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## Diagnostic value of blood biomarkers for the diagnosis of lung cancer

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### Abstract

**Aim:** to evaluate the diagnostic value of 20 biomarkers in lung cancer and to determine their informative value for potential use in clinical practice.

**Material and methods.** The study included 85 patients with non-small cell lung cancer (NSCLC) and 190 healthy volunteers. Biomarker levels were measured using modern immunological and biochemical methods. Statistical analysis included the Mann–Whitney U test, and diagnostic performance was assessed by the area under the receiver operating characteristic curve (AUC). For markers showing an inverse association, an additional ROC analysis was performed with inversion of the outcome variable. Optimal biomarker cut-off values were determined using the Youden's index.

**Results.** Patients with NSCLC demonstrated statistically significant changes in the concentrations of most of the studied biomarkers after strict Bonferroni correction. Increased levels of CEA, CA 125, HE4, B2M,

high-sensitivity C-reactive protein (hsCRP), D-dimer, CYFRA 21-1, and LRG-1 were observed, along with decreased levels of ApoA1, ApoA2, TTR, ApoA4, RANTES, and VEGFR1. The highest AUC values were shown by HE4 (0.903), ApoA2 (0.860), CYFRA 21-1 (0.836), ApoA1 (0.795), D-dimer (0.793), TTR (0.790), ApoA4 (0.784), B2M (0.765), and LRG-1 (0.757).

**Conclusion.** Certain blood biomarkers demonstrate high AUC values, indicating their potential utility for the diagnosis of NSCLC. The combined use of multiple biomarkers may improve the effectiveness of minimally invasive lung cancer diagnostics, which warrants further investigation. Validation of these findings in multicenter studies is required.

**Keywords:** lung cancer, diagnosis, biomarkers, HE4, CYFRA 21-1.

**Conflict of interest:** nothing to disclose.

### Citation

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## Диагностическая ценность биомаркеров крови для диагностики рака легкого

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### Аннотация

**Цель:** оценить диагностическую ценность 20 биомаркеров при раке легкого и определить их информативность для возможного использования в клинической практике.

**Материал и методы.** В исследование были включены 85 пациентов с немелкоклеточным раком легкого (НМРЛ) и 190 здоровых добровольцев. Уровни биомаркеров определялись современными иммунологическими и биохимическими методами. Статистическая обработка включала U-критерий Манна – Уитни, а диагностическая ценность оценивалась по площади под ROC-кривой (AUC). Для оценки информативности маркеров с обратной ассоциацией проведен дополнительный ROC-анализ с инверсией переменной состояния. Определены пороговые значения биомаркеров с использованием индекса Юдена.

**Результаты.** У пациентов с НМРЛ отмечены статистически значимые изменения концентраций большинства исследованных биомаркеров с учетом строгой поправки Бонферрони: повышение уровней CEA, CA

125, HE4, B2M, вчСРБ, D-димер, CYFRA 21-1, LRG-1, а также снижение ApoA1, ApoA2, TTR, ApoA4, RANTES и VEGFR1. Наибольшие значения площади под кривой показали HE4 (0,903), ApoA2 (0,86), CYFRA 21-1 (0,836), ApoA1 (0,795), D-димер (0,793), TTR (0,79), ApoA4 (0,784), B2M (0,765), LRG1 (0,757).

**Выводы.** Отдельные биомаркеры крови демонстрируют высокие значения площади под кривой, что указывает на потенциал их применения с целью диагностики НМРЛ. Комплексное использование биомаркеров может повысить эффективность малоинвазивной диагностики рака легкого, что требует дальнейшего исследования. Для подтверждения полученных данных требуется валидация в многоцентровых исследованиях.

**Ключевые слова:** рак легкого, диагностика, биомаркеры, HE4, CYFRA 21-1.

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НМРЛ – немелкоклеточный рак легкого; НДКТ – низкодозная компьютерная томография.

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## INTRODUCTION

Lung cancer remains one of the most important problems of modern oncology. According to the Global Cancer Observatory (GCO) project of the World Health Organization, in 2022, lung cancer was the leader in morbidity (2,480,675 new cases) and mortality from cancer (1,817,469 lethal outcomes) [1]. From the biological standpoint, lung cancer is characterized by a variety of morphological types, and the non-small cells lung cancer (NSCLC) accounts for approx. 85% of all cases of the disease [2].

