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The Role of Nitrosative Stress and Endothelial Dysfunction in the Development of Pneumonia in Patients with Ischemic Stroke

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Abstract--- *In 78 patients with pneumonia (average age 59 ± 7 years), the levels of endothelin-1 (ET-1), nitrite and nitrate anions (NO2- and NO3-), 3-nitrotyrosine (3-NO-Tyr) in the blood serum were determined. Of these, the first (main) group included 41 patients with pneumonia who had a history of ischemic stroke (IS) for more than six months (P + IS), the second group (comparisons) was 37 with a history of pneumonia without a stroke (P). The control group consisted of 20 relatively healthy donors, comparable in age and gender with the studied groups. In all patients, the severity of the symptoms of pneumonia was evaluated, the data of clinical, laboratory, instrumental research methods were analyzed. The content of NO2- and NO3- in the selected and frozen blood sera of patients was determined using the Griss reagent (Reakhim, Russia) spectrophotometrically, the content of 3-NO-Tyr was determined spectrophotometrically by the reaction with hydrochloric acid, estimating the maximum light absorption at a wavelength of 370 nm, and the level of ET- 1 (1-21) was determined by enzyme-linked immunosorbent assay using a commercial set of Biomedica (Austria). In patients with P + IS, compared with patients with P without a history of stroke, the symptoms of the disease were more pronounced due to the more severe course of pneumonia. All patients included in the study revealed a significant decrease in the content of NO2-, NO3- in the blood, as well as a significant increase in the concentration of 3-NO-Tyr, which indicated a violation of the metabolism of nitric oxide (NO). Serum ET-1 was significantly higher than control values. In the blood, these changes were more pronounced in patients with P + IS. Indicators of NO metabolism had a correlation with the state of lung function and serum ET-1 content. Thus, the course of the disease in patients with P + IS and in patients with P without stroke is characterized by the development of nitrosative stress and endothelial dysfunction. Their intensity is more pronounced in patients with P + IS. The high frequency of changes in the levels of the studied parameters in patients with pneumonia, as well as in the case of transferred ischemic stroke, suggests the role of endothelial dysfunction and nitrosative stress in the development and worsening of pneumonia. The study of markers of endothelial dysfunction and nitrosative stress (ET-1, NO metabolites, 3-NO-Tyr) in patients with pneumonia provides additional information on the state of the inflammatory process and the effectiveness of the treatment, taking into account extrapulmonary comorbid conditions.*

Keywords--- *Pneumonia, Ischemic Stroke, Endothelial Dysfunction, Nitrosative Stress, Endothelin-1, Nitric Oxide, Nitrite and Nitrate Anions, 3-nitrotyrosine, Blood Serum.*

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I. INTRODUCTION

According to WHO (2016), over the past decade, the main diseases that claimed the most human lives were circulatory system diseases (coronary heart disease and stroke), respiratory infections of the lower respiratory tract and chronic obstructive pulmonary disease (COPD) [28]. The urgency of the problem of pneumonia is that in addition to the prevalence, this disease is characterized by high mortality and the presence of comorbid conditions (competing, background and associated diseases), and sometimes it is difficult to determine the cause of death directly associated with pneumonia. Pneumonia causes every second death in the geriatric population and 90% of deaths from respiratory infections in people over 64 years of age [12, 28].

According to the literature, nosocomial (including post-stroke) and community-acquired pneumonia play a significant role in the thanatogenesis of acute vascular accidents [12]. The incidence of pneumonia in patients with acute stroke is about 30% [27]. Patients who have had an ischemic stroke have risk factors and comorbid diseases that predispose to more frequent pneumonia. Regardless of the type and form of pneumonia, they often worsen the course of stroke in both acute and recovery periods [27]. This leads to an increase in hospitalization, economic losses, as well as an increase in mortality.

There are few works in the literature on the role of pneumonia in the course and prognosis of patients with cardiovascular catastrophes. There are few works in which it was shown that it was pneumonia that caused death in patients with stroke — about 5% [20, 27].

According to different authors, the frequency of infectious complications in patients with strokes both in the acute phase and with the consequences varies significantly (5–65% of cases), which is associated with differences between patient samples, study design, and methods for determining infection [12, 20], therefore, reliable statistics on the incidence of infections in patients with strokes are absent. The literature also discusses the complexity of diagnosing nosocomial pneumonia in patients with cardiovascular disasters in the intensive care unit and intensive care unit (ICU) [12].

