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INFECTIOUS EXTRACTION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: PLACE AND ROLE OF RESPIRATORY VIRAL PATHOGENS

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Ключові слова: хронічне обструктивне захворювання легень, інфекційне загострення, респіраторні вірусні збудники, швидкі імунохроматографічні тести, мультиплексна ПЛР у реальному часі

Ключевые слова: хроническое обструктивное заболевание легких, инфекционное обострение, респираторные вирусные возбудители, быстрые иммунохроматографические тесты, мультиплексная ПЦР в реальном времени

Abstract. Infectious extraction of chronic obstructive pulmonary disease: place and role of respiratory viral pathogens. Feshchenko Y.I., Gavrisyuk V.K., Dziublyk I.V., Dziublyk O.Ya., Gumeniuk G.L., Gumeniuk M.I., Kapitan G.B., Yachnik V.A. To determine the prevalence and spectrum of viral pathogens in patients with infectious exacerbation (IE) of chronic obstructive pulmonary disease (COPD), 106 patients were examined, who underwent a virological examination using fast immunochromatographic tests (IC-tests), as well as real-time multiplex polymerase chain reaction (PCR) with the simultaneous identification of 12 respiratory viruses. Respiratory viruses, as the etiological factor of IE COPD, were identified in (65.9 ± 4.7)% of patients. Among viral pathogens was adenovirus and rhinovirus - in (24.5±6.1)% of cases each and parainfluenza virus - in (18.4±4.6)% were of greatest etiological significance for the entire observation period. The influenza A and B virus was detected much less frequently - in (8.2±3.9)% and in (4.1±2.8)% of cases, respectively; respiratory syncytial virus and bocavirus – in (6.1±3.4)% and metapneumovirus – in (8.2±3.9)% of cases. The last two types of viruses were detected in Ukraine for the first time. The spectrum of viral pathogens of IE COPD depended on the seasonal circulation of a particular type of virus, the epidemiological situation in the region, and the characteristics of the examined population. The use of modern virological diagnostic technologies testifies to the leading role of respiratory viruses in the etiology of IE COPD and also allows the etiological diagnosis to be established in a short period of time – from 10-15 minutes when using fast IC-tests and up to 6-8 hours – when using real-time multiplex PCR, which contributes to the timely administration of adequate etiotropic treatment.

Реферат. Инфекционное обострение хронического обструктивного заболевания легких: место и роль респираторных вирусных возбудителей. Фещенко Ю.И., Гаврисюк В.К., Дзюблик И.В., Дзюблик А.Я., Гуменюк Г.Л., Гуменюк Н.И., Капитан Г.Б., Ячник В.А. Для установления распространенности и спектра вирусных возбудителей у больных с инфекционным обострением (ИО) хронического обструктивного заболевания легких (ХОЗЛ) обследовано 106 пациентов, которым было проведено вирусологическое обследование с использованием быстрых иммунохроматографических тестов (ИХ-тестов), а также мультиплексной полимеразной цепной реакции (ПЦР) в реальном времени с одновременной идентификацией 12 респираторных вирусов. Респираторные вирусы, как этиологический фактор ИО ХОЗЛ, идентифицированы у

65,9±4,7% больных. Наибольшую этиологическую значимость за весь период наблюдения среди вирусных возбудителей имели аденовирус и риновирус – в 24,5±6,1% случаев каждый и вирус парагриппа – в 18,4±4,6%. Значительно реже выявляли вирус гриппа А и В – в 8,2±3,9% и в 4,1±2,8% случаев соответственно; респираторно-синцитиальный вирус и бокавирус – в 6,1±3,4% и метапневмовирус – в 8,2±3,9% случаев. Последние два вида вирусов были выявлены в Украине впервые. Спектр вирусных возбудителей ИО ХОЗЛ зависел от сезонной циркуляции того или иного вида вируса, эпидемиологической ситуации в регионе и особенностей обследованной популяции. Использование современных технологий вирусологической диагностики свидетельствует о ведущей роли респираторных вирусов в этиологии ИО ХОЗЛ, а также позволяет установить этиологический диагноз в короткий промежуток времени – от 10-15 мин. при применении быстрых ИХ-тестов и до 6-8 ч. при использовании мультиплексной ПЦР в реальном времени, что способствует своевременному назначению адекватного этиотропного лечения.

