ENGLISH VERSION: FEATURES OF ETIOLOGY AND PATHOGEN IDENTIFICATION IN PATIENTS WITH SEVERE COMMUNITY ACQUIRED PNEUMONIA[*]

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The importance of identifying the causative agent in patients with severe community-acquired pneumonia (CAP) is out of doubt. Different data on the leading role of respiratory pathogens in the etiology of severe CAP are pronted, as well as the roles of the various methods of there identifying the aim of study was to determine the spectrum of pathogens of severe CAP, as well as the effectiveness of the use of such non-culture method of sputum as multiplex PCR. To do this 62 patients with verifiable severe CAP were examined. Identification of the etiologic pathogen and HIV status for all patients were performed after hospitalization before prescribing of antibiotic therapy. According to the study it was found that due to a combination of two methods of sputum diagnostic etiology of severe CAP was identified in 61.2% of all cases. There were designated 3 cohorts of patients: with identified Gr(+) bacteria, with identified Gr(-) bacteria and with identified opportunistic flora. According to the analysis of the structure of bacterial pathogens from all identified patients with severe CAP pneumococcus prevails, we revealed a high frequency of opportunistic and multiresistant Gr(-) flora. Due to the high risk of atypical and Gr(-) flora as respiratory pathogens of severe CAP identification of its etiology should be included in the list of mandatory measures in diagnostic algorithm at this pathology. "Gold standard" for identification of respiratory pathogens in patients with severe CAP microbiological research with revealing sensitivity to antibiotics remains, but if it cannot be conducted, as well as by suspected atypical pathogens using of sputum PCR is an efficient approximate, but fast method.

Keywords: severe community-acquired pneumonia, etiology, pathogens

There is no doubt about relevance of management of patients with severe community acquired pneumonia (CAP) due to constant rise of its morbidity and mortality in our country and around the world [3, 9, 15, 17]. Because of constant increase of severe CAP in recent years, some researchers have attributed it most of all to changing of etiological patterns of the disease, namely to increaseof the frequency in occurrence of staphylococci, Legionella and grammnegative (Gr (-)) are microorganisms that lead to more severe course of the disease [1, 2, 16].

According to the literature, the leading etiologic role in severe CAP play such microorganisms as *Staphylococcus pneumoniae* (*S. pneumoniae*) (approximately 30% of cases), *Legionella pneumophila (L. pneumophila)* (1–15% of cases), *Staphylococcus aureus* (*S. aureus*) (7–8% of cases), *Pseudomonas aeruginosa (P. aeruginosa)* (10–15% of cases), the family of *Enterobacteriaceae* (*Esherichia coli, Klebsiella rneumoniae* (*K. pneumoniae*), *Proteus*) (22% of cases) [1, 18]. Rarely severe CAP is caused by *Haemophilus influenza* (*H. influenza*) (4–5% of cases), atypical pathogens *Mycoplasma Pneumoniae* (*M. pneumoniae*), *Chlamydia pneumoniae* (*C. pneumoniae*) (2–2.5% of cases), viruses (5% of cases) [20, 21]. The most common pathogens of fatal pneumonia are *K. pneumoniae, S. aureus, S. pneumoniae* and *H. influenzae* (31,4, 28,6, 12,9 and 11,4% of all isolates respectively) [5].

In addition, there is a steady increase of pneumonia in patients suffering from various immunodeficiency states, not only due to the continuous increase in HIV-positive patients [19] but also due to conditions that lead to a reduction in the activity of cellular immunity (diabetes, alcoholism, cancer, etc.) [4, 7]. The most common causative agents of such severe CAP are *S. aureus, Streptococcus viridians, P. aeruginosa, Streptococcus ryogenes, Streptococcus anhaemolyticus, Pneumocystis jirovecii (P. jirovecii), Cytomegalovirus, Candida* fungi, *Aspergilus,* viruses, *L. pneumophila, Mycobacterium avium-intercellulare, Enterobactenaceae* [8, 10].