The term currently used, stroke-associated pneumonia (SAP), or post-stroke pneumonia, includes the spectrum of lower respiratory tract infections that occurred within seven days of the onset of a stroke. SAP most often develops during the first week from the onset of a stroke, possibly reflecting the period of the highest risk of pneumonia due to dysphagia, impaired consciousness, a decreased immune response and immobility [25].

In accordance with the WHO requirements set out in ICD-10, pneumonia is rarely considered as the main disease - the initial cause of death, and is regarded as a fatal complication - the immediate cause of death. This complicates the statistical accounting of pneumonia, which requires special studies, and, to a certain extent, makes it difficult to understand its true value in vascular accidents. X-ray studies do not show the degree of damage to the lung tissue with a sufficiently pronounced process [10, 12]. In this regard, recently, to assess the severity of pathological processes in medical practice, new biochemical methods are increasingly being introduced that provide a significant part of diagnostic information and are most promising in verifying biological defects in the early stages of the disease, which is of great importance in preventing them development [2].

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Recently, in Uzbekistan there is a tendency to increase various respiratory infections of the lower respiratory tract, while the registration of pneumonia remains low. The decrease in the level of respiratory diseases is mainly due not to the emergence of new methods of treatment, but to the development and implementation of effective preventive measures based on the etiopathogenetic features of various forms of this group of diseases. For a clearer understanding of the pathogenetic mechanisms of the development and progression of pneumonia, there is a need to monitor markers of endothelial dysfunction, nitrosative stress and the immune response at various stages and forms of the disease, as well as to analyze the relationships between the considered indicators and risk factors for this pathology. The search for new sensitive and specific markers that allow us to study the pathomechanisms of the development of the disease, taking into account the comorbid background, will allow us to develop effective methods of pharmacological correction. One of these areas may be the effect on endothelial dysfunction (ED) [2, 8]. It can be assumed that ED can be an early sign of hemodynamic disturbances in the pulmonary circulation. The search for new targets for therapeutic effects in pneumonia, taking into account extrapulmonary disorders, is a promising area of modern pulmonology and intensive care.

In this publication, we present the results of a study performed at the Department of Faculty of Internal Medicine, Occupational Diseases, Military Field Therapy, Hospital Internal Medicine and Propaedeutics of Internal Medicine at Tashkent Pediatric Medical Institute based on City Clinical Hospital #5 in Tashkent. The aim of the work was to study the content of nitric oxide (NO) and endothelin-1 (ET-1) metabolites as markers of endothelial dysfunction and nitrosative stress in pneumonia in patients after ischemic stroke (IS) more than six months ago (late recovery and period of persistent consequences).

II. MATERIALS AND METHODS

A retrospective, prospective, cohort, case-control study was conducted. A total of 78 patients with community-acquired and nosocomial pneumonias at an average age of 59 ± 7 years (from 38 to 83 years) were examined. Of these, 43 (55.1%) are men and 35 (44.9%) are women, the ratio is 1: 1.2.

Patients were selected for the study in accordance with inclusion and exclusion criteria. Criteria for inclusion of patients in the study: age over 18 years, radiologically confirmed diagnosis of pneumonia (infiltrates in the lung tissue), the presence of cough with or without sputum, fever, a typical auscultatory picture in the lungs, and the absence of antibacterial therapy until diagnosis. Exclusion criteria: acute, early recovery period of stroke, patients with active tuberculosis, hemorrhagic stroke, severe diseases of other organs and systems in the stage of decompensation, immunodeficiency states, patients who received antibiotic therapy in the previous four weeks before the development of pneumonia or who used antimicrobial agents the previous three months.

Depending on the history of stroke, the patients were divided into two groups: the first (main) group consisted of patients with pneumonia who had a history of ischemic stroke for more than six months (n = 41; 52.6%) (P + IS), i.e. late recovery and the period of persistent consequences of a stroke, the second (comparison group) - patients with a history of pneumonia without stroke (n = 37; 47.4%) (P). The control group consisted of 20 healthy donors, comparable in age and gender with the studied groups.

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All patients underwent general clinical examination (collection of complaints, individual and family history, physical examination, examination by organs and systems, neurological status, clinical blood test with leukocytogram), instrumental (chest x-ray, pulse oximetry), microbiological (microscopic examination of sputum with staining by staining standard methods, the isolation of pure cultures of microorganisms from the material taken) research. The severity of pneumonia was assessed on a M.J. Fine scale I - V classes (PSI - Pneumonia Severity Index, or PORT scale - Pneumonia Outcomes Research Team, 1997). The degree of impaired consciousness was assessed using the Glasgow com scale (Teasdale G.M., Jennett B., 1974.); the severity of ischemic stroke was determined using the Scandinavian stroke scale (1985).