In the Russian Federation, the situation is further aggravated by the fact that 42.2% of the patients are diagnosed at advanced stages, which makes the prognosis considerably poorer [3]. The 5-year overall survival (OS) median varies from 68.4% at stage I to 5.8% at stage IV of the disease [4]; therefore, the development of new affordable methods of early diagnostics of lung cancer still has high clinical significance.

The only method of early identification of lung cancer with a proven efficiency is the low-dose computed tomography (LDCT) in high-risk groups. The NLST study showed that the annual LDCT screening among high-risk groups decreases mortality from lung cancer by approx. 20% [5]. Based on these data, LDCT screening is implemented in some countries [1]. In Russia, only the pilot projects are underway, and the national lung cancer screening has not been implemented so far. The LDCT method has serious limitations: it involves only the smokers and provides a very high level of false-positive results (above 90% of identified foci, according to NLST data, were benign); besides, multiple irradiation poses extra risk [1, 5]. Therefore, the search for other non-invasive diagnostic methods supplementing the screening and identifying tumors at early stages is highly important.

One of prospective areas of study is the use of tumor biomarkers identified in the blood test. The tumor produces fragments of DNA, RNA, proteins, and exosomes into the bloodstream, i.e. products that may serve as indicators of a malign tumor and that may be viewed as potential biomarkers [6, 7].

In the context of studying the biomarkers to diagnose lung cancer, especially interesting are the biomarkers of different groups depending on their biological function and chemical origin. In the context of lung cancer diagnostics, tumor-associated including carcino-fetal antigens were studied; inflammation and immune response markers, or

chemokines; metabolism markers and apolipoproteins; coagulation and angiogenesis markers [8].

Thus, the problem of late diagnostic of lung cancer and the lack of a simple screening test necessitate further study of the role of blood biomarkers in the NSCLC diagnostics. The search for reliable non-invasive markers capable of diagnosing the tumor at an early stage will potentially improve treatment outcomes and decrease the disease mortality.

## MATERIAL AND METHODS

### *Patients, taking of samples, measurements of biomarkers*

The study included 85 patients with histologically confirmed non-small cells lung cancer and 190 healthy individuals. Men accounted for 63.5% (54) patients in the study group, and 52.1% (99) in the control group. The median age of patients in the study group was 62.14 years, and 48.86 years in the control group.

Levels of 20 biomarkers were measured including the following: apolipoproteins A1, A2, A4, B (ApoA1, ApoA2, ApoA4, ApoB), alpha fetoprotein (AFP), beta-2-microglobulin (B2M), carbohydrate antigen 19-9 (CA 19-9), cancer antigens 15-3 and 125 (CA 15-3, CA 125), carcinoembryonal antigen (CEA), cytokeratin fragments 19 (CYFRA 21-1), epididymis protein 4 (HE4), highly-sensitive human C-reactive protein (hsCRP), D-dimer, total prostate-specific antigen (tPSA), soluble form of vascular cell adhesion molecule 1 (sVCAM 1), transthyretin (TTR), regulator of activity of normal T-lymphocyte expression and secretion (RANTES), vascular endothelium growth factor receptor 1 (VEGFR-1), leucine-rich  $\alpha$ -2 glycoprotein 1 (LRG-1). The analysis of tPSA was only performed among men (the tables provide metrics for the male subgroup). The levels of ApoA4, RANTES, VEGFR-1 and LRG-1 were not altered in 10 patients die to lack of solutes of interest at the moment of serum analysis.

The levels of AFP, CA 15-3, CA 19-9, CA 125, HE4, CEA, CYFRA 21-1 and tPSA were measured by electrochemiluminescence assay on the Cobas e411 analyzer (Roche Diagnostics, Germany); levels of hsCRP, ApoA1, ApoA4, ApoB, TTR were measured on the Advia 1800 analyzer (Siemens Healthcare, Germany) by immunoturbidimetric technique; levels of sVCAM-1, RANTES, VEGFR-1 and LRG-1 were identified by enzyme-linked immunosorbent assay (ELISA) (QuantikineR kits, R&D systems, US) using a Biochrom

Anthos 2020 microplate reader (Biochrom, UK); B2M and D-dimer were measured by chemiluminescent immunoassay on the Immulite 2000 analyzer (Siemens Medical Solutions, USA); and the ApoA2 level was measured using the fermentative colorimetric method (Randox laboratories, UK).

