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Blood laboratory parameters can predict relapse-free survival of patients with advanced squamous cell lung cancer and adenocarcinoma

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Abstract

The aim of the work was to study the relationship between the concentration of cells and proteins in the blood of patients with Stage III squamous cell lung cancer (SCLC) and adenocarcinoma (AC) before surgical treatment and the duration of the relapse-free period after tumor resection to develop prognostic models for relapse-free survival in these diseases. Using logistic regression equations, the models incorporated variables included cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1) concentration, the proportion of lymphocytes expressing the C-X-C motif chemokine receptor 1 (CXCR1), and monocytes expressing the C-X-C motif chemokine receptor 2 for SCLC. For AC, the models included the CYFRA 21-1 concentration, lymphocytes expressing the CXCR1 receptor, and the eosinophil-to-monocyte ratio. These models can predict the probability of tumor recurrence based on measurements of blood parameters in the pre-operative period, with a prediction efficiency of 87.7% for SCLC and 89.0% for AC.

Keywords: Squamous cell lung cancer; Adenocarcinoma; Relapse; Prognosis; CYFRA 21-1; CXCR1; CXCR2; Stage III

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1. Introduction

Lung cancer (LC) is one of the most common forms of malignant tumors. According to the histological structure, 80 – 85% of LC cases are classified as non-small cell LC (NSCLC).[1] Within the NSCLC category, two main histological types are distinguished: adenocarcinoma (AC) and squamous cell LC (SCLC).[2]

In most patients, NSCLC is diagnosed at an advanced stage, often with disease metastases present. About 30% of all NSCLC cases are patients with Stage III disease.[1,2] According to the TNM classification,[3] Stage III NSCLC is a heterogeneous group of tumors that differ in size, the presence of invasion into surrounding mediastinal structures, and damage to the mediastinal lymph nodes. These are categorized as stage IIIA (T1-2N2, T3N1, T4N0-1), Stage IIIB (T1-2N3, T3-4N2), and Stage IIIC (T3-4N3), each of which determines the particular treatment regimen. Surgical treatment is

typically performed for stage IIIA and some stage IIIB cases, often supplemented with neoadjuvant and/or adjuvant chemotherapy, sometimes in combination with radiation therapy.[2]

Different treatment regimens are aimed at preventing disease recurrence. Despite this, the prognosis in patients with Stage III NSCLC remains poor. For patients with T1-4N0-2, the overall 5-year survival rate after treatment varies from 36% to 82%.[1-3] The median survival rate for stage III NSCLC generally does not exceed 20 months, with no more than 20% of patients surpassing the 5-year survival barrier.[2,3] At the same time, patients with the same TNM stage may have different outcomes and the likelihood of relapse. One of the approaches to optimizing the effectiveness of treatment in this category of patients is the ability to predict those at high risk of disease relapse. They have a high risk of retaining hidden metastases after surgical removal of the tumor, which significantly contributes to disease recurrence, referred to as "relapse."

Early prediction of rapid relapse after treatment would allow for the timely and targeted implementation of neoadjuvant and adjuvant therapy, in addition to surgical treatment. Therapeutic treatments are associated with various side effects, but when targeted, they can provide maximum benefit,[2] thereby increasing the survival of patients with Stage III NSCLC. Therefore, predicting the risk of tumor recurrence in patients with Stage III (T1-4N0-2) NSCLC before treatment is highly relevant.

There is considerable evidence supporting the relationship between systemic inflammation and cancer.[4] On one hand, the inflammatory reaction creates conditions for the development of cancer, and on the other hand, it is a consequence of metabolic changes in tumor cells.[5] Inflammation in the tumor microenvironment plays a role in the proliferation and survival of malignant tumor cells, angiogenesis in tumor tissue, and metastasis.[6] Signs of tumor-associated inflammation are the presence of cells and inflammatory mediators (chemokines, cytokines) in tumor tissue, similar to those observed in chronic inflammation and reparation.

During transformation, many cells of epithelial or mesenchymal origin begin to express chemokine receptors, thereby utilizing these factors for migration and survival at sites distant from the primary tumor. In particular, the proinflammatory C-X-C motif chemokine ligand 8 (CXCL8) exerts its effects by signaling through two seven-transmembrane-segment receptors, C-X-C motif chemokine receptor 1 (CXCR1) and receptor 2 (CXCR2). Activation of the CXCL8-CXCR1/2 signaling pathway in the tumor microenvironment of numerous cancers enhances tumor progression by promoting proliferation, angiogenesis, migration, invasion, cell survival, and involvement in organ-specific metastasis.[6] Another chemokine that binds to the CXCR2 receptor is C-X-C motif chemokine ligand 5 (CXCL5), which serves as an attractant for granulocytes. The CXCL5/CXCR2 axis has been shown to be important in the development of many human cancers. The serum CXCL5 protein concentration was significantly increased in NSCLC compared to healthy volunteers. CXCL5 expression correlated with tumor size and stage of NSCLC, lymph node metastases, and decreased patient survival.[7] Our previous studies also showed changes in the levels of these proteins in the blood of NSCLC patients.[8-10] Their relationship with tumor process descriptors was established, and the diagnostic efficiency of their determination in this disease was calculated, which in some cases exceeded that of classical markers.