The pathogenetic role of ED and nitrosative stress was judged by the nitroxide and endothelin-producing functions of the vascular endothelium. The serum NO content was determined by the sum of nitrate and nitrite metabolites (NO2- and NO3-) using the Griss reagent (Reakhim, Russia) by spectrophotometric method. To 200 μl of the test sample, 50 μl of Griss reagent was added, a pink color developed within 10 minutes. The optical density was measured on a spectrophotometer at a wavelength of 540 nm. A calibration curve was constructed using known concentrations of sodium nitrite [7, 13, 14]. The content of 3-nitrotyrosine (3-NO-Tyr) in blood serum was determined spectrophotometrically by reaction with hydrochloric acid, evaluating the maximum light absorption at a wavelength of 370 nm [5, 6]. The level of ET-1 (1-21) was determined by enzyme-linked immunosorbent assay in selected and frozen blood sera of patients using a commercial set of Biomedica (Austria) according to the manufacturer's instructions. All reagents were thoroughly mixed before analysis and brought to room temperature. The optical density was measured on a horizontal scanning photometer at a wavelength of 450 nm. For calculations, we used the formula:

$$\frac{B - Bm}{Bo - Bm} \cdot 100\%$$

Where B is the average optical density in the wells containing calibration or test samples

B_0 is the average optical density in the wells containing the calibration sample “0 nmol / L”

B_m is the average optical density of the wells A1 and A2. In the “logit-log” coordinates, a plot of the concentration of ET-1 (1-21) (fmol / ml) in calibration samples was constructed for calibration samples [15].

All patients with pneumonia underwent standard antibacterial therapy - 3rd generation cephalosporins parenterally + inside macrolides for 10 days; detoxification and symptomatic therapy. Antibacterial therapy was prescribed empirically until the etiology of pneumonia was established in accordance with the principles of rational antibacterial therapy.

The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2012 software package. We used methods of variational parametric and nonparametric statistics with calculation of the arithmetic mean of the studied indicator (M), standard deviation (σ), standard error of the mean (m), relative values (frequency, %), linear correlation coefficient (r) Pearson. The statistical significance of the measurements obtained when comparing the average values was determined by the

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Student t-test with the calculation of the probability -of error (P) when checking the normality of the distribution (by the excess criterion) and the equality of the general variances (F is the Fisher test). For statistically significant changes, the confidence level P <0.05 (95% significance level) was taken.

III. Results And Discussion

Clinical features of pneumonia in patients after ischemic stroke. The results of the study showed the largest number of cases of pneumonia in the age group of 45-75 years (31%) and in people older than 75 years (26%). The smallest proportion of severe pneumonia in young patients (22%), aged 20-39, 40-51 years and older than 60 years. In the P + IS group, male patients predominated (58.5%) (24 men and 17 women), while female patients predominated among patients with pneumonia without stroke (54%) (20 women and 17 men).

The study included patients with pneumonia of various etiologies. Of the total number of patients with pneumonia (n = 68), the majority were patients with nosocomial pneumonia - 26 patients (38.3%), community-acquired pneumonia - 23 patients (33.8%), aspiration pneumonia - 19 patients (27,9%) (Fig. 1). In patients who underwent IS, nosocomial and aspiration pneumonia were more often observed.