The study was approved by the local ethical committee of the Sechenov First Moscow State Medical University. All patients provided an informed consent for the participation in the study.

**Statistical processing of data**

The statistical processing was performed after formalization and conversion of the obtained data into electronic spreadsheets by various methods of the SPSS Statistics v. 23.0 software suite (IBM, USA).

The distribution of the main analyzed variables was determined using the Kolmogorov–Smirnov test and deviated from normality; therefore, nonparametric statistical methods were employed. For descriptive statistics of quantitative variables, the median and interquartile range or the mean and standard deviation were used. Comparisons between categories of independent quantitative variables were performed using the Mann–Whitney U test. Differences were considered statistically significant at a p-value <0.05. Qualitative (categorical) indicators are presented as absolute and relative frequencies (n, %).

On the next stage of the study, to assess the diagnostic significance of each of the biomarkers, the analysis of Receiver Operating Characteristic was performed (ROC analysis). The binary variable “presence of lung cancer” (1 – disease, 0 – control group) was used as the state variable. For each biomarker, a receiver operating characteristic curve was plotted, and the area under the curve (AUC), standard error, statistical significance level (p-value), and 95% confidence interval were calculated. Depending on the direction of the association between the marker and the diagnosis, higher or lower biomarker values were used as predictive markers during ROC analysis.

The analysis was performed in the SPSS Statistics using its ROC Curve Analysis built-in function. The interpretation of the diagnostic value of the AUC was performed in compliance with the following criteria: AUC < 0.6 – low diagnostic value, 0.6–0.75 – moderate value, 0.75–0.9 – high value, > 0.9 – very high value. In order to assess the optimal threshold value of biomarkers, the Youden’s index was used.

**RESULTS**

**Characteristics of the studies sample**

The study included 275 patients, of which 85 were the NSCLC group (study group), and 190 were healthy individuals (control group). In the entire cohort, men prevailed (n=153; 55.6%) over women (n=122; 44.4%).

The clinical and demographic characteristics of the studies population are shown in **Tables 1** and **2**. The control group included 91 women aged from 41 to 68 years and 99 men aged from 40 to 64 years. The NSCLC group included 31 women aged from 42 to 80 and 54 men aged from 36 to 78 years. The median age of the control group

Metric	Category	Value
<b>T-status:</b>	T1	24 (28.2%)
	T2	42 (49.4%)
	T3	9 (10.6%)
	T4	5 (5.9%)
	Tx	5 (5.9%)
<b>N-status:</b>	N0	39 (45.9%)
	N1	5 (5.9%)
	N2	8 (9.4%)
	Nx	33 (38.8%)
<b>M-status:</b>	M0	61 (71.8%)
	M1	6 (7.1%)
	Mx	18 (21.2%)

**Table 1.** Clinical characteristics of patients in the study group (NSCLC)

**Таблица 1.** Клинические характеристики пациентов исследуемой группы (НМРЛ)

was 48.86 [95% CI 47.97–47.94] years, the average age in the study group was 62.14 [95% CI 60.07–64.21] years.

The analysis of clinical characteristics of the study group showed the following distribution in the T-status: T1 in 24 (28.2%), T2 in 42 (49.4%), T3 in 9 (10.6%), T4 in 5 (5.9%) patients. In 5 (5.9%) patients, the T-status was not specified (Tx).

Distribution in the N-status: lack of metastases in regional lymph nodes (N0) was seen in 39 (45.9%) patients, N1 in 5 (5.9%), N2 in 8 (9.4%), while in 33 (38.8%) patients the N-status was not specified (Nx).

Distribution in the M-status: lack of remote metastases (M0) was diagnosed in 61 (71.8%) patients, the presence of metastases (M1) in 6 (7.1%), while in 18 (21.2%) cases the M-status was not specified (Mx).

