Cancer Screening and Prevention **2024** vol. 3(2) | 75–84
DOI: 10.14218/CSP.2024.00004

Original Article

Combined Determination of CYFRA 21-1 and CXCR1/2 Levels for Detecting Recurrence in Stage III Squamous Cell Lung Carcinoma

Anatoli D. Tahanovich[1*], Mikalai M. Kauhanka[1], Elizabeth M. Barabanova[1], Oxana V. Gotko[2] and Violetta I. Prokhorova[2]

[1]*Department of Biological Chemistry, Medical Faculty of International Students, Belarusian State Medical University, Minsk, Belarus;* [2]*Department of Laboratory Diagnostics, N.N. Alexandrov National Cancer Center of Belarus, Minsk, Belarus*

Received: February 06, 2024 | **Revised:** March 12, 2024 | **Accepted:** May 14, 2024 | **Published online:** June 18, 2024

Abstract

Background and objectives: Despite efforts, tumor recurrence is diagnosed in 35–40% of patients with stage III squamous cell lung carcinoma (SCLC) during the first year after treatment. The purpose of the present investigation was to determine the levels of cytokeratin-fragment 19 (CYFRA 21-1) in blood serum, the percentages of lymphocytes containing chemokine receptor 1 (CXCR1, %, lymphocytes), and the percentages of monocytes containing chemokine receptor 2 (CXCR2, %, monocytes), as well as their combined model before and after treatment for the early detection of recurrence.

Methods: Forty-eight patients (29 men and 19 women) with newly diagnosed stage III SCLC were examined. Serum levels of CYFRA 21-1, CXCR1, %, lymphocytes, and CXCR2, %, monocytes in peripheral blood were measured before treatment and at three weeks, three months, and six months after treatment using a chemiluminescence immunoassay analyzer and a flow cytometer, respectively.

Results: The levels of all determined indicators, which were elevated before treatment, decreased sharply three weeks after treatment. Subsequently, three months and six months after treatment, the levels steadily increased in patients with diagnosed tumor recurrence. The differences in these indicators in three weeks to three months, three months to six months, and three weeks to six months after treatment, when included in a regression equation, corresponded to the presence of recurrence with accuracies of 83.3%, 91.7%, and 95.8%, respectively.

Conclusions: Determining the combination of CYFRA 21-1 levels, CXCR1, %, lymphocytes, and CXCR2, %, monocytes in the blood of patients with stage III SCLC is important for assessing the probability of recurrence after treatment.

Introduction

According to the World Health Organization, in 2023, lung cancer was the second most common newly diagnosed cancer and the leading cause of cancer mortality.[1] The most common type of lung cancer, comprising 85% of cases, is non-small cell lung cancer (NSCLC), which can be classified into adenocarcinoma at 50%, squamous cell lung carcinoma (SCLC) at 47%, and large cell cancer at 3%.[2] These subtypes differ not only in their histological structure. For example, adenocarcinoma is often associated with mutations in the epidermal growth factor receptor and anaplastic lymphoma kinase (CD246) genes, while SCLC more often develops in smokers and is not typically associated with these mutations.[1] Therefore, the study of the pathogenesis and diagnosis of these subtypes is often conducted separately.[2–4] Stage III SCLC is heterogeneous and is divided into IIIA (T1N2M0, T2N2M0, T3N1M0, T4N0M0, T4N1M0), IIIB (T1N3M0, T2N3M0, T3N2M0, T4N2M0 and IIIC (T3N3M0, T4N3M0).[4]

Patients with lymph node lesions in the contralateral lung (N3) are not candidates for surgical treatment (IIIC and partially IIIB

Keywords: Squamous cell lung carcinoma; Stage III; CYFRA 21-1; CXCR1; CXCR2; Recurrence; Prognosis.

***Correspondence to:** Anatoli D. Tahanovich, Department of Biological Chemistry, Medical Faculty of International Students, Belarusian State Medical University, Dzerzhinskii av.,83, Minsk 220116, Belarus. ORCID: https://orcid.org/0000-0002-0668-2888. Tel: +375-17-3741764, E-mail: ataganovich@gmail.com

How to cite this article: Tahanovich AD, Kauhanka MM, Barabanova EM, Gotko OV, Prokhorova VI. Combined Determination of CYFRA 21-1 and CXCR1/2 Levels for Detecting Recurrence in Stage III Squamous Cell Lung Carcinoma. *Cancer Screen Prev* 2024;3(2):75–84. doi: 10.14218/CSP.2024.00004.

stages). The remaining stage III SCLC patients undergo tumor removal with R0 surgery, which ensures complete tumor excision along with lymph node dissection. Patients with tumor characteristics T1N2M0, T2N2M0, T3N1M0, and T3N2M0 undergo adjuvant (postoperative) polychemotherapy to suppress remaining cancer cells. Those with T4N0M0, T4N1M0, and T4N2M0 receive neoadjuvant polychemotherapy before surgery.[5] Despite these efforts, the risk of relapse due to micrometastases after surgery remains high. Tumor recurrence is diagnosed in 20% of stage I SCLC patients during the first year after treatment,[3] and in 35–40% of stage III patients.[4]

All patients undergo medical examinations every three months for two years post-treatment.[5] If the patient is asymptomatic, computed tomography (CT) is performed at six and 12 months after treatment. However, relapses may occur earlier and initially be asymptomatic.

Circulating components of tumor metabolism could serve as informative tools for additional diagnosis and prognosis of NSCLC outcomes. The use of blood concentrations of cytokeratin fragment 19 (CYFRA 21-1), cancer embryonic antigen (CEA), squamous cell carcinoma antigen, neuron-specific enolase, tissue polypeptide antigen, and others is being explored for this purpose.[6–14] Only CYFRA 21-1 and CEA have been associated with overall and relapse-free survival, and only in early-stage (I-II) patients.[8,9,12] However, these markers have low sensitivity and specificity, and studies have only examined stage III SCLC patients in mixed groups with early stages.[13,14]

Based on previous studies, we selected three biochemical markers in stage III SCLC patients: the levels of CYFRA 21-1, the percentages of lymphocytes with CXCR1 receptors (CXCR1, %, lymphocytes), the percentages of monocytes with CXCR2 receptors (CXCR2, %, monocytes) in blood cell populations—for relapse-free survival prediction.[15] Their combined determination in a regression equation was shown to be the most informative as a prognostic factor, suggesting a relationship with the likelihood of tumor relapse after treatment.