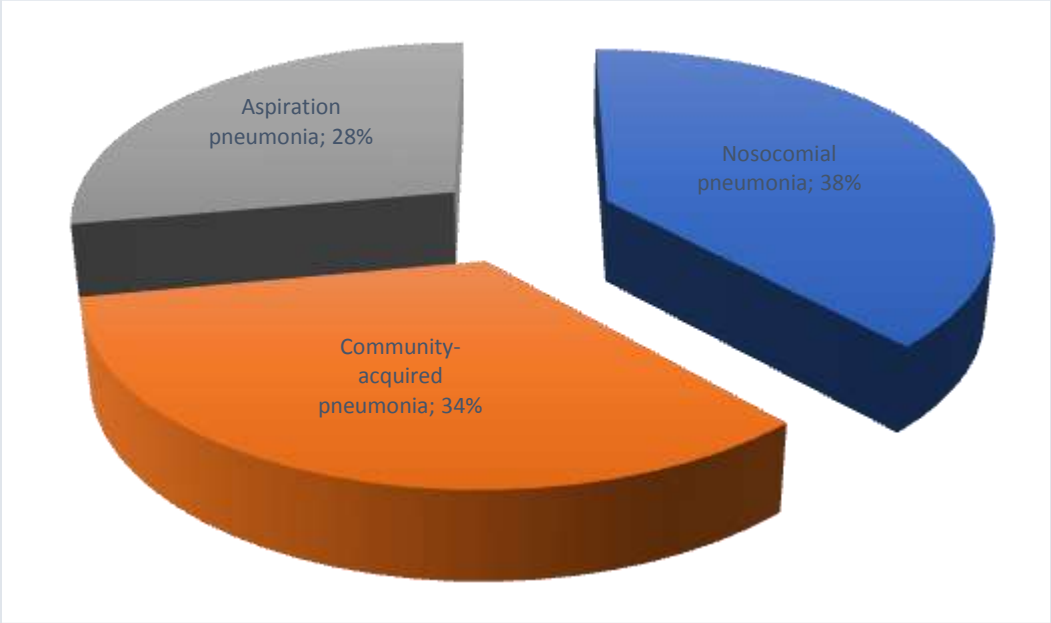

Fig. 1: The etiological structure of patients with pneumonia (n = 68)

The analysis of the clinical picture of pneumonia in hospitalized patients generally reflected the general patterns of the course of the disease. An analysis of the nature and frequency of clinical signs showed that complaints in both groups are similar in nature, but significantly differ in severity. Symptoms of the disease consisted of intoxication (general weakness, headache, shortness of breath), general inflammatory reaction (chills, sweating, fever) and syndromes of inflammatory changes in the lungs (cough with or without sputum, pleural pain, shortening of percussion sound, aggravation voice trembling, increased bronchophony, weakened breathing, small-, medium- and large-bubbly, crepitating rales).

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Table 1: Analysis of clinical symptoms in patients with pneumonia, depending on the presence of IS in anamnesis, %

Indicator	**P+IS (n=41)**	**P (n=37)**
Cough	64,3*	94,0
Dyspnea	46,0*	60,3
Chest pain	32,9	37,7
Wheezing auscultation	65,0	70,0
Respiratory rate greater than 20 per minute	48,0	56,4
Heart rate over 90 per minute	47,2	31,0
Body temperature over 37.7 ° C	39,0	42,0

Note: * - significant significance of differences between groups (P <0.05).

Analysis of Table 1 showed that the incidence of cough and shortness of breath in patients with pneumonia who underwent IS was significantly lower than in patients without a history of stroke, which may be associated with a decrease in the cough reflex and inhibition of the respiratory center due to a neurological disorder.

The complexity of the diagnosis of pneumonia in patients with acute cerebrovascular accident (stroke), especially in the acute period, is due to the fact that individual signs of pneumonia (clinical, laboratory and radiological) have an unsatisfactory sensitivity. Chest x-ray is of limited value in the early stages of pneumonia, as it takes time for the development of infiltrate. The forced horizontal position of patients, the restriction of a full breath due to impaired consciousness affects the quality and interpretation of radiographs, therefore, the diagnosis of pneumonia is based mainly on clinical data [26]. Clinical signs typical of pneumonia in other cases, such as fever, purulent sputum, usually do not occur in the early stages of pneumonia in patients with stroke. This is due to impaired consciousness, the absence of a cough reflex, paresis of the respiratory muscles and a violation of thermoregulation. In a study by A. Warusevitane et al. (2016), shortness of breath, decreased oxygen saturation of less than 90%, and wheezing were the most common manifestations of pneumonia in stroke. However, these symptoms may be a manifestation of other cardio-respiratory diseases, such as lung atelectasis, pulmonary thromboembolism, and heart failure. Therefore, there is a risk of late diagnosis of pneumonia in this group of patients, because these clinical signs are non-specific. The study shows the need to develop special guidelines for the diagnosis of pneumonia in stroke, since key diagnostic signs of pneumonia rarely occur in patients with stroke in the early stages of pneumonia. In some manuals, a fever of more than 38 ° C is mandatory in the diagnosis of pneumonia [26]. In our study, fever in patients with IS was observed only in 39% of cases. This is due to the fact that most patients with a history of IS were elderly, and in the elderly, acute infection does not always manifest a high temperature. In 86% of patients, the temperature increased to 37.7 ° C, but brain damage with stroke can cause a temperature increase of up to 37.5 ° C within 12 hours from the onset of the disease even in the absence of infection. Therefore, if pneumonia is suspected, it is recommended to consider the temperature of 37.7 ° C as a threshold temperature, which is consistent with the recommendations of other researchers [2, 12, 26].