Because of the retrospective character of the analysis the possibility of specifying clinical data of the patients was limited, therefore, the “x” code in the TNM status was used to identify the non-specified status of the T, N and M.

**Diagnostic value of isolated biomarkers**

On the first stage of the analysis, median concentrations were identified between the groups (NSCLC vs. control). The significance of differences was assessed using the Mann – Whitney non-parametric test for two independent samples, which was accounted for by the distribution of indicators different from normal. The obtained data is shown in **Table 3**.

Comparative analysis of biomarker concentrations revealed statistically significant differences between the study and control groups for the majority of the investigated

Metric	Control group	Lung cancer	p-value
<b>Sex:</b>			0.103
Female	91 (47.9%)	31 (36.5%)	
Male	99 (52.1%)	54 (63.5%)	
<b>Age, years</b>			<0.001
Median, (Q1-Q3)	47 (45–53)	63 (56–68)	

**Table 2.** Demographic characteristics (sex, age) of the study sample  
**Таблица 2.** Демографические характеристики (пол, возраст) исследуемой выборки

Indicator	Unit	Control group	NSCLC group	p-value
AFP	IU/mL	2.40 [1.60; 3.40]	2.02 [1.50; 3.00]	0.131
CEA	ng/mL	1.60 [1.00; 2.40]	2.95 [1.40; 5.50]	<0.001*
CA 19-9	IU/mL	4.75 [3.00; 8.00]	6.50 [3.92; 12.43]	0.012
CA 125	IU/mL	8.70 [6.70; 12.80]	16.50 [9.75; 25.04]	<0.001*
HE4	pmol/L	48.45 [42.50; 57.70]	95.60 [71.70; 125.10]	<0.001*
tPSA	ng/mL	0.880 [0.620; 1.230]	0.937 [0.570; 2.010]	0.247
CA 15-3	IU/mL	14.75 [10.80; 18.60]	17.71 [12.50; 24.90]	0.001
B2M	ng/mL	1441.00 [1297.00; 1637.00]	1801.00 [1526.00; 2233.00]	<0.001*
hsCRP	mg/L	0.00 [0.00; 2.00]	3.00 [1.00; 11.00]	<0.001*
D-dimer	ng/mL	83.50 [57.50; 140.00]	210.00 [123.00; 340.00]	<0.001*
CYFRA 21-1	ng/mL	1.26 [1.00; 1.64]	2.52 [1.62; 3.76]	<0.001*
ApoA1	g/L	1.57 [1.42; 1.76]	1.29 [1.09; 1.49]	<0.001*
ApoA2	g/L	0.289 [0.266; 0.321]	0.218 [0.181; 0.249]	<0.001*
ApoB	g/L	1.01 [0.86; 1.18]	0.93 [0.79; 1.12]	0.016
TTR	mg/dL	26.00 [22.00; 29.00]	19.00 [15.00; 24.00]	<0.001*
sVCAM-1	ng/mL	640.00 [565.00; 743.00]	683.00 [567.00; 897.00]	0.031
ApoA4	g/L	71.00 [56.40; 79.90]	44.15 [29.05; 64.00]	<0.001*
RANTES	pg/mL	51853.00 [40784.00; 68671.00]	44249.50 [22911.50; 62450.50]	0.001*
VEGFR1	pg/mL	121.00 [107.00; 135.00]	94.50 [78.50; 143.00]	<0.001*
LRG-1	pg/mL	52902.00 [39539.00; 68016.00]	74278.50 [61214.00; 103649.00]	<0.001*

Notes: \* – statistically significant differences after correction for multiple comparisons.

Примечания: \* – статистически значимые различия после выполнения поправки на множественные сравнения.

**Table 3.** Biomarker values in the studied groups (median, [Q1;Q3])

**Таблица 3.** Значения биомаркеров в исследуемых группах (медиана, [Q1;Q3])

parameters. No statistically significant differences were found for AFP ( $p = 0.131$ ) or tPSA ( $p = 0.247$ ).