Recently, we demonstrated the role of biomarkers in predicting relapse in stage I-II SCLC after surgical treatment. We found that the likelihood of relapse based on a difference in the concentrations of SCC antigens, the percentages of lymphocytes with CXCR2, and monocytes with CD44v6 receptors in the populations of corresponding blood cells during three weeks to three months, three to six months, and three weeks to six months post-surgery can be predicted with accuracies ranging from 68.4% to 89.5%. Subsequent regression analysis and the development of a combined model that included these parameters increased the predictive value of tumor recurrence to 96.5% (specificity – 95.6%, sensitivity – 100%). These results indicated the usefulness of the combined model in stage I-II SCLC patients as an additional marker for predicting postoperative relapse.[16,17]

Thus, surgical treatment of stage III SCLC is associated with chemotherapy. Despite these efforts, some patients develop tumor relapses, mostly within the first year after surgery, which are associated with high mortality. Early detection of relapse leads to more effective anti-relapse treatment. Therefore, identifying effective criteria for predicting relapse remains crucial.[6–14] Although biochemical criteria have not yet found practical application due to their nonspecificity and insufficient sensitivity, numerous studies continue to explore new biomarkers.

The purpose of the present investigation was to determine the levels of CYFRA 21-1, the percentages of lymphocytes with CXCR1 receptors, and the percentages of monocytes with CXCR2 receptors, as well as their combination after treatment to predict relapse in stage III SCLC patients.

Materials and methods

Research object

The study involved 48 patients (29 men and 19 women) admitted to the clinic of the N.N. Alexandrov National Cancer Center of Belarus between 2021 and 2022. The inclusion criteria for patients were as follows: newly diagnosed stage IIIA or IIIB SCLC. The exclusion criteria were the presence of metachronous or secondary cancer, and patient refusal to participate in the study. During the first year of observation, there were no patient dropouts. Patients with T1N2M0, T2N2M0, T3N1M0, or T3N2M0 underwent surgical resection of the tumor (surgical volume – R0) followed by 4 courses of adjuvant polychemotherapy consisting of a combination of vinorelbine (V) 25–30 mg/m^2 and cisplatin (C) 80 mg/m^2. Patients with T4N0M0, T4N1M0, or T4N2M0 received two courses of neoadjuvant chemotherapy consisting of V + C, followed by surgical resection of the tumor and two additional courses of adjuvant polychemotherapy with V + C. The post-treatment monitoring algorithm for all patients included a physical examination every three months for the first year after surgery. In the absence of complaints and symptoms of the disease based on the results of a physical examination, a CT scan was performed at six and 12 months after treatment. This schedule is critical for early relapse detection. Information on the development of relapse in the examined patients after surgery was obtained based on CT data from the Cancer Register of the Republic of Belarus (N.N. Alexandrov National Cancer Center of Belarus).

Ethical approval and consent

The study received approval from the Biomedical Ethics Committee of Belarusian State Medical University (Committee meeting No. 2 dated 10/04/2021). All patients provided written voluntary consent to participate in the study in accordance with the Declaration of Helsinki as revised in 2013.

Study design and sample collection

Before treatment and at three weeks, three months, and six months after surgery, candidate biomarkers were measured in all patients. Blood was collected from the cubital vein of patients on an empty stomach into a vacutainer with EDTA-K2 as an anticoagulant. To obtain serum, blood was collected into a tube with thrombin and separating gel.

Analysis of samples

The concentration of CYFRA 21-1 antigen in blood serum was determined using the electrochemiluminescent method on a Cobas e411 automated analyzer (Roche Diagnostics GmbH, Germany) using original Elecsys CYFRA 21-1 kits (Roche Diagnostics GmbH, Germany). To assess the concentrations of CXCR1 and CXCR2 receptors in leukocyte cells, a Navios flow cytometer (Beckman Coulter, USA) was used. For this, 100 µL of blood and a solution containing a mixture of antibodies with fluorescent labels were placed in a test tube: CD181 (CXCR1)-PE-Cy5 (BioLegend, USA), CD182 (CXCR2)-PE (BioLegend, USA), and CD45-Pacific Orange (Exbio, Czech Republic). After 15 m of incubation in the dark with antibodies containing a fluorescent label, 1 ml of VersaLyse lysis solution (Beckman Coulter, France) was added to the mixture. Antibodies were fixed on the

Table 1. Comparison of general conditions between the relapse and nonrelapse patients

Characteristics	Relapse group (n = 17)	Nonrelapse group (n = 31)	*P*-value
Age (M ± σ years)	55.3±19.3	58.1 ± 18.5	0.215
Gender, n (%)			0.198
Males	10 (58.8)	19 (61.3)	
Females	7 (41.2)	12 (38.7)	
Smoking status	2/8/0	3/15/1	
Males n (%)			0.107
former	2 (20.0)	3 (15.8)	
current	8 (80.0)	15 (78.9)	
never	0 (0.0)	1 (5.2)	
Females n (%)			0.125
former	1 (14.3)	2 (16.7)	
current	5 (71.4)	8 (66.6)	
never	1 (14.3)	2 (16.7)	

cell surface using IQTest 3 fixing solution (Beckman Coulter, France).

Statistical analysis

The combined model for predicting relapse-free survival included the result of a regression equation (Y) for determining three indicators: the concentration of the CYFRA 21-1 antigen in blood serum (X1); the relative amount (%) of CXCR1 receptor in lymphocytes (X2); and the relative amount (%) of CXCR2 receptor in monocytes (X3):[15]

$$Y = \frac{\exp(-5,315 + 0,116 * X1 + 1,901 * X2 + 0,279 * X3)}{1 + \exp(-5,315 + 0,116 * X1 + 1,901 * X2 + 0,279 * X3)}$$

The relationship between changes in the levels of the determined indicators and disease-free survival was assessed using single- and multivariate Cox proportional hazard models.