The next step in our work was to assess the severity of the clinical course of pneumonia and the relationship with the course and severity of the stroke. In 69% of cases, pneumonia developed in severe IS with impaired consciousness, gross neurological deficit (gross paresis, paralysis, swallowing disorders, bulbar, pseudobulbar syndrome). Depending on the location of the focus of the cerebral infarction, pneumonia was more common with

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vertebro-basilar (stem) stroke, which may also cause disturbances in the central regulation of respiratory function, along with hemorheological, microcirculatory and other disorders. Assessment of the severity of pneumonia made it possible to regard the course of pneumonia in patients with IS as a whole as severe. The results of our study showed that among patients with pneumonia after an IS, pneumonia was more common with lesions of more than two pulmonary segments, and hospitalization in the intensive care unit and intensive care was more often required. In the structure of pneumonia complications, lung abscesses were noted - 1.7%, pleural empyema - 1.7%, pleurisy - 17%, sepsis, acute respiratory distress syndrome (ARDS) and multiple organ failure syndrome - 19.4%. The proportion of patients with complicated pneumonia in the P + IS group was higher, due to the greater number of severe pneumonia in this group. The severe course of pneumonia observed in 50% of cases directly correlated with the severity of the stroke, prolonged forced horizontal position and swallowing disorder in patients with IS. Also in the P + IS group, 2 patients (4.9%) with a protracted course of pneumonia were noted, where the treatment duration was 1-1.5 months. Pneumonia was qualified as a fatal complication (the direct cause of death) in the P + IS group in only 3 (7.3%) cases.

According to D.V. Odintsova et al. (2017), pneumonia develops much faster (on average 3 days) in patients with acute myocardial infarction than in patients with stroke (on average 11 days) [12]. Apparently, pneumonia in patients with stroke is more dependent on dysphagia due to neurological pathology, which increases the risk of aspiration, and according to the literature [1, 9, 21], among patients with aspiration pneumonia develops in 30% of cases.

The literature discusses the predictors of pneumonia in patients with acute myocardial infarction and stroke, since early diagnosis and treatment of this complication improves the prognosis and survival of these patients. These predictors include old age, male gender, duration of hospitalization, etc. It has been shown that the development of pneumonia is associated with old age [24]. In some works, there is evidence that pneumonia with stroke is more common in men and is also associated with old age [20]. Prolonged hospitalization also increases the risk of pneumonia [10, 12].

Our study of risk factors showed that COPD was diagnosed in 57 (73.1%) patients. A comparison of the frequency of COPD in patients with P + IS and P without stroke was performed. In 32 (78%) cases, out of 41 patients with P + IS, there was COPD, while in 25 (67.6%) cases, COPD was in patients with P without IS. Thus, there was no statistically significant difference (P = 0.436).

Type 2 diabetes was observed in 19 (24.4%) patients. The presence of diabetes did not affect the increase in the frequency of pneumonia. DM was noted in 10 (24.4%) of 41 patients with P + IS, and in 9 (24.3%) of 37 patients with pneumonia without IS (P = 0.976).

Thus, COPD and diabetes did not affect the incidence of pneumonia in patients with ischemic stroke, which is consistent with data from other authors [2, 10, 12].

It should also be noted that the presence of bad habits (chronic alcoholism (24.4%) and smoking (49%) in patients with P + IS group was of leading importance in the development and aggravation of the course of pneumonia).

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According to published data, the risk factors for pneumonia in stroke are the severity of neurological disorders, dysphagia, dysarthria, and not the type of stroke [10, 12, 24].

A prospective cohort study by C. Sellars et al. (2007) showed that pneumonia after stroke is associated with older age, dysarthria, the severity of neurological impairment after stroke, cognitive impairment, or an impaired water swallow test [4, 24]. A simple assessment of these indicators can be used to highlight a group of patients with a high risk of developing pneumonia after stroke. According to the authors, these five key factors form the basis of simple instrumental screening, which can be easily introduced into the clinical practice of nurses and medical staff who seek to identify patients with a higher risk of developing pneumonia [24]. It can also provide the basis for trial interventions that are thought to reduce the risk of pneumonia, including early movement activation, treatment of swallowing disorders, alternative feeding methods to reduce the effects of dysphagia, and early antibiotic treatment of oral pathogens [2, 4, 9, 10, 12].

In recent years, the A2DS2 scale has been proposed in the literature for assessing the risk of developing pneumonia in ischemic-type patients with stroke. She estimates age, gender, presence of atrial fibrillation, dysphagia and severity of stroke in points. With a value of more than 10, the risk of pneumonia is considered high and requires active preventive measures [22]. Timely diagnosis of aspiration pneumonia leads to effective therapy and improves prognosis [21].