In view of the above, we want to elect attention to the importance of identifying the pathogen in patients with severe CAP for opportunities of individualization of antibiotic therapy (ABT). However, Russian experts in the field of pulmonology indicate that the etiology of severe CAP can not be determined in 40-60% of patients [1, 14]. Lack of productive cough in patients with weakened inhibition of cough reflex, inadequate requirements of biological material, widely irrational use of antibiotics in out-patient, violation of the rules for collecting, storing and shipping of specimens, long time diagnostic, the inability to identify intracellular pathogens are the most important reason for the negative results of microbiological studies of expectorant.

At the same time, there is evidence that in case of combination of modern diagnostic methods, identification of the causative agent of severe CAP can bring closer to 80% [2]. In the presence of severe CAP as a quick search of the etiological agent comes to the fore, it is important to introduce fast, versatile, highly specific methods for rapid diagnosis of respiratory pathogens into medical practice, which include the rapid testing of specimens using multiplex polymerase chain reaction (PCR) [22]. This method makes possible to detect in few hours common pathogens of severe CAP including intracellular microorganisms and Pneumocystis [11].

Given different data about the leading role of respiratory pathogens in the etiology of severe CAP, as well as the role of the various methods of their identifying the aim of the work was to determine the spectrum of pathogens in patients with severe CAP in our region, and efficiency of such method of nonculture sputum diagnostic as multiplexed PCR in patients of this category.

Materials and methods

We have examined 62 patients with verified (according to the criteria of the Order of the Ministry of Health of Ukraine №128 as of 19.03.2007 [12]) severe CAP, which

accounted to the main group. All patients after admission before appointment of ABT underwent etiological agent identification and determination of HIV status, according to the results of which they were divided into groups and subgroups:

- group 1, which included 51 patient with severe CAP without HIV infection, divided into subgroups according to the etiological factor:

- subgroup A – 17 patients with severe CAP (mean age – 57,5 ± 4,3 years, men – 13 (76.5%)) with isolated Gr(+) bacteria;

- subgroup B – 10 patients with severe CAP (mean age – 50,2 ± 5,2 years, men – 7 (70.0%)) with isolated Gr(-) bacteria;

- group 2 , which included 11 patients with severe CAP (mean age – 35,8 ± 2,5 years, men – 4 (36.4%)) with HIV infection.

Assessment of HIV status was performed by rapid testing of patients using CITO TEST HIV 1/2 ("Pharmasco", Ukraine).

Identification of pathogens in sputum, induced sputum or bronchoalveolar washings performed by microbiological research material and method for determining bacterial DNA by PCR. For this spontaneous or induced sputum was carried in the morning on an empty stomach after pre-treatment of the patients teeth and rinsing the mouth with boiled water. Induction of sputum was carried out by prior inhalation of nebulized of hypertonic (3–5%) sodium chloride solution during 7–10 minutes, followed by rinsing the mouth and sputum collection.

Collection of bronchoalveolar flush performed during diagnostic or sanation bronchoscopy. Delivery of diagnostic material in the laboratory was not more than 2 hours with material stored under normal conditions.

For the culture and identification of pathogenic causative agent in sputum we used classical culture media. The sensitivity of microorganisms was evaluated by disco-diffusion method.

Identification of DNA of S. pneumoniae, Neisseria meningitides (N. meningitides), H. influencia, L. pneumophila, M. pneumoniae, C. pneumoniae, P. jirovecii was performed by multiplex PCR using kits of series "Multy-Praym" ("YnterLabServys", Russia) for one-stage DNA amplification of S. pneumoniae, N. meningitides, H. influencia, for one-stage DNA amplification of M. pneumoniae, C. pneumoniae and by using specific primers to amplify DNA pAZ102E of P. jirovecii using bio-analyzer Agilent 2100 («Agilent Technologies", United States).

All patients gave written consent for research.

Statistical analysis of the results of research was carried out by the methods of biometric analysis, implemented in software packages EXCEL-2003 (№ 74017-641-9475201-57075) and STATISTICA 6.0 (№ 31415926535897) [6, 13].