The integral diagnostic information content of the laboratory tests was evaluated by constructing characteristic receiver operating characteristic (ROC) curves and calculating the area under the ROC curve. The diagnostic value of the analyzed indicators was determined by calculating the following diagnostic sensitivity: the number of actual relapses in patients whose indicator values exceeded the threshold value (TV), diagnostic specificity (the number of patients without relapse, whose laboratory indicator levels were below the TV, the predictive value of positive and negative results, and the overall diagnostic accuracy among all examined patients. For this purpose, these calculations utilized the true positive, true negative, false positive, and false negative results of the diagnostic test, employing generally accepted formulas.[18] The threshold value was determined as the optimal combination of sensitivity and specificity of the test by plotting the dependence of sensitivity on the probability of false-positive results. For all types of statistical analysis, the critical significance level was set at 5%.

Results

A comparison of the general situation between the patient groups with relapse and without relapse during one year after treatment shows there was no statistically significant difference ($P > 0.05$) between these two groups, suggesting comparability (Table 1).

Relapse developed in 17 out of 48 patients examined within one year after treatment: one patient at 3.8 months, three patients within a period of up to six months, and 13 patients within a period of six months to one year (Table 2).

Three weeks after treatment, the levels of all determined indicators sharply decreased (Table 2), nearing the threshold values (Table 3). In 11 out of 17 patients who relapsed, the levels of CYFRA 21-1, despite decreasing, remained above the TV (Tables 2 and 3). Similar changes were observed for other parameters. The percentages of lymphocytes with CXCR1 receptors and monocytes with CXCR2 receptors in the total population of these cells, as well as the value of the combined model, were higher than the TV in nine, 11, and 13 out of 17 patients with tumor relapse, respectively (Tables 2 and 3).

Evidence of the relationship between the decrease in the levels of indicators and relapse-free survival of patients three weeks after treatment is demonstrated by Cox proportional hazard analysis. All measured indicators are significantly associated with the development of relapse (Table 3). This is evidenced, in particular, by the confidence interval of the hazard ratio, whose values do not include 1, as well as by the value of the parameter $P < 0.05$ for all measured indicators.

In patients without relapse, the median values at three and six months did not differ from the levels at three weeks after treatment (Table 4). In patients with relapse, the levels of these indicators at three weeks, three months, and six months after treatment steadily increased. The values of any of the determined indicators after three months are significantly higher than after three weeks, and after six months, they are higher compared to the value after three months. The value differences between three months and three weeks, three months and six months, and three weeks and six months in patients with identified relapse demonstrate the rise even more clearly (Table 5). At the same time, in patients without relapse, the difference is minimal and not significant in the specified periods after treatment.

Data from Cox proportional hazard analysis show a significant relationship between the differences in the levels of determined

Table 2. Levels of CYFRA 21-1 and percentages of lymphocytes with CXCR1, and percentages of monocytes with CXCR2 in patients with relapsed SCLC before and after treatment

Patients	Index	Before treatment	After completion of treatment			Time to relapse, months
			Three weeks	Three months	Six months	
1	CYFRA 21-1, g/l, $\times10^{-6}$	6.64	5.29	6.41	8.07	3.8
	CXCR1, %, lymphocytes	3.83	2.27	2.72	3.42	
	CXCR2, %, monocytes	2.19	1.2	1.46	1.91	
	Combined model[a]	0.738	0.249	0.325	0.454	
2	CYFRA 21-1, g/l, $\times10^{-6}$	7.94	3.59	4.75	6.23	4.3
	CXCR1, %, lymphocytes	5.94	2.37	2.77	3.31	
	CXCR2, %, monocytes	1.83	1.01	1.24	1.71	
	Combined model[a]	0.639	0.261	0.337	0.464	
3	CYFRA 21-1, g/l, $\times10^{-6}$	5.99	3.14	4.30	5.82	5.1
	CXCR1, %, lymphocytes	3.73	1.86	2.42	3.07	
	CXCR2, %, monocytes	1.7	1.41	1.63	2.12	
	Combined model[a]	0.639	0.231	0.315	0.448	
4	CYFRA 21-1, g/l, $\times10^{-6}$	7.32	3.64	4.72	6.16	5.4
	CXCR1, %, lymphocytes	4.78	2.27	2.70	3.25	
	CXCR2, %, monocytes	1.62	1.29	1.52	1.98	
	Combined model[a]	0.627	0.240	0.313	0.439	
5	CYFRA 21-1, g/l, $\times10^{-6}$	6.56	4.21	5.23	6.67	6.1
	CXCR1, %, lymphocytes	4.38	2.17	2.53	3.12	
	CXCR2, %, monocytes	1.42	1.25	1.48	1.93	
	Combined model[a]	0.623	0.251	0.314	0.438	
6	CYFRA 21-1, g/l, $\times10^{-6}$	7.27	3.47	4.65	6.19	6.3
	CXCR1, %, lymphocytes	4.28	2.17	2.81	4.14	
	CXCR2, %, monocytes	2.19	0.7	0.97	1.42	
	Combined model[a]	0.686	0.257	0.324	0.453	
7	CYFRA 21-1, g/l, $\times10^{-6}$	7.20	3.62	4.60	5.95	7.2
	CXCR1, %, lymphocytes	3.63	1.76	2.11	2.67	
	CXCR2, %, monocytes	2.16	1.49	1.73	2.18	
	Combined model[a]	0.732	0.227	0.289	0.422	
8	CYFRA 21-1, g/l, $\times10^{-6}$	6.42	3.95	4.66	6.09	7.9
	CXCR1, %, lymphocytes	4.18	2.27	2.52	3.07	
	CXCR2, %, monocytes	1.36	0.75	1.01	1.47	
	Combined model[a]	0.843	0.235	0.274	0.414	
9	CYFRA 21-1, g/l, $\times10^{-6}$	6.21	3.96	4.64	5.92	8.4
	CXCR1, %, lymphocytes	4.13	2.06	2.40	2.98	
	CXCR2, %, monocytes	2.32	1.44	1.65	2.15	
	Combined model[a]	0.619	0.259	0.319	0.432	
10	CYFRA 21-1, g/l, $\times10^{-6}$	5.85	3.29	3.61	4.96	8.9
	CXCR1, %, lymphocytes	3.68	2.37	2.52	3.26	
	CXCR2, %, monocytes	2.12	1.06	1.29	1.77	