The study included an analysis of over 20 biomarkers, which increases the possibility of type I error due to multiple comparisons. Under the strict Bonferroni correction (significance threshold  $p < 0.0025$ ), statistical significance was maintained for the majority of biomarkers, including HE4, D-dimer, CYFRA 21-1, CA 125,  $\beta$ 2-microglobulin, hs-CRP, ApoA1, ApoA2, ApoA4, TTR, LRG-1, and VEGFR1. In contrast, differences for CA 19-9 ( $p = 0.012$ ), CA 15-3 ( $p = 0.001$  at borderline), ApoB ( $p = 0.016$ ), and sVCAM-1 ( $p = 0.031$ ) no longer reached the level of statistical significance.

Patients with NSCLC demonstrated significantly higher levels of (2.95 vs. 1.6 ng/mL), CA 125 (16.5 vs. 8.7 IU/mL), HE4 (95.6 vs. 48.45 pmol/L), hsCRP (3 vs. 0 mg/L), D-dimer (210.00 vs. 83.5 ng/mL), CYFRA 21-1 (2.52 vs. 1.26 ng/mL), and LRG-1 (74278.5 vs. 52902.00 pg/mL). Some indicators, conversely, demonstrated lower values in the patients of the control group versus control group, including the ApoA2 (0.226 vs. 0.296 g/L), ApoB (0.96 vs. 1.03 g/L), TTR (19.09 vs. 25.81 mg/dL) and ApoA4 (46.98 vs. 68.71 g/L).

Next stage involved the ROC-analysis (Table 4) that revealed significant differences in the diagnostic value of the studied biomarkers.

The highest diagnostic indicators were seen in HE4, CYFRA 21-1, ApoA1, D-dimer, TTR, ApoA4, B2M and LRG-1. This might point at the potential of use of these biomarkers in order to diagnose the NSCLC. Moderate predictive value was seen in CA 125, hsCRP, CEA, VEGFR1, RANTES and CA 15-3.

To determine the optimal threshold concentrations of biomarkers for most effectively differentiating patients with NSCLC from control group individuals, Youden's index was used. The optimal thresholds, sensitivity (Se),

specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and test accuracy are presented in **Table 4**. For most biomarkers, diagnostic relevance was observed when the concentration exceeded the threshold; for ApoA1, ApoA2, ApoA4, TTR and VEGFR1, a decrease in the level was associated with the presence of the disease.

## DISCUSSION

In the recent years, various minimally invasive approaches to early diagnostics of NSCLC based on the analysis of blood biomarkers received much attention. Hundreds of candidates are described in the literature, from tumor markers (CEA, CYFRA 21-1, CA 125 etc.) to genetic markers (circulating tumor DNA, mRNA, exosomes), biochemical and proteomic indicators. The results of our study match the literature data with respect to CYFRA 21-1, CA 125, D-dimer and HE4 as the most informative biomarkers for the lung cancer diagnostics. It is to be noted that the majority of lipid proteins (ApoA1, ApoA2, ApoA4, ApoB, TTR) and the RANTES, B2M, LRG-1 and VEGFR1 molecules have not been hitherto analyzed in key publications on biomedicine as markers for lung cancer diagnostics. High levels of AUC for ApoA2, LRG-1, B2M, ApoA1 and TTR in the early identification of lung cancer for such metrics as sensitivity and specificity demonstrated in our study open new opportunities to decode molecular mechanisms of tumor progression of lung cancer and may facilitate development of new diagnostic approaches.

Increased levels of CEA, CYFRA 21-1 and CA 125 in the NSCLC group match the findings of earlier studies of M. Li et al. (2015) [9]. The marker CA 19-9, specific for the gastrointestinal tract tumors, may still be produced by bronchial glands and is moderately increased in approx. one third of patients with NSCLC. Meanwhile, T. Kodama