Results and discussion

The study proved that due to the combination of two methods of sputum diagnostic the etiology of severe CAP was identified in 61.2% of cases, with apparent 3 cohorts of patients: with isolated Gr(+) bacteria, with isolated Gr(-) bacteria and isolated opportunistic flora (Fig. 1), which underlay the principle of patients' dividing into groups and subgroups.
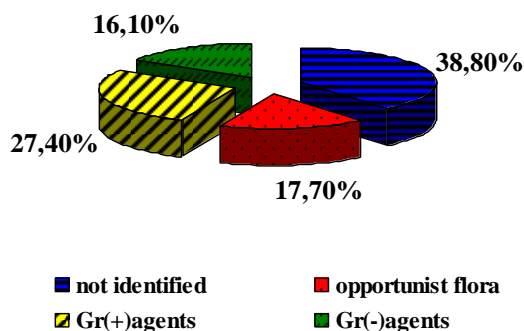

Fig. 1. Structure of identified pathogens in patients with severe CAP

As for the patients of group 1, among the 51 patients without HIV infection pathogens were identified in 27 (52.9%) cases (Table 1).

Table 1
Identified pathogens in patients of group 1, abs. (%)

Agent	Group 1 (n=51)
S. aureus	5 (18,5)
S. pneumoniae	12 (44,4)
K. pneumoniae	3 (11,1)
P. aeruginosa	3 (11,1)
N meningitides	2 (7,5)
Acinetobacter	1 (3,7)
Enterobacteriacae	1 (3,7)
P. jirovecii	-
C. pneumoniae	-
M. pneumoniae	-
L. pneumophila	-
Total	27

Thus in 17 (62.9%) patients included in subgroup A, Gr(+) bacteria (*S. pneumoniae* (n = 11), *S. aureus* (n = 6)) were identified (Fig. 2).
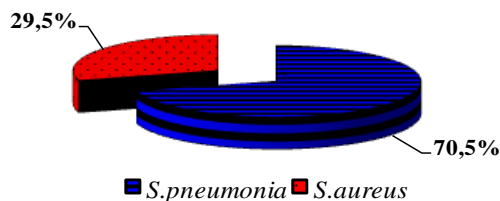

Fig. 2. Structure of identified pathogens in patients with severe CAP of subgroup A

Subgroup B included 10 (19.6%) patientsin whom Gr(-) bacteria (*P. aeruginosa* (n=3), *K. pneumonia* (n=3), *N. meningitides* (n=2), *Acinetobacter* (n=1), *Enterobacteriacae* (n=1))were detected (Fig. 3).
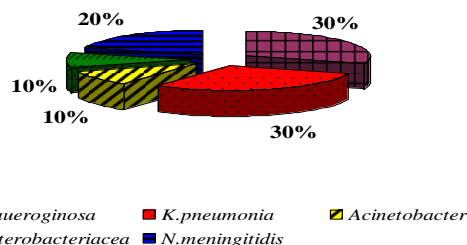

Fig. 3. Structure of identified pathogens in patients with severe CAP of subgroup B

According to the analysis of the structure of identified bacterial pathogens in 27 patients of group 1, it was found that almost in a half of cases *S. pneumoniae* was detected (Table 1). Attention is drawn by high frequency

of Gr(-) pathogens identification, which were diagnosed in 10 (37.0%) of cases.

The results about the superiority of pneumococcus, *S. aureus*, *K. pneumonia*, *P. aeruginosa* in the structure of the etiologic agents of severe CAP consistent with other data of domestic scientists, while the detection of *N. meningitides*, *Enterobacteriacae* and *Acinetobacter* as an etiological factor needs discussion.

According to the literature, meningococcal DNA can be recovered by PCR in patients with nasopharingeal bacteria carrier state of this pathogen that does not indicate the etiology of severe CAP. However, given the state of severe course in these patients and the effectiveness of ABT with high activity against Gr(-) flora, it can be argued that *N. meningitides* acted as the causative agent of severe CAP.