(continued)

Table 2. *(continued)*

Patients	Index	Before treatment	After completion of treatment			Time to relapse, months
			Three weeks	Three months	Six months	
	Combined model[a]	0.567	0.241	0.280	0.390	
11	CYFRA 21-1, g/l, $\times10^{-6}$	6.03	3.29	4.24	5.80	9.3
	CXCR1, %, lymphocytes	3.83	1.81	2.14	2.73	
	CXCR2, %, monocytes	1.5	1.1	1.33	1.81	
	Combined model[a]	0.607	0.237	0.295	0.430	
12	CYFRA 21-1, g/l, $\times10^{-6}$	6.29	3.92	4.37	6.30	10.1
	CXCR1, %, lymphocytes	3.32	1.66	1.84	2.67	
	CXCR2, %, monocytes	2.54	1.2	1.48	1.91	
	Combined model[a]	0.418	0.229	0.248	0.402	
13	CYFRA 21-1, g/l, $\times10^{-6}$	7.84	3.24	3.54	4.86	10.6
	CXCR1, %, lymphocytes	3.98	1.66	1.78	2.44	
	CXCR2, %, monocytes	2.33 _	1.37	1.61	2.09	
	Combined model[a]	0.707	0.253	0.289	0.437	
14	CYFRA 21-1, g/l, $\times10^{-6}$	4.14	3.09	4.06	5.67	10.8
	CXCR1, %, lymphocytes	2.47	1.61	1.93	2.69	
	CXCR2, %, monocytes	1.69	1.16	1.42	1.85	
	Combined model[a]	0.537	0.225	0.285	0.421	
15	CYFRA 21-1, g/l, $\times10^{-6}$	4.57	3.57	4.43	5.93	11.0
	CXCR1, %, lymphocytes	3.70	1.80	2.15	2.70	
	CXCR2, %, monocytes	1.99	1.02	1.28	1.73	
	Combined model[a]	0.411	0.201	0.258	0.392	
16	CYFRA 21-1, g/l, $\times10^{-6}$	6.58	3.29	4.15	5.64	11.2
	CXCR1, %, lymphocytes	4.25	1.85	2.20	2.75	
	CXCR2, %, monocytes	1.96	1.32	1.55	2.01	
	Combined model[a]	0.403	0.195	0.253	0.382	
17	CYFRA 21-1, g/l, $\times10^{-6}$	5.12	3.68	4.54	6.03	11.7
	CXCR1, %, lymphocytes	3.55	1.95	2.30	2.85	
	CXCR2, %, monocytes	1.92	1.13	1.39	1.84	
	Combined model[a]	0.392	0.190	0.248	0.380	

[a]Combined model, the result of a regression equation; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; SCLC, squamous cell lung carcinoma.

Table 3. Threshold values and relationship of the levels of CYFRA 21-1, lymphocytes with CXCR1, and monocytes with CXCR2 in patients with relapsed SCLC three weeks after treatment with relapse-free survival in stage III SCLC (according to the Cox proportional hazard model)

Index	TV	Univariate model		Multivariate model	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
CYFRA 21-1, g/l, $\times10^{-6}$	3.30	1.069 (1.005–1.133)	0.035	1.062 (1.003–1.121)	0.039
CXCR1, %, lymphocytes	1.90	1.059 (1.008–1.110)	0.037	1.055 (1.005–1.105)	0.041
CXCR2, %, monocytes	1.10	1.032 (1.009–1.055)	0.038	1.027 (1.006–1.048)	0.043
Combined model[a]	0.225	1.197 (1.027–1.167)	0.027	1.091 (1.018–1.164)	0.031

[a]Combined model, the result of a regression equation; CI, confidence interval; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; HR, hazard ratio; SCLC, squamous cell lung carcinoma; TV, threshold value.

Table 4. Levels of CYFRA 21-1, lymphocytes with CXCR1, and monocytes with CXCR2 in groups of patients with and without SCLC relapse

Index	Relapse	Before treatment	After treatment		
			Three weeks	Three months	Six months
CYFRA 21-1, g/l, $\times 10^{-6}$	No	4.19 [3.51;5.13]	2.96 [0.90; 3.79]	2.97 [1.01; 3.81]	2.99 [1.03; 3.86]
	Yes	6.42 [5.99;7.20]	3.59* [3.29; 3.92]	4.54# [4.24; 4.66]	5.95# [5.80; 6.19]
CXCR1, %, lymphocytes	No	2.45 [1.75;3.20]	1.70 [0.95;2.00]	1.75 [1.00; 2.10]	1.80 [1.05;2.20]
	Yes	3.83 [3.68;4.25]	1.95* [1.80; 2.27]	2.40# [2.14; 2.53]	2.98# [2.70; 3.25]
CXCR2, %, monocytes	No	1.50 [0.55;2.45]	0.90 [0.30;1.40]	0.95 [0.35;1.65]	1.00 [0.50;1.95]
	Yes	1.92 [1.62;2.16]	1.20* [1.06; 1.32]	1.46# [1.29; 1.55]	1.91# [1.77; 2.01]
Combined model[a]	No	0.290 [0.221;0.296]	0.193 [0.159;0.237]	0.199 [0.161; 0.241]	0.201 [0.164;0.245]
	Yes	0.623 [0.537; 0.686]	0.237* [0.227; 0.251]	0.290# [0.274; 0.315]	0.430# [0.402;0.439]

[a]Combined model, the result of a regression equation; *significant differences in patients with relapse compared to patients without relapse; #significant differences after three and 6 months compared to three weeks after treatment; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; SCLC, squamous cell lung carcinoma.

indicators presented in Table 5 and the development of tumor relapse. This is evidenced by hazard ratios in both univariate and multivariate Cox models for each of the indicators in the compared time intervals after treatment of patients (Table 6). The highest hazard ratio is observed for the interval from 3 weeks to 6 months. This indicates that the strength of the relationship between changes in the levels of any of the indicators during this time interval and relapse is the greatest.