Biomarker	AUC	95% CI	Threshold value	Direction of correlation	Sensitivity, Se	specificity, Sp	PPV	NPV	Accuracy	p-value
HE4	0.903	0.857–0.948	68.445	≥	0.835	0.879	0.755	0.923	0.866	< 0.001
ApoA2	0.86	0.807–0.914	0.235	≤	0.718	0.968	0.910	0.885	0.891	< 0.001
CYFRA 21-1	0.836	0.780–0.893	2.24	≥	0.612	0.937	0.813	0.844	0.836	< 0.001
ApoA1	0.795	0.733–0.857	1.395	≤	0.694	0.805	0.615	0.855	0.771	< 0.001
D-dimer	0.793	0.731–0.855	116.5	≥	0.8	0.679	0.527	0.884	0.716	< 0.001
TTR	0.79	0.727–0.852	20.5	≤	0.576	0.863	0.653	0.82	0.775	< 0.001
ApoA4	0.784	0.717–0.850	45.95	≤	0.553	0.899	0.689	0.833	0.8	< 0.001
B2M	0.765	0.700–0.830	1594	≥	0.682	0.732	0.532	0.837	0.716	< 0.001
LRG-1	0.757	0.688–0.826	56105	>	0.842	0.593	0.454	0.903	0.664	< 0.001
CA 125	0.749	0.683–0.816	14.5	≥	0.576	0.832	0.605	0.814	0.753	< 0.001
hsCRP	0.735	0.667–0.802	2.105	≥	0.576	0.853	0.636	0.818	0.767	< 0.001
CEA	0.671	0.599–0.742	2.925	≥	0.506	0.842	0.589	0.792	0.738	0.039
VEGFR1	0.658	0.582–0.733	98	≤	0.553	0.873	0.636	0.829	0.781	< 0.001
RANTES	0.628	0.551–0.704	29274	≤	0.368	0.915	0.636	0.783	0.759	0.001
CA 15-3	0.623	0.549–0.696	19.725	≥	0.424	0.811	0.5	0.759	0.691	0.01
CA 19-9	0.595	0.521–0.669	5.7	≥	0.576	0.616	0.402	0.765	0.604	0.02
ApoB	0.591	0.516–0.665	0.975	≤	0.624	0.553	0.384	0.766	0.575	0.007
sVCAM-1	0.581	0.507–0.656	808.5	≥	0.4	0.878	0.597	0.765	0.73	0.006
tPSA	0.557	0.460–0.653	1.59	≥	0.389	0.859	0.6	0.72	0.693	0.337
AFP	0.557	0.483–0.631	2.58	≤	0.682	0.489	0.374	0.775	0.549	0.087

**Table 4.** ROC analysis of biomarkers indicating direction of association, optimal cutoff value, and diagnostic characteristics

**Таблица 4.** ROC-анализ биомаркеров с указанием направления связи, оптимального порогового значения и диагностическими характеристиками

et al. (2007) note that the increased level of CA 19-9 is seen in approx. 40% of patients with non-malignant lung diseases such as idiopathic interstitial pneumonia, collagen-associated pulmonary fibrosis, diffuse panbronchiolitis and bronchoectases [10]. The values of sensitivity (57.6%) and specificity (61.6%) of this biomarker identified in our study align with literature data.

In our study, the marker HE4 (human epididymis secretory protein 4) was one of the most perspective biomarkers to diagnose NSCLC with sensitivity and specificity values of 83.5 and 87.9, respectively. These data align with findings of Y He et al. (2019), according to which the values of sensitivity and specificity of this biomarker for the diagnostics of NSCLC are 73% and 86%, respectively. The same study also notes that in NSCLC patients the concentrations of HE4 are statistically significantly higher than in healthy individuals, and the increase of HE4 is registered in early stages and does not depend on the size of the tumor [11].

NSCLC patients demonstrate activation of systemic inflammation and coagulation. According to J. Torrecilla et al. (2014), the highly sensitive C-reactive protein (hsCRP) is several times higher in NSCLC patients (in our study, the median hsCRP is 3 mg/L vs. 0 mg/L in healthy individuals,  $p < 0.001$ ). CRP is an acute-phase protein whose synthesis is stimulated by proinflammatory cytokines (IL-6) in response to the presence of the tumor. The increase of its level points at an active inflammatory process; for example, it is known that  $CRP > 40$  mg/L is associated with the presence of metastases in NSCLC [12]. Another important biomarker is the D-dimer, a product of fibrin proteolysis. In the event of lung cancer, its levels are often elevated due to tumor-induced hypercoagulation. In our study, in the NSCLC group the median level of the D-dimer was 210 ng/mL vs. 83.5 ng/mL in the control group ( $p < 0.001$ ). The elevated

level of the D-dimer is characteristic of malignant tumors and reflects activation of coagulation and fibrinolysis. According to N. De Pooter et al. (2021), the basal levels of D-dimer increase over age, and the especially high levels in elderly NSCLC patients are to be interpreted with allowances for age [13]. Nevertheless, the high levels of D-dimer in NSCLC correlate with advanced stages of the disease and act as an independent poor prognosis factor [14]. Thus, the CRP and the D-dimer are not specific of lung cancer but indicate the severity of the disease and the systemic processes.