Enterobacteriacae and *Acinetobacter* can be considered as nosocomial infections, which joined in the taking of specimens. However, given that biological materials in these patients were collected before the use of respiratory support devices in disposable tableware, which almost eliminates contamination, we can assume that these organisms were also etiological agents in these patients with severe CAP.

It should also be noted that no cases of atypical intracellular pathogens (*C. pneumoniae, M. pneumoniae* and *L. pneumophila*) by PCR were observed.

During the analysis of the diagnostic value of diagnostic methods of etiological factors in patients with severe CAP that were used in this study, it was found that out that from 27 strains of microorganisms that have been identified in patients of group 1, 8 strains were identified only by microbiological studies (*S. aureus* (n=5), *P. aeruginosa* (n=3), *K. pneumonia* (n=3), *Acinetobacter* (n=1), *Enterobacteriacae* (n=1)), 15 strains – only by PCR (*S. pneumoniae* (n=13), *N. meningitides* (n=2), 4 strains – by both methods simultaneously (*S. pneumoniae* (n=4)).

It is necessary to point out that the pneumococcus was isolated by PCR in all cases of its overall detection and by microbiological – only 17 4 (23.5%) out of cases. Other advantages of the multiplex PCR were low intelligibility to the number of specimens, obtaining results within a day and the possibility of verification of intracellular pathogens. However, the inability to identify a lot of Gr(-) microorganisms, lack of information about pathogen susceptibility to antibiotics, the possibility of false-positive results were the main disadvantages of the method. Thus, a comparison of different methods for identification of respiratory pathogens has no effect, while the smart choice and their combination come to the fore in severe patients with CAP.

Regarding the sensitivity of respiratory pathogens to antibiotics in 4 patients the results of microbiological examination of sputum were identified *S. aureus*, *S. pneumonia* which sensitive only to moxifloxacin and carbopenems, in 3 patients the results of microbiological examination of sputum were identified as *K. pneumonia* which was weakly sensitive only to imipenem, indicating a fairly high degree of respiratory pathogens multiresistence in patients with severe CAP without HIV infection.

As for the patients in group 2 the results of the identification of the pathogen reveal the etiologic agent succeeded in 100% of cases. Absolutely dominated pneumonia caused by *P. jirivecii* (n=9), whereas others (n=2) were identified as pneumococcus (Fig. 4).
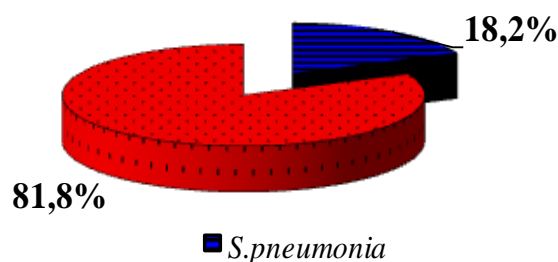

Fig. 4. Structure of identified pathogens in patients of group 2

Moreover, all strains were found in the sputum (in 2 (18.2%) cases) and induced sputum (in 9 (81.8%) cases) using PCR, the advantages of which was the possibility of verification atypical opportunistic pathogen *P. jirivecii* and in small number of samples of biological material.

Conclusions

1. In spite of the fact that *S. pneumoniae* dominated in the structure of all bacterial pathogens identified at patients with severe CAP in Dnipropetrovsk region, we found a high frequency of opportunistic (in 11 (17.7%) cases) and multiresistant Gr(-) (10 (16.1%) cases) flora.

2. Due to high risk of atypical and Gr(-) respiratory pathogens in patients with severe CAP identification of its etiology should be included in the mandatory measures of the diagnostic algorithm at this pathology, aided to sputum induction and flush water intake during sanation or diagnostic bronchoscopy.

3. "Gold standard" of respiratory pathogens identification in patients with severe CAP is microbiological research with identification of susceptibility to antibiotics, however with the inability of it, as well as by suspected atypical pathogens the use of sputum PCR is efficient approximate but rapid method.

4. PCR studies of induced sputum are on the first place in the verification of etiologic pathogen in HIV-infected patients with severe CAP.

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