Chronic obstructive pulmonary disease (COPD) remains one of the most important health problems [8]. First of all this is due to the high prevalence of this pathology, sufficiently high rates of disability and mortality, as well as the huge moral and material losses suffered by patients, their families and the economies of countries around the world [9], [11]. Important episodes of COPD course are exacerbations of the process that adversely affect health, increase the frequency of hospitalizations, lead to disease progression and impair the quality of life of patients [14]. COPD exacerbation is a complex of events that is usually associated with increased respiratory tract inflammation, increased mucus production, and marked pulmonary hyperinflation. These changes enhance dyspnea which is a key sign of exacerbation. Other respiratory symptoms include increased virulence and amount of sputum, as well as aggravated coughing and wheezing [8]. In about 80% of cases exacerbations are infectious and in others ones are related to environmental pollution, temperature factors, low compliance, or cancellation of basic therapy [9, 14, 15].

According to recent data, infectious exacerbation (IE) of COPD is mainly caused by viral respiratory pathogens, although bacterial etiopathogens can initiate and/or enhance them [4, 12]. The most common viral pathogens are human rhinoviruses and adenoviruses, which can be detected within a week after the onset of exacerbation. Exacerbations associated with viral infections are more severe, lasting, they require more frequent hospitalizations, mainly in winter [15].

Unfortunately, even when traditional microbiological methods are used, at the best case the pathogen is identified in 50% of cases [6, 7]. This indicates both the limited possibilities of routine etiologic diagnosis of pulmonary infections and the lack of information on all potential etiopathogens of these diseases.

Laboratory diagnostics, which can be performed by classical virological, immunological and molecular-genetic methods is currently used to identify viral pathogens [6]. In our opinion, molecular-

genetic studies that are based on the identification of the DNA/RNA of the pathogen genome are the most informative and promising for the etiologic diagnosis of COPD [5]. Although these methods are still complex, they require high-quality specialists, appropriate diagnostic agents and specialized laboratory equipment, they have very high potential and are characterized by the highest levels of sensitivity and specificity [1], they not only can identify the pathogen, but also establish the presence of resistance genes to certain antimicrobial chemotherapeutic agents [13]. In addition, they enable to identify viral and bacterial pathogens simultaneously, which is especially important during epidemics of acute respiratory infections and can have a decisive influence on the choice of treatment and administration of antimicrobial chemotherapy [2, 10].

The aim of the work is to determine the prevalence and spectrum of viral pathogens in patients with COPD.

MATERIALS AND METHODS OF RESEARCH

Object of the study – 106 COPD patients who were examined and treated in the accredited clinic of the State Institution “National Institute of Phthysiology and Pulmonology named after F.G. Yanovsky of National Academy of Medical Sciences of Ukraine” (NIPP of NAMSU) (Director – Academician of NAMS of Ukraine, Doctor of Medical Sciences, Professor Yu.I. Feshchenko) in 2014-2016. Virological studies were conducted in the laboratory of virology department of National Medical Academy of Postgraduate Education named after P.L. Shupik of Ministry of Health of Ukraine (Head of the Department – Doctor of Medical Sciences, Prof. I.V. Dzublik). The research was done at the expense of the state budget.

The complex of methodical approaches was used in the work, which included modern express methods of indication of viruses in clinical material - rapid tests based on immunochromatographic analysis (ICh-tests) and molecular-genetic diagnostics by multiplex polymerase chain reaction (PCR) with real-time fixation (realtime-PCR) [5].

Material for laboratory tests were smears or swabs from the nasopharynx. On admission all patients underwent express diagnostics of influenza A and B, adenovirus and PC virus with using express ICh tests: "CITO TEST INFLUENZA A+B" (Farmasco, Ukraine), "CITO TEST ADENO RESPI" (Farmasco, Ukraine) and CITO TEST RSV (Farmasco, Ukraine). They are based on the specific interaction of antigens and antibodies on the chromatographic membrane of the test after its wetting with the liquid of the test sample taken from the patient. This interaction is due to the diffuse movement of the indicative immune component, stained with colloidal gold pre-applied onto the membrane, and the antigens of the test sample after applying the latter onto the membrane. Having obtained positive results of these tests, molecular-genetic study was not performed at a later date. In all other cases (88 patients) multiplex real-time PCR using the Seeplex® RV12 ACE Detection test system (Seegen, Korea) was performed, which simultaneously determined the presence of DNA/RNA markers of 12 respiratory viruses: adenovirus; bocavirus; metapneumovirus; influenza A virus; influenza B virus; PC viruses A and B; rhinovirus; parainfluenza 1, 2, 3, 4; coronavirus 229E/NL63. The RotorGene 6000 amplifier (Sorbett Research, Australia) was used for real-time PCR.

Descriptive statistics (number of observations, mean, error of the mean, frequency, percent) are given for all indicators of analysis, taking into account their type according to the recommendations of S.M. Lapach et al. [3].