Statistically significant results were found for the biomarkers from the acute phase protein group and for apolipoproteins. We found that in the event of NSCLC, levels of some transport proteins and blood apolipoproteins decrease. For example, the levels of transthyretin (TTR, prealbumin), the transport protein of thyroxin and retinol, was significantly lower in lung cancer patients than in healthy individuals (19.0 vs. 26.0 mg/dL,  $p < 0.001$ ). The decrease of TTR may reflect the nutritive status: deficient prealbumin is often seen in some oncological diseases due to inflammation-induced malnutrition [15]. Among patients in the NSCLC group, apolipoprotein levels were reduced: ApoA1, ApoA2, ApoA4 and ApoB. According to R. Xu et al. (2023), in the event of NSCLC, reduction of ApoA1 and ApoA2 are seen with simultaneous increase of ApoB in comparison with the control group, which partially aligns with our findings [16].

Changes of levels of some cytokines and vascular factors are also noteworthy. The level of sVCAM-1 was elevated in the NSCLC group (median 683 vs. 640 ng/mL,  $p=0.031$ ). Among cytokines, most interesting is the RANTES chemokine (CCL5). In our study, the RANTES level was lower among patients with NSCLC than in the group of healthy volunteers (median 44249 vs. 51853 pg/mL,  $p=0.001$ ). CCL5 (RANTES) is a

proinflammatory cytokine attracting lymphocytes; the decrease of its systemic level in lung cancer may reflect exhaustion of the immune system or binding of CCL5 in the tissues by tumor microenvironment. Some studies report an ambiguous role of CCL5: on the one hand, the high level of RANTES was found in some tumors and related to their progression, on the other hand, in some localizations (e.g., breast cancer) paradoxically high concentrations of RANTES in the blood are associated with a more favorable prognosis, likely due to an active anti-tumor immune response [17]. Data on systemic CCL5 for lung cancer are limited; it can be supposed that a decrease of the circulating RANTES reflects its absorption by tumor and stromal cells in the lungs and the immunosuppressive action related to it. Another important factor of angiogenesis in cancer is the VEGF and its receptors. We measured the levels of VEGFR1 (sFlt-1) and found a mild but reliable increase of this receptor in NSCLC patients (94.5 vs. 121 pg/mL,  $p < 0.001$ ). The sVEGFR1 is an endogenous inhibitor of angiogenesis binding the excessive VEGF-A; its growth may reflect a compensatory reaction to the excessive production of VEGF by the tumor. In general, the increase of sVCAM-1, CRP, D-dimer and the decrease of RANTES may indicate a presence of a systemic inflammation, activation of the endothelium and alteration of the immune regulation in NSCLC.

The comprehensive analysis of the literature and our own data enabled the identification of both known and potentially novel biomarkers with high diagnostic potential for lung cancer diagnosis. Existing scientific evidence and our findings indicate the possibility of improving the diagnostic accuracy of NSCLC by developing panels comprising a combination of biomarkers. This approach requires validation in multicenter studies involving larger patient cohorts.

Another key result of our study was the quantitative determination of optimal cut-off values of 20 studies biomarkers to diagnose the NSCLC using the Youden's index. This finding comprises an important scientific contribution: before, many publications and meta-analyses did not provide cut-off values or varied greatly, which complicated comparison of results of different studies and their clinical applications. Thus, in the meta-analysis of

Y. He et al. (2019) studying the diagnostic value of HE4 to diagnose lung cancer, the cut-off values varies from 32.45 to 150 pmol/L [11]. The wide scattering of cut-off values may complicate reproducibility of results and interpretation of scientific data.