The established relationship between the differences in the studied parameters at selected time intervals after treatment forms the basis for assessing the likelihood of relapse in patients with SCLC. A generally accepted argument and objective criterion for such an assessment is to determine the diagnostic value or accuracy of the selected parameter. A necessary requirement for such an assessment is information about the threshold value for the time interval difference. Such data can be obtained from the results of ROC analysis (Fig. 1). TV for each of the indicators is presented in Table 7.

Based on the results of determining the level difference during the period from three weeks to three months after treatment, the diagnostic accuracy of relapse probability ranged from 70.8% (CXCR2, %, monocytes) to 75.0% (CYFRA 21-1). Using the results of the combined model calculation within the specified period, the accuracy increases to 83.3%.

Based on the level difference three months and six months after treatment, the determination of CXCR2, %, monocytes, CXCR1, %, lymphocytes, and CYFRA 21-1 demonstrates higher accuracy for predicting relapse (72.9%, 75.0%, and 81.3%, respectively) (Table 7). For the combined model, accuracy increased to 91.7%. The predictive values of a positive result and a negative result were 84.2% and 96.6%, respectively.

The greatest efficiency was demonstrated by determining the level difference between three weeks and six months after treatment. This approach makes it possible to predict the presence of relapse after treatment with an accuracy ranging from 83.3% (CXCR2, %, monocytes) to 89.6% (CYFRA 21-1) (Table 7). Using the combined model calculation increases the accuracy to 95.8%.

Discussion

Our study focused on three biochemical parameters in the blood:

Table 5. Differences in the levels of studied indicators in patients with stage III SCLC between three weeks and three months, three and six months, and three weeks and six months after treatment

Index	Relapse	Before treatment	After treatment		
			Three weeks – three months	Three months – six months	Three weeks – six months
CYFRA 21-1, g/l, $\times 10^{-6}$	No	1.27 [0.91; 2.03]	0.02 [0.03; 0.68]	0.03 [0.02; 1.19]	0.04 [0.01; 1.29]
	Yes	2.56 [2.25; 3.58]	0.95* [0.71; 1.08]	1.49# [1.43; 1.54]	2.38# [2.33; 2.58]
CXCR1, %, lymphocytes	No	1.25 [1.00; 1.85]	0.03 [0.01;0.35]	0.03 [0.01; 0.45]	0.05 [0.01;0.70]
	Yes	1.91 [1.66; 2.21]	0.35* [0.32; 0.40]	0.59# [0.55; 0.70]	0.92# [0.90; 1.01]
CXCR2, %, monocytes	No	0.55 [0.35; 0.75]	0.02 [0.01;0.19]	0.03 [0.01; 0.37]	0.05 [0.01;0.60]
	Yes	0.79 [0.53; 0.97]	0.24* [0.23; 0.26]	0.46# [0.45; 0.48]	0.71# [0.69; 0.71]
Combined model[a]	No	0.209 [0.107;0.271]	0.002 [0.001;0.031]	0.003 [0.001; 0.119]	0.005 [0.001;0.153]
	Yes	0.372 [0.312; 0.429]	0.060* [0.057; 0.067]	0.132# [0.127; 0.135]	0.191# [0.184; 0.196]

[a]Combined model, the result of a regression equation; *significant difference in patients with and without relapse; #significant difference during the period of three months – six months and three weeks – six months in comparison to three weeks – three months after treatment; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; SCLC, squamous cell lung carcinoma.

Table 6. Association of relapse-free survival of patients with lung SCLC with differences of the levels of CYFRA 21-1, CXCR1, %, lymphocytes and CXCR2, %, monocytes at selected time intervals after treatment (according to the Cox proportional hazard model)

Index	Time interval	Univariate model		Multivariate model	
		HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
CYFRA 21-1, g/l, $\times 10^{-6}$	Three weeks to three months	1.123 (1.013–1.233)	0.041	1.114 (1.008–1.220)	0.038
	Three months to six months	1.219 (1.095–1.343)	0.037	1.203 (1.011–1.395)	0.035
	Three weeks to six months	1.375 (1.117–1.633)	0.023	1.314 (1.035–1.593)	0.021
CXCR1, %, lymphocytes	Three weeks to three months	1.023 (1.003–1.043)	0.033	1.023 (1.001–1.045)	0.031
	Three months to six months	1.051 (1.005–1.197)	0.026	1.051 (1.002–1.100)	0.024
	Three weeks to six months	1.096 (1.006–1.186)	0.017	1.091 (1.004–1.178)	0.016
CXCR2, %, monocytes	Three weeks to three months	1.036 (1.004–1.068)	0.031	1.031 (1.002–1.060)	0.028
	Three months to six months	1.041 (1.005–1.077)	0.028	1.040 (1.003–1.077)	0.027
	Three weeks to six months	1.055 (1.007–1.103)	0.024	1.054 (1.006–1.102)	0.022
Combined model[a]	Three weeks to three months	1.219 (1.098–1.340)	0.018	1.207 (1.093–1.321)	0.016
	Three months to six months	1.262 (1.108–1.416)	0.014	1.224 (1.095–1.353)	0.013
	Three weeks to six months	1.334 (1.133–1.535)	0.013	1.315 (1.111–1.519)	0.011

[a]Combined model, the result of a regression equation; CI, confidence interval; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; HR, hazard ratio; SCLC, squamous cell lung carcinoma.