RESULTS AND DISCUSSION

On examination of 106 COPD patients with express ICh tests, respiratory viruses were identified in 18 (17.0±3.7%) of patients (Fig. 1). Adenoviruses (55.6%) and influenza A and B viruses (33.3%) were the leading etiopathogens identified.

Multiplex real-time PCR was performed in 88 patients, which enabled the establishment of viral pathogens in 43 (48.9±5.4%) of patients. The adenovirus and rhinovirus had the highest etiologic significance for the entire observation period - 24.5±6.1% each, and parainfluenza virus - 18.4±4.6%. Influenza A and B virus were found less frequently - in 8.2±3.9% and 4.1±2.8%, respectively; respiratory syncytial virus and bocavirus each - in 6.1±3.4% and metapneumovirus - in 8.2±3.9% of cases.

It should be noted that 2 or more viral pathogens simultaneously were identified in 4 patients. Thus, in 2 patients the combination of boca- and adenovirus was detected, in 1 patient - boca- and metapneumovirus and in 1 patient - respiratory syncytial virus, influenza B and A virus.

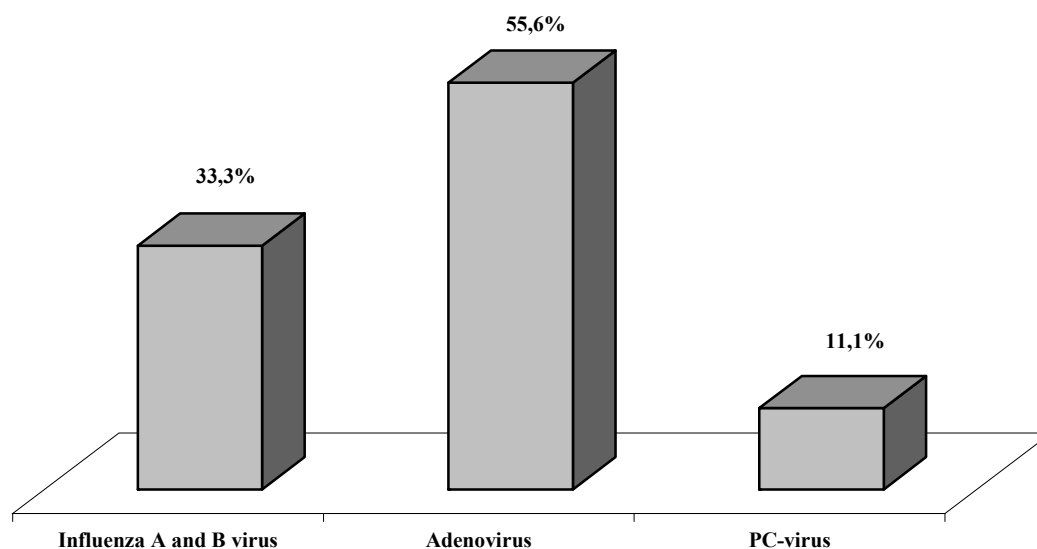


Fig. 1. The spectrum of respiratory viruses detected by ICh tests

In patients with COPD, viral pathogens were detected mainly in the autumn-winter and winter-spring periods: in September-December - in 37.5% of the patients examined, in January-March - in

46.3%, which was generally the same as seasonality of influenza and SARS. As can be seen from the figures presented, the spectrum of COPD viral pathogens had a clear dependence on the survey

period. Thus, in 2014 (Fig. 2), rhinoviruses (55.0%) had a predominant role in the development of COPD; influenza A and B viruses were detected in 27.0% of patients. A feature of this year was the appearance of metapneumovirus (9.0%) in the spectrum of viral pathogens. In 2015 (Fig. 3), adenoviruses (52.2%) predominated and were iden-

tified along with influenza A and B viruses and metapneumovirus and PC virus parainfluenza. In January-March 2016 (Fig. 4) parainfluenza viruses (47.0%) were dominant, with bocavirus (20.0%) and metapneumovirus (13.0%) detected in a significant percentage.

