisms other than those determined in PNP. In the present study, resistance to carbapenems, not resulting from the production of carbapenemases, was found in *P. aeruginosa*. Therefore, due to the large variety of bacterial resistance patterns, especially in hospitalized patients, and the different mechanisms of their occurrence, it is necessary to perform conventional culture and susceptibility tests in parallel with the PNP test. When choosing empiric therapy based on the results of the PNP test, local microbiological maps and the risk of multidrug-resistant microorganisms should also be taken into account (Murphy et al. 2020).

Viruses are the second most common group of pathogens in lower respiratory tract infections. With respiratory viral infections, mainly of community origin, caused by influenza, parainfluenza, adenoviruses, rhinoviruses/enteroviruses, and RSV, their detections by the PNP test were very useful in the choice of oncologic patient treatment, especially during the infectious season. Using the PNP test allowed the detection of bacterial and viral co-infection in 3.8% of the tested samples. Also, the ability to detect infections caused by atypical bacteria, including *Legionella pneumophila*, without other tests and collection of further samples is an advantage of the PNP test. Although our work aimed not to compare methods for detecting atypical bacteria, it is known that culture, which is the gold standard for detecting *Legionella* infections, is characterized by low sensitivity and takes a long time (Cristovam et al. 2017). The PNP test, characterized by greater sensitivity and a short time for obtaining results, may be an alternative to a standard diagnostic procedure in oncologic patients with impaired cellular immunity (Bai et al. 2023).

The PNP test does not detect infections caused by *Candida* spp., *Aspergillus* spp., and *Pneumocystis jirovecii*, which is a limitation in the use of the test in diagnosing pneumonia in oncologic patients. However, pulmonary candidiasis is a rare disease entity. It occurs mainly during neutropenia, in the case of using immunosuppressive drugs, and in severely ill patients with solid tumors treated with broad-spectrum antibiotics for a long time (Schmiedel and Zimmerli 2016). However, very often in cancer patients, the respiratory tract is colonized by fungi. Our research confirmed this. Growth of fungi was obtained in slightly less than 41.0% of the samples tested, but only in one sample at a significant level. In prolonged neutropenia, severe infections caused by *Aspergillus* and other molds may also develop. We found the growth of *Aspergillus fumigatus* in about 10.0% (7/69) of the tested samples. Therefore, among patients with an increased risk of developing an infection caused by *Aspergillu*s, especially among patients with acute leukemia and recipients of hematopoietic stem cell transplantation (HSCTs), available mycological diagnostics should additionally be performed aimed at timely diagnosis of infections (Latgé and Chamilos 2019).

Non-infectious pneumonia caused by neoplastic changes, post-radiation complications, pulmonary edema, and drug-induced pneumonia may have a similar course to infectious pneumonia (Black 2016). Since the PNP test is characterized by a high NPV, reaching 90–100%, which is also confirmed by our research, a negative PNP test result may likely indicate a possible cause of pneumonia other than an infectious one. It should be noted, however, that a negative result in the PNP test does not confirm the existence of a non-infectious cause of pneumonia. However, it can be a good tool in the hands of a clinician and complement the diagnostic process and thus influence the direction of the treatment (Azoulay et al. 2020).

The PNP test allows for quick identification of pneumonia pathogens among oncologic patients. It can be used to assess bacterial etiology and to diagnose especially community-acquired viral infections. Due to the various levels of immunosuppression and different stages of cancer in oncologic patients, which are associated with the presence of a specific profile of microorganisms responsible for infections, the benefits of molecular diagnostics should be considered after validations in specific groups of patients with cancer. Similarly, in other works, the authors emphasize that rapid molecular tests should be an additional tool, which, at

the present stage of clinical effectiveness, assessment should be supplemented with the standard microbiological tests (Alnimr 2023). Due to the difficulty in predicting etiology, diagnosis of opportunistic pneumonia requires extensive diagnostics, going beyond the microorganisms covered by the panel, appropriate for the type of immunodeficiency, clinical course, and additional tests performed. Therefore, until studies are conducted in groups of oncologic patients who may receive particular benefits from performing molecular tests, tests should be performed in parallel.

This study presents some limitations. The limitation is the lack of clinical characteristics of the patients. Due to the large diversity in the group of patients who were tested (outpatients and hospitalized patients), and the relatively small number of tests performed and the possible impact of local epidemiology, the results cannot be generalized.

In conclusion, the PNP test is a good tool for determining the etiology of bacterial pneumonia in oncologic patients with solid tumors. It provides important diagnostic information, which may support the care of an oncologic patient. However, further large-sample studies are needed to research in strictly defined groups of oncologic patients.

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Author contributions

All authors contributed to the final version of the manuscript: M. Szymankiewicz designed the study and prepared the manuscript draft; E. Stefaniuk and M. Szymankiewicz conducted the statistical analysis; A. Szczepańska collected the test results. M. Szymankiewicz and E. Stefaniuk critically reviewed the final version of the manuscript.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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