The study of endothelial dysfunction and nitrosative stress in the development of pneumonia in patients after ischemic stroke. Due to the small literature data and the "scatter" of the norms of ET-1, NO2- and NO3- levels, adopted for various laboratory kits to determine the values of these indicators, to obtain the values of the norm, we examined a group of relatively healthy donors, as well as a group of patients with sharp ischemic stroke. The level of ET-1 in the blood of practically healthy ones was 9.5 ± 2.87 fmol / ml, and the content of NO products in the control group was 22.14 ± 0.61 μmol / L. The content of 3-NO-Tyr in the blood of healthy volunteers was 4.22 ± 1.25 ng / ml.

A study of the content of stable NO metabolites — nitrite and nitrate anions, 3-nitrotyrosine, and ET-1 levels in the blood serum of patients with pneumonia included in the study showed the following (Table 2).

Table 2: The content of NO metabolites and the level of ET-1 in serum in patients with pneumonia, depending on the presence of ischemic stroke in anamnesis

Indicators	Control (n=20)	Total (n=68)	P+IS (n=31)	P (n=37)
NO (μmol / L)	22,14±0,61	13,86±2,61*	11,16±1,23*#	16,56±1,37*
3-NO-Tyr (ng / ml)	4,22±1,25	11,72±3,57**	13,58±5,11**#	9,86±2,03*
ET-1 (fmol / ml)	9,5±2,87	38,79±7,11**	45,32±6,18**#	32,26±8,05**

Note: * - differences relative to the data of the control group are significant (* - P <0.05, ** - P <0.01); # - differences relative to the comparison group are significant (# - P <0.05)

Compared with the control group in patients with pneumonia, the course of the disease was accompanied by a statistically significant decrease in the level of active NO metabolites in the blood by 1.6 times (13.86 ± 2.61 μmol /

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L) (P <0.05). Moreover, in patients with P + IS, the content of NO metabolites was lower (11.16 ± 1.23 μmol / L) than in patients with P without stroke (16.56 ± 1.37 μmol / L), an average of 1.48 times (P <0.05). Such changes may indicate a violation of NO metabolism and a pronounced violation of the dilatation properties of the vascular wall [14, 15].

The content of 3-NO-Tyr in the blood serum of patients with pneumonia compared with the control was increased on average by 2.78 times (P <0.01). Significant differences between the studied parameter between patients P and P + IS were revealed. So, in patients with P + IS, the content of 3-NO-Tyr in the blood serum (13.58 ± 5.11 ng / ml) was higher than the values of patients with P without stroke (9.86 ± 2.03 ng / ml) on average 1,38 times (P <0.05). The data obtained indicate that pneumonia is accompanied by the development of nitrosative stress in patients. The intensity of nitrosative stress in the blood according to the studied parameters was more pronounced in patients with P + IS.

In patients with pneumonia, an increase in ET-1 level was determined in comparison with the control group (38.79 ± 7.11 and 9.5 ± 2.87 fmol / ml, respectively; P <0.01). The level of ET-1 in the blood serum of patients with P + IS was 45.32 ± 6.18 fmol / ml, which was significantly 1.4 times higher than the value of patients P without stroke - 32.26 ± 8.05 fmol / ml (P < 0.05).