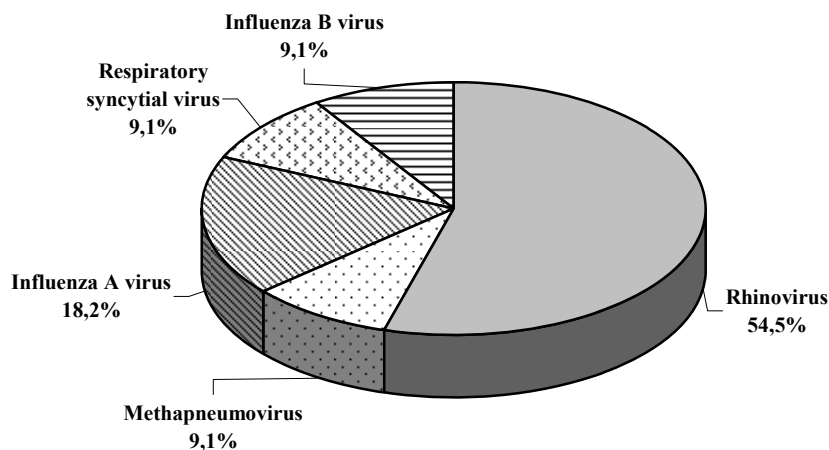


Fig. 2. The spectrum of identified COPD viral pathogens in 2014 by PCR

Thus, the use of the proposed methods of diagnostics enabled to establish the etiological pathogen of COPD in a total of $65.9 \pm 4.7\%$ of patients, therewith with multiplex PCR diagnostics viruses were identified in 48.9% of cases, and with ICh tests – in 17.0% of cases. At the same time, convenience of performance and the availability of express tests for the identification of influenza viruses A and B

leave this method of diagnostics as one of the most important for the diagnosis of influenza, especially in the epidemiological period. The data obtained also indicate the expansion of the spectrum of pathogens and the emergence in it of new respiratory viruses – metapneumovirus and bocavirus, which leads to the search and development of new approaches in the treatment of COPD patients.

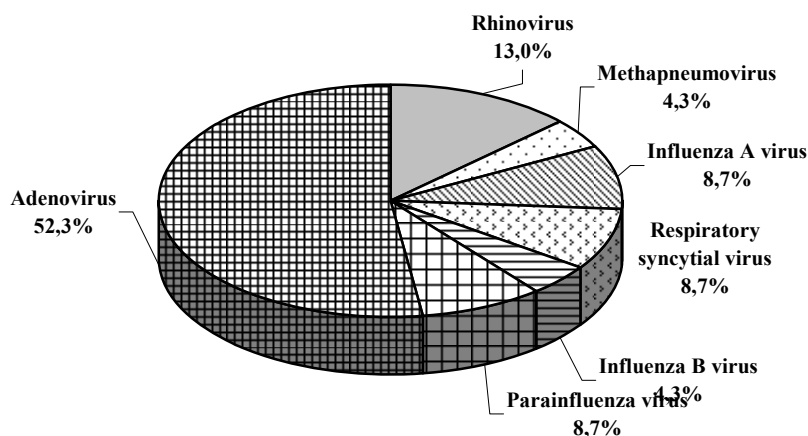


Fig. 3. The spectrum of identified COPD viral pathogens in 2015 by PCR

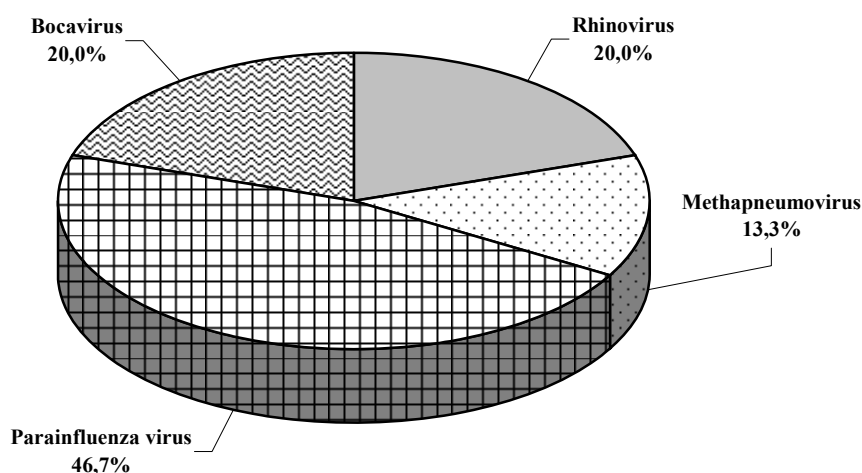


Fig. 4. The spectrum of identified COPD viral pathogens in 2016 by PCR

CONCLUSIONS

1. Respiratory viruses occupy a leading position in the structure of COPD pathogens and play an important role in the origin and development of this pathological condition.
2. The simultaneous use of express ICh tests and real-time PCR in multiplex format allows to increase the efficiency of etiological diagnosis of COPD and

to establish features of the spectrum of respiratory viruses.

3. New respiratory viruses (metapneumovirus and human bocavirus) have been identified for the first time in Ukraine in the etiology of COPD, which needs further research in this area.

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