The cut-off values proposed by us allow for alignment and standardization of the approach towards NSCLC diagnostics. The specific cut-off values identified with the aid of the Youden's index, may serve as the single basis for screening and differential diagnostics. This reduces the variance of diagnostic characteristics between different centers and studies, improved result reproducibility and simplifies integration of biomarker screening data into clinical practice. In this way, unification of cut-off values of markers simplifies their use in the event of suspected NSCLC and facilitates standardization of diagnostic algorithms.

**Limitations of study.** Our study was a single-center study characterized with an age imbalance between the groups, which might affect the level of specific biomarkers, especially inflammatory and metabolic ones. Besides, the results were not checked against an external cohort, which decreases the possibility of their generalization and necessitates validation versus independent populations. Besides, we assessed the biomarkers using the ROC-analysis, without construction of combined models that could have potentially increased diagnostic accuracy. These factors must be taken into account in the interpretation of data and in the planning of future multi-center studies utilizing multi-marker panels.

■ **CONCLUSION**

The greatest AUC values we demonstrated by the following biomarkers: HE4 (0.903), ApoA2 (0.86), CYFRA 21-1 (0.836), ApoA1 (0.795), D-dimer (0.793), TTR (0.79), ApoA4 (0.784), B2M (0.765) and LRG1 (0.757). The combination of the identified changes indicates systemic reactions associated with oncological diseases, emphasizing the significant role of these proteins in the pathogenesis of lung cancer. The obtained data point to the potential for developing multi-marker panels based on the identified biomarkers for NSCLC diagnosis. Validation on independent samples in multicenter studies is required to confirm the findings. ■

ADDITIONAL INFORMATION	ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ
<p><b>Ethics approval.</b> The study was approved by the Local Ethics Committee of the First Moscow State Medical University named after I.M. Sechenov (№ 16-21 from 16.09.2021).</p>	<p><b>Этическая экспертиза.</b> Исследование было одобрено локальным этическим комитетом Первого Московского государственного медицинского университета имени И.М. Сеченова (№ 16-21 от 16.09.2021 г.).</p>
<p><b>Consent for publication.</b> All patients signed a written informed consent form.</p>	<p><b>Согласие на публикацию.</b> Все пациенты подписывали добровольное информированное согласие.</p>
<p><b>Study funding.</b> The study was the authors' initiative without external funding.</p>	<p><b>Источник финансирования.</b> Работа выполнена по инициативе авторов без привлечения финансирования.</p>
<p><b>Conflict of interest.</b> The authors declare that there are no obvious or potential conflicts of interest associated with the content of this article.</p>	<p><b>Конфликт интересов.</b> Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с содержанием настоящей статьи.</p>
<p><b>Contribution of individual authors.</b> Zhilenkova A.V.: study design and concept, statistical analysis, writing of the manuscript. Voronova V.M.: study design and concept, statistical analysis. Orlova E.V., Istranov A.L., Sekacheva M.I.: review and editing. All authors gave their final approval of the manuscript for submission, and agreed to be accountable for all aspects of the work, implying proper study and resolution of issues related to the accuracy or integrity of any part of the work.</p>	<p><b>Участие авторов.</b> Жиленкова А.В. – разработка концепции и дизайна исследования, проведение статистического анализа, написание текста. Воронова В.М. – разработка концепции и дизайна исследования, проведение статистического анализа. Орлова Е.В., Истранов А.Л., Секачева М.И. – рецензирование и редактирование. Все авторы одобрили финальную версию статьи перед публикацией, выразили согласие нести ответственность за все аспекты работы, подразумевающую надлежащее изучение и решение вопросов, связанных с точностью или добросовестностью любой части работы.</p>

<b>Statement of originality.</b> No previously published material (text, images, or data) was used in this work.	<b>Оригинальность.</b> При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).
<b>Data availability statement.</b> The editorial policy regarding data sharing does not apply to this work.	<b>Доступ к данным.</b> Редакционная политика в отношении совместного использования данных к настоящей работе не применима.
<b>Generative AI.</b> No generative artificial intelligence technologies were used to prepare this article.	<b>Генеративный искусственный интеллект.</b> При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.
<b>Provenance and peer review.</b> This paper was submitted unsolicited and reviewed following the standard procedure. The peer review process involved 2 external reviewers.	<b>Рассмотрение и рецензирование.</b> Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали 2 внешних рецензента.

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