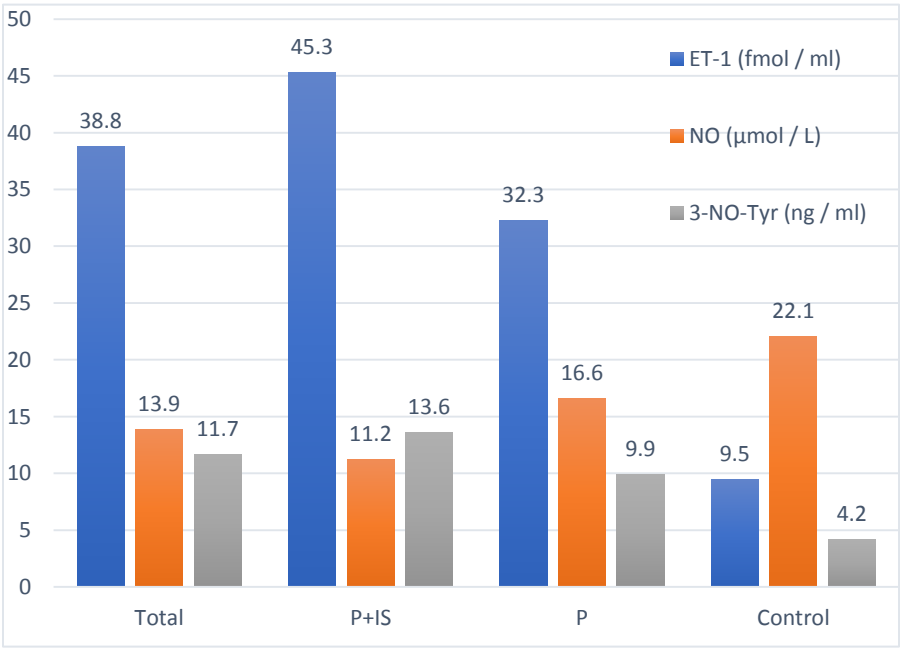

Fig. 2: Serum levels of ET-1, NO and 3-NO-Tyr in both groups of patients with pneumonia and in healthy donors.

When studying the correlation relationships of the data obtained during the study in patients with pneumonia, significant statistically significant gender and age differences in the levels of the studied markers of ED and nitrosauric stress were not detected. The correlation between these markers and clinical scores evaluating the severity of stroke and pneumonia was very weak. Nevertheless, it can be noted that there is a positive correlation between the level of 3-NO-Tyr and ET-1 (r = 0.53; P <0.01) and a negative relationship between the level of NO and 3-NO-Tyr (r = -0.420; P <0, 05), NO, and ET-1 (r = -0.534; P <0.01). This suggests that ED and nitrosative

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stress are more pronounced as pneumonia progresses and worsens in all groups of patients, especially against the background of transferred IS (Table 3).

Table 3: Correlation relationships of factors of endothelial dysfunction and nitrosative stress in pneumonia in patients after IS(r)

Factors	**NO**	**3-NO-Tyr**	**ET-1**
Age	-0,112	0,012	0,206
Glasgow Scale Scores	0,028	-0,121	-0,105
Scandinavian Scale Scores	-0,044	0,307	0,024
Fine Scale Scores	0,019	-0,187	-0,029
NO		-0,534	-0,83
3-NO-Tyr	-0,420		0,53
ET-1	-0,534	0,53	

This study showed that the level of ET-1 in the blood of patients with pneumonia is one of the most important indicators of endothelial dysfunction, as well as one of the independent factors in predicting the course of the disease in these patients. Deterioration of the clinical picture of the disease is accompanied by a multiple increase in the level of ET-1. Given the foregoing, we can conclude about the prognostic value of determining the level of ET-1. The correlation of ET-1 level with the presence of ischemic stroke noted by us is consistent with the data of other authors on the relationship of this ED marker with cardiovascular diseases [2, 14, 15].

As a result of the studies, a significant increase in the concentration of 3-NO-Tyr in patients with pneumonia was established in comparison with practically healthy people. Such a change may indicate a pronounced oxidation of the thiol groups of membrane peptides, leading to destruction of the lipid layer of cell membranes, as well as a significant severity of nitrosative stress. A decrease in the level of nitric oxide, determined by the content of its nitrite anion, indicated a violation of the synthesis of NO in endothelial cells and confirmed the development of ED in pneumonia.

With the development of oxidative stress, an excess of the active forms of oxygen and nitrogen accumulates. Active forms of oxygen are generated by cell mitochondria during hypoperfusion, ischemia / reperfusion (for example, in patients with heart failure, coronary artery disease, or IA) and reoxygenation (for example, with myocardial revascularization as a result of coronary artery bypass grafting, CABG). The main active form of nitrogen - NO - is a unique molecule that performs a number of extremely important functions in the body both in physiological conditions and in pathology. One unpaired electron makes NO a very reactive free radical [3, 19, 23]. Nevertheless, most researchers believe that the cytotoxic effects attributed to NO belong to peroxynitrite (ONOO-), which is formed in the reaction of NO with a superoxide molecule (O2–) [3, 29].

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Regarding the active forms of nitrogen, the data are more controversial today. According to V.Yu. Titova et al. (2014), normally the majority of living tissues, including blood, contain a minimal (less than 100 nM) nitrite concentration. The data on the nitrite content in the blood plasma of healthy people at higher concentrations are apparently the result of the insufficient selectivity of the methods used [16]. A number of studies have shown that in the blood of patients suffering from inflammatory diseases, there is an increase in the content of nitrite [17]. The formation of nitrite (NO2) and non-thiolate nitroso compounds in human blood upon activation of leukocytes occurs mainly due to the destruction of plasma compounds - donors NO, and not the intensification of NO synthesis [16].