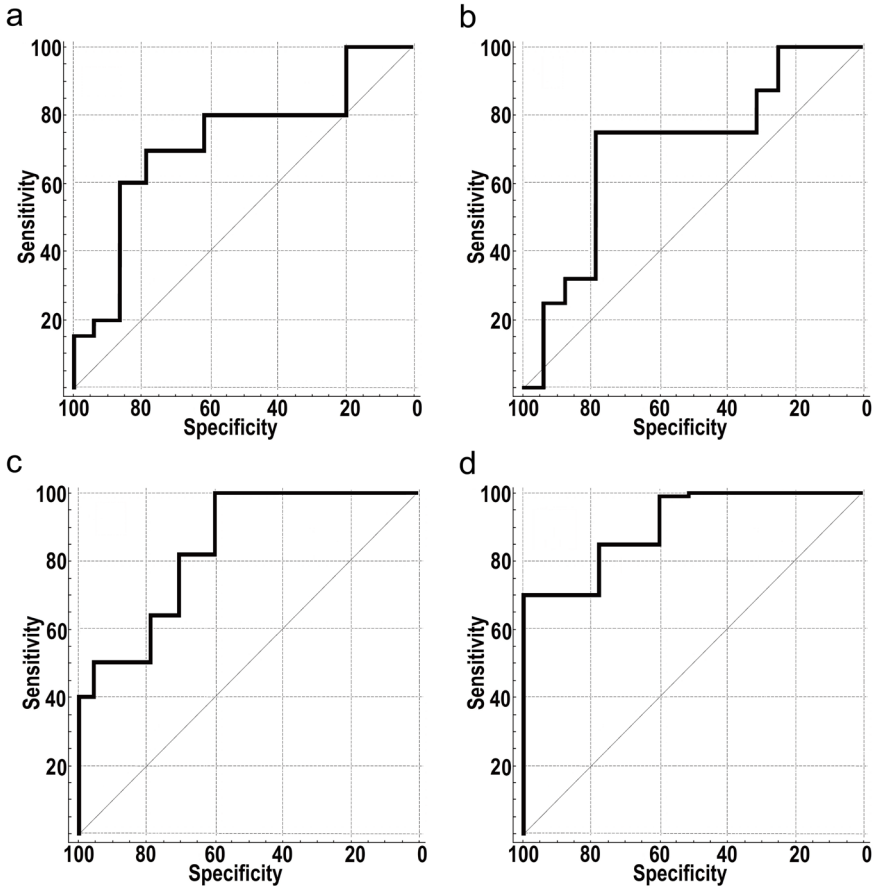

Fig. 1. ROC curves for the level difference between three weeks and three months after treatment. (a) CYFRA 21-1; (b) CXCR1, %, lymphocytes; (c) CXCR2, %, monocytes; (d) combined model- the result of a regression equation. CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; ROC, receiver operating characteristic.

Table 7. Diagnostic efficiency of the level difference after treatment to predict the development of SCLC relapse

Index	TV	SE	SP	PPV	NPV	AUC	ACC
From three weeks to three months							
CYFRA 21-1, g/l, $\times 10^{-6}$	0.95	76.5	74.2	61.9	85.2	0.726	75.0
CXCR1, %, lymphocytes	0.35	64.7	77.4	61.1	80.0	0.698	72.9
CXCR2, %, monocytes	0.24	70.6	71.0	57.1	81.5	0.681	70.8
Combined model[a]	0.060	82.4	83.9	73.7	89.7	0.811	83.3
From three to six months							
CYFRA 21-1, g/l, $\times 10^{-6}$	1.49	82.4	80.6	70.0	89.3	0.787	81.3
CXCR1, %, lymphocytes	0.58	70.6	77.4	63.2	82.8	0.729	75.0
CXCR2, %, monocytes	0.47	70.6	74.2	60.0	82.1	0.693	72.9
Combined model[a]	0.132	94.1	90.3	84.2	96.6	0.892	91.7
From three weeks to six months							
CYFRA 21-1, g/l, $\times 10^{-6}$	2.38	94.1	87.1	80.0	96.4	0.865	89.6
CXCR1, %, lymphocytes	0.90	94.1	80.6	72.7	96.2	0.831	85.4
CXCR2, %, monocytes	0.70	88.2	80.6	71.4	92.6	0.829	83.3
Combined model[a]	0.190	100.0	93.5	89.5	100.0	0.933	95.8

[a]Combined model, the result of a regression equation; ACC, accuracy; AUC, area under ROC-curve; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; CYFRA 21-1, cytokeratin 19 fragment antigen 21-1; NPV, predictive value of a negative result; PPV, predictive value of a positive result; SCLC, squamous cell lung carcinoma; SE, sensitivity; SP, specificity; TV, threshold value.

the concentrations of CYFRA 21-1, the percentages of lymphocytes with the CXCR1 receptor in the total lymphocyte population, and the percentages of monocytes with the CXCR2 receptor in the total monocyte population. Their ability to diagnose and predict disease-free survival based on preoperative assessment was previously proven, as was the advantage of using a combined model that included these parameters.[15] Based on the comparison of the results obtained in this work with the calculated TV, it was found that 16 patients were included in the group with low relapse-free survival. Fourteen of them actually developed tumor recurrence within one year after treatment, as confirmed by CT data. This amounts to 87.5%, which is in good accordance with the predictive value of a positive result of regression equation calculation (90%). Thirty-two patients were included in the group with high relapse-free survival. Relapse developed in three of them. This indicates that the remaining 29 out of 32 patients were correctly predicted to have a low risk of relapse, representing 90.6%, with a calculated negative predictive value of 85.0%. Thus, the data obtained confirmed the performance of the previously proposed multivariate prognostic model.

In our current investigation, while tracking the dynamics of changes in the determined parameters within one year after treatment, several trends attracted attention. The first is that within three weeks after surgical resection of the tumor, the values of all indicators in all patients decreased to values comparable to the TV. However, in some patients, the amplitude of the decrease did not reach the TV, remaining above this value. The majority of those patients (76.5%) subsequently developed tumor relapse during the year of observation.

Other researchers have also observed a decrease in blood concentrations of CYFRA 21-1 and CEA, although to different degrees. In some patients, it decreased compared to the levels before treatment but did not reach the threshold values.[7–9] In these studies, patients had early stages of NSCLC and underwent only surgical treatment, so the phenomenon of a sharp decrease in the levels of these indicators after surgery was due to the resection of tumor tissue. In our study, patients received neoadjuvant and adjuvant therapy in addition to surgical treatment, which also aimed at destroying tumor cells and reducing their metabolites.