According to some authors, as a result of the action of active forms of NO, either oxidative stress or nitrosative stress develops with the formation of nitrites, nitrosamines, 3-NO-Tyr. The latter affects the formation of a subpopulation of T-regulatory cells and, as a result, a special pattern of inflammation, which results in remodeling of the bronchial wall, irreversible bronchial obstruction, and therapeutic resistance. The key role in the regulation of the immune response and the development of inflammatory, allergic reactions is played by T-regulatory cells, which under the conditions of nitrosative stress mature in the presence of high concentrations of 3-NO-Tyr [11]. 3-NO-Tyr has been identified as a marker of inflammation and NO production. Nitrotyrosine is formed in the presence of an active metabolite of NO. Various pathways lead to nitrotyrosine production, including the formation of peroxynitrite. Since nitrotyrosine is a stable end product of oxidation of peroxynitrite, an assessment of its plasma concentration may be useful as a marker of in vivo NO-dependent damage. Since NOX is only an indicator of enhanced NO production, the associated protein nitrotyrosine may be a more suitable marker for assessing damage induced by reactive nitrogen intermediates, NO derivatives. Moreover, most proteins circulating in the bloodstream have a longer half-life than NOX levels [3]. The presence of nitrotyrosine has been shown in various inflammatory processes, including the formation of atherosclerotic plaques, celiac disease, rheumatoid arthritis, chronic renal failure, and septic shock. Normally, low, undetectable levels of nitrotyrosine are present in plasma. Despite the fact that some authors report the absence of significant changes in the plasma content of nitrotyrosine in patients after operations on the heart and coronary vessels, as well as the absence of a correlation between the level of nitrotyrosine and the incidence of postoperative complications [3], the role of peroxynitrite, which is evaluated in the clinic by the level of nitrotyrosine, in the pathogenesis of oxidative and nitrosative stress is not in doubt.

Thus, the study of indicators of nitrosative stress and ED, indicating signs of inflammation, is justified and necessary, because it contributes to the early detection and prediction of such a serious and socially significant disease as pneumonia. At the same time, the severity of these indicators (high concentrations of ET-1 and 3-NO-Tyr, low concentrations of NO metabolites) against the background of transferred ischemic stroke, as well as in cases of increased pneumonia, can be a prognostically unfavorable factor determining the further tactics of management of such patients (administration of NO donors, inhaled nitric oxide [18], etc.).

IV. CONCLUSIONS

1. Patients with stroke are at increased risk of developing pneumonia due to the frequent development of dysphagia, the presence of aspiration, forced horizontal position, respiratory failure. However, the diagnosis of pneumonia in this group of patients is difficult due to the frequent absence of signs of the disease in the early stages,

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which requires a comprehensive assessment of clinical, laboratory and radiological data, early detection of development predictors. At the same time, fever, leukocytosis, and auscultatory data may indicate the development of pneumonia in this group of patients, which requires the timely start of adequate antibiotic therapy.

2. The course of pneumonia in patients who have undergone ischemic stroke is more difficult than in patients without a history of stroke, aggravates the course of the underlying disease, increasing mortality, and in some cases, pneumonia is the direct cause of death of patients.

3. In addition to the transferred ischemic stroke, important predictors that increase the risk of developing and worsening pneumonia include old age, male gender, background and comorbid conditions (COPD, diabetes, cardiovascular diseases), the presence of bad habits (chronic alcoholism, smoking), as well the severity of neurological deficit (stem stroke, impaired consciousness and breathing, gross paresis, paralysis, bulbar, pseudobulbar syndrome, the presence of dysphagia, aspiration, prolonged forced horizontal position).

4. With pneumonia, there is a natural increase in the level of endothelin-1 and 3-nitrotyrosine in the blood serum with a decrease in the level of NO metabolites, which indicates the development of nitrosative stress, inflammation and endothelial dysfunction, as well as impaired hemodynamics in the pulmonary circulation. Severe and prolonged production disturbances of these markers depend on the severity of pneumonia, the presence and severity of an ischemic stroke, complications and concomitant diseases.

5. The levels of endothelin-1, 3-nitrotyrosine and NO metabolites in blood serum in patients with pneumonia of various etiologies can be used as a criterion for assessing the severity of the disease, prognosis, the possibility of a protracted course, the addition of complications and the fullness of recovery.

Conflict of interest. The authors declare that there is no conflict of interest in the preparation of this article.

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