The next observed event was a subsequent increase in the levels of measured parameters in some patients after a decrease. The majority of these patients (89.5%) subsequently developed a relapse. Other researchers have also noted that serum concentrations of CYFRA 21-1 and CEA in patients with NSCLC and resected tumors often increase further after a decrease. Moreover, such dynamics are associated with the development of relapse.[12–14] Based on these data, the researchers concluded that monitoring serum CEA concentrations in patients with NSCLC predicts relapse with a sensitivity of 74.7% and specificity of 69.8%.[12] Comparable results were obtained for CYFRA 21-1, where the sensitivity and specificity of measuring this marker for response to treatment were 79.1% and 60.6%, respectively.[13] Moreover, according to another study, of five serum tumor markers (CYFRA 21-1, CEA, neuron-specific enolase, and carbohydrate antigen 125 and 19–9), only CYFRA 21-1 was the most sensitive for predicting response to chemotherapy, and an increase in its level after an initial decrease correlates with a high likelihood of tumor relapse.[14] Another study reported an observed increase in the concentrations of CYFRA 21-1 in the blood serum of patients with SCLC who developed tumor recurrence within one year.[19,20] Studies of cytokine receptors in blood cells in SCLC have not been carried out before our studies, but it was known that their concentrations were increased in the tumor microenvironment of patients with NSCLC.[21–23]

During the study, we concluded that the level differences of the determined indicators in time intervals of three weeks to three months, three to six months, and three weeks to six months characterized the dynamics of changes and provided valuable insights. This technique did not bring fundamentally new information, but

it clearly demonstrated an increase in the values of indicators over the observation period, which was characteristic only for patients with a diagnosed relapse. The results of the Cox proportional hazard analysis showed a relationship between the increase in the levels of the analyzed indicators in each time interval and the development of relapse after treatment. Therefore, the increase in the indicator with the prolongation of time after treatment was involved in assessing the diagnostic efficiency of the studied parameters for detecting the probability of tumor relapse.

The accuracy of the determination of selected indicators obtained in our investigation significantly exceeded the accuracy of the prediction based on CYFRA 21-1 and CEA determinations observed by other investigators. During the time interval from three weeks to three months, the sensitivity and specificity of the determination of CYFRA 21-1 were the highest (76.5% and 74.2%, respectively) compared to CXCR1, %, lymphocytes and CXCR2, %, monocytes, while they were 82.4% and 83.9% for the combined model assessment. The sensitivity and specificity of CYFRA 21-1 determination in the time interval from three to six months were 82.4% and 80.6%, respectively, and for the combined model, they were 94.1% and 90.3%. This is significantly higher than the diagnostic characteristics of CYFRA 21-1 determination for relapse prognosis obtained by other researchers. Not surprisingly, the determination of CYFRA 21-1 was even more informative over the period from three weeks to six months because of the broader time interval (sensitivity and specificity – 94.1% and 87.1%). However, the combined model showed the most prominent diagnostic characteristics in this time interval, –with 100% and 93.5%, respectively.

Conclusions

The study showed that, in addition to CYFRA 21-1, the determination of the percentages of lymphocytes with CXCR1 and the percentages of monocytes with CXCR2 in the blood population of those cells undergo significant changes after treatment. Soon after tumor resection, these percentages decrease, but due to remaining micrometastases or resistance to chemotherapy drugs, they exceed the TV. Subsequently, as the relapse progresses, the values of these indicators increase. Determining the increase and comparing it with the TV has diagnostic value.

Without complaints, the patient can be examined after three months, and CT can be carried out only six months after treatment. Therefore, determining the levels of CYFRA 21-1, the percentages of lymphocytes with CXCR1 receptors in the total population of lymphocytes, and the percentages of monocytes with CXCR2 receptors in the total population of monocytes in the blood at stage III SCLC seems to be important to carry out at three weeks, three months, and six months after the end of treatment. If the difference in the estimated values of the combined model at the specified time intervals exceeds the TV, the standard treatment regimen should be adjusted due to the high probability of tumor recurrence.

It should be noted that the study included 48 patients. In the future, to validate the proposed model, it is necessary to increase the number of examined patients and test it on an examination sample.

Acknowledgments

We appreciate Professor Victor T. Malkevich, the head of the Laboratory of Thoracic Oncopathology at N.N. Alexandrov National Cancer Center of Belarus, for his kind and fruitful cooperation and discussion of the study design.

Funding

The work was carried out with financial support from the Ministry of Health of the Republic of Belarus (grant number: 2.17/20220385).

Conflict of interest

All authors declare no conflict of interests.

Author contributions

Conceptualization (ADT, VIP); formal analysis, methodology, and original draft writing (ADT, MMK); investigation (MMK, EMB, OVG); review and editing (ADT, MMK, VIP). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

The study was approved by the decision of the Biomedical Ethics Committee of the Belarusian State Medical University (Committee meeting No. 2, dated 10/04/2021). All patients provided written voluntary consent to participate in the study in accordance with the Declaration of Helsinki as revised in 2013.

Data sharing statement

Data supporting the research article are available from the corresponding author at ataganovich@gmail.com.

References

[1] Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics 2023. CA Cancer J Clin 2023;73(1):17–48. doi:10.3322/caac.21763, PMID:36633525.

[2] Araghi M, Mannani R, Heidarnejad Maleki A, Hamidi A, Rostami S, Safa SH, *et al*. Recent advances in non-small cell lung cancer targeted therapy; an update review. Cancer Cell Int 2023;23(1):162. doi:10.1186/s12935-023-02990-y, PMID:37568193.

[3] Wang C, Yu Q, Song T, Wang Z, Song L, Yang Y, *et al*. The heterogeneous immune landscape between lung adenocarcinoma and squamous carcinoma revealed by single-cell RNA sequencing. Signal Transduct Target Ther 2022;7(1):289. doi:10.1038/s41392-022-01130-8, PMID:36008393.

[4] Wang BY, Huang JY, Chen HC, Lin CH, Lin SH, Hung WH, *et al*. The comparison between adenocarcinoma and squamous cell carcinoma in lung cancer patients. J Cancer Res Clin Oncol 2020;146(1):43–52. doi:10.1007/s00432-019-03079-8, PMID:31705294.

[5] Chen JW, Dhahbi J. Lung adenocarcinoma and lung squamous cell carcinoma cancer classification, biomarker identification, and gene expression analysis using overlapping feature selection methods. Sci Rep 2021;11(1):13323. doi:10.1038/s41598-021-92725-8, PMID:34172784.

[6] Zhang Y, Vaccarella S, Morgan E, Li M, Etxeberria J, Chokunonga E, *et al*. Global variations in lung cancer incidence by histological subtype in 2020: a population-based study. Lancet Oncol 2023;24(11):1206–1218. doi:10.1016/S1470-2045(23)00444-8, PMID:37837979.

[7] Holdenrieder S. Biomarkers along the continuum of care in lung cancer. Scand J Clin Lab Invest Suppl 2016;245:S40–S45. doi:10.1080/00365513.2016.1208446, PMID:27542002.

[8] Zissimopoulos A, Stellos K, Permenopoulou V, Petrakis G, Theodorakopoulos P, Baziotis N, *et al*. The importance of the tumor marker CYFRA 21-1 in patients with lung cancer after surgery or chemotherapy. Hell J Nucl Med 2007;10(1):62–66. PMID:17450257.

[9] Yeh JJ, Liu FY, Hsu WH, Wang JJ, Ho ST, Kao A. Monitoring cytokeratin

fragment 19 (CYFRA 21-1) serum levels for early prediction of recurrence of adenocarcinoma and squamous cell carcinoma in the lung after surgical resection. Lung 2002;180(5):273–279. doi:10.1007/s004080000101, PMID:12489021.

[10] Sawabata N, Maeda H, Yokota S, Takeda S, Koma M, Tokunaga T, *et al*. Postoperative serum carcinoembryonic antigen levels in patients with pathologic stage IA nonsmall cell lung carcinoma: subnormal levels as an indicator of favorable prognosis. Cancer 2004;101(4):803–809. doi:10.1002/cncr.20421, PMID:15305413.

[11] Ozeki N, Fukui T, Taniguchi T, Usami N, Kawaguchi K, Ito S, *et al*. Significance of the serum carcinoembryonic antigen level during the follow-up of patients with completely resected non-small-cell lung cancer. Eur J Cardiothorac Surg 2014;45(4):687–692. doi:10.1093/ejcts/ezt424, PMID:23979987.

[12] Duan X, Cui Y, Li H, Shi G, Wu B, Liu M, *et al*. High preoperative and postoperative levels of carcinoembryonic antigen and CYFRA 21-1 indicate poor prognosis in patients with pathological Stage I nonsmall cell lung cancer. Indian J Cancer 2015;52(Suppl 3):E158–E163. doi:10.4103/0019-509X.186564, PMID:27453414.

[13] Barak V, Holdenrieder S, Nisman B, Stieber P. Relevance of circulating biomarkers for the therapy monitoring and follow-up investigations in patients with non-small cell lung cancer. Cancer Biomark 2010;6(3-4):191–196. doi:10.3233/CBM-2009-0129, PMID:20660964.

[14] Holdenrieder S, Wehnl B, Hettwer K, Simon K, Uhlig S, Dayyani F. Carcinoembryonic antigen and cytokeratin-19 fragments for assessment of therapy response in non-small cell lung cancer: a systematic review and meta-analysis. Br J Cancer 2017;116(8):1037–1045. doi:10.1038/bjc.2017.45, PMID:28278517.

[15] Tahanovich AD, Kauhanka NN, Murashka DI, Kolb AV, Prohorova VI, Gotko OV, *et al*. Prediction of recurrence-free survival of patients with stage III lung squamous cell carcinoma after surgical treatment. Healthcare 2022;11:44–50.

[16] Tahanovich AD, Kauhanka MM, Rutkovskaya ZA, Khotko EA, Gotko OV, Prokhorova VI. Prognostic evaluation of relapse based on squamous cell carcinoma antigen, CXCR2, and CD44V6 blood levels in patients with Stage I-II squamous cell lung cancer. Global Transl Med 2023;2(4):2209. doi:10.36922/gtm.2209.

[17] Tahanovich AD, Kauhanka NN, Prohorova VI, Murashka DI, Gotko OV. Predicting the risk of tumor progression in patients with early stages of adenocarcinoma and squamous cell lung carcinoma based on laboratory parameters. Biomed Khim 2021;67(6):507–517. doi:10.18097/PBMC20216706507, PMID:34964445.

[18] Parikh R, Mathai A, Parikh S, Chandra Sekhar G, Thomas R. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol 2008;56(1):45–50. doi:10.4103/0301-4738.37595, PMID:18158403.

[19] Muley T, He Y, Rolny V, Wehnl B, Escherich A, Warth A, *et al*. Potential for the blood-based biomarkers cytokeratin 19 fragment (CYFRA 21-1) and human epididymal protein 4 (HE4) to detect recurrence during monitoring after surgical resection of adenocarcinoma of the lung. Lung Cancer 2019;130:194–200. doi:10.1016/j.lungcan.2019.02.017, PMID:30885344.

[20] Pang L, Wang J, Jiang Y, Chen L. Decreased levels of serum cytokeratin 19 fragment CYFRA 21-1 predict objective response to chemotherapy in patients with non-small cell lung cancer. Exp Ther Med 2013;6(2):355–360. doi:10.3892/etm.2013.1171, PMID:24137188.

[21] Zhang W, Wang H, Sun M, Deng X, Wu X, Ma Y, *et al*. CXCL5/CXCR2 axis in tumor microenvironment as potential diagnostic biomarker and therapeutic target. Cancer Commun (Lond) 2020;40(2-3):69–80. doi:10.1002/cac2.12010, PMID:32237072.

[22] Lagiou P, Trichopoulos D. Inflammatory biomarkers and risk of lung cancer. J Natl Cancer Inst 2011;103(14):1073–1075. doi:10.1093/jnci/djr220, PMID:21685358.

[23] Spaks A. Role of CXC group chemokines in lung cancer development and progression. J Thorac Dis 2017;9(Suppl 3):S164–S171. doi:10.21037/jtd.2017.03.61, PMID:28446981.