An aggressive, rapidly growing tumor with multiple metastases produces and secretes a large number of these proteins into the blood serum, which indicates a poor prognosis.[11] Therefore, blood, being a minimally invasive and the most accessible material, plays a crucial role in the search for oncobiomarkers, including in patients with NSCLC. Tumor cell components, or molecules involved in the development of tumor tissue, circulating in the bloodstream, have been studied as candidates for malignant growth markers. These include the well-established cytokeratin 19 fragment antigen 21-1 (CYFRA 21-1), squamous cell carcinoma (SCC) antigen, and cancer embryonic antigen (CEA).[11] Subsequent studies have shown that CEA and CYFRA 21-1, in addition to their diagnostic value, also hold prognostic significance in NSCLC.[12-17] However, determining the level of each of these markers separately in blood serum has not demonstrated sufficient specificity and sensitivity.

Researchers are increasingly focusing on other systemic inflammatory markers in the blood, such as lymphocytes (L),[18] neutrophils (N), platelets (P),[19] C-reactive protein (CRP), and albumin,[20] as well as their ratios,[21-23] as prognostic markers in cancer. Interest in such indicators is understandable, given that the quantitative and semi-quantitative assessment of blood cells is a routine and relatively inexpensive test, which is usually carried out for every patient admitted to a clinic. Evaluating these results to predict patient survival is a critical issue. For these same purposes, the calculation of the systemic immune-inflammatory index (SII), which has proven effective in determining treatment strategies for a wide variety of cancers,[24] and the inflammatory prognostic index (IPI),[25] have been proposed. The advantage of these laboratory indicators lies not only in their low cost but also in the stability and reproducibility of the results. However, the data obtained often contradict each other, and the

correspondence between their concentration in the blood and the tumor response in NSCLC is only 40 – 70%.[26]

To increase the diagnostic and prognostic efficiency of these markers, there have been attempts to create multianalytical panels incorporating these and other indicators of tumor tissue metabolism.[27] However, to date, there is still no informative single biomarker or combination of biomarkers that can help in predicting the recurrence of LC after diagnosis and before treatment initiation. This is believed due to the lack of a standardized study designs, patient stratification criteria, and the low diagnostic sensitivity and/or specificity of the markers.[27]

As histological subtypes of NSCLC, AC and SCLC differ in etiology and course.[28] Thus, SCLC develops significantly more often in smoking patients, while AC is more associated with obesity. Compared to AC, SCLC is more prevalent in men, whereas AC is more frequently seen in women.

Both subtypes are characterized by symptoms, including hemoptysis, dyspnea, chest pain, cough, and general weakness. Changes in the level of some laboratory parameters in the blood of patients, such as CRP, fibrinogen, and haptoglobin, also show similar patterns in both subtypes.[29] In both cases, the disease often proceeds without any clinical manifestations for extended periods, leading to a late diagnosis (stage III or IV) in 45% of patients with AC and SCLC.[30]

Compared with SCLC, AC is slightly more often detected in patients with early-stage disease (I or II).[31] In patients with Stage I SCLC, the 5-year survival rate is 47%, while for AC, this figure is almost twice as high (79%).[28] For Stage II AC and SCLC, the prognosis of the disease worsens significantly. During this period, the 5-year survival rate for patients with SCLC (32%) is also significantly lower than for those with AC (50%). In patients with Stage IV SCLC and AC, this figure drops to only 2% and 6%, respectively.[28]

In SCLC, serum levels of CYFRA 21-1 and CEA have been reported to associate with overall and relapse-free survival.[12] However, these studies were also conducted only in patients with early-stage disease (I–II). Patients with Stage III were examined only in a mixed group with early stages.[32] Information on the role of blood laboratory parameters in predicting relapse-free survival in patients with Stage III SCLC, as well as with Stage III AC, was lacking.

Therefore, the aim of this study was to investigate the potential use of pre-operative levels several indicators, which characterize the cellular composition and metabolism in the blood of patients with Stage III AC and SCLC, to predict their relapse-free survival and make a decision on the therapeutic strategy. A key condition for selecting these indicators was to compare their prognostic value with known marker, including CYFRA 21-1, SCC antigen, and tissue polypeptide antigen (TPA).

2. Materials and methods

2.1. Study population

To substantiate the risk groups for tumor recurrence in patients with operable Stage III (T1-4N0-2) SCLC, a retrospective study was initially undertaken using information from the Belarusian Cancer Registry database. The study included 416 patients with newly diagnosed SCLC (Table 1) and 451 patients with Stage III (T1-4N0-2) AC (Table 2), between January 1, 2015, and December 31, 2021. The period of relapse development after treatment was analyzed based on the results of 1-year observation, due to the majority of NSCLC relapses develop within the first year following treatment.[33]

The investigation of laboratory parameters was carried out in a "study group" of 73 patients with newly diagnosed SCLC (Table 1) and 77 patients with Stage III (T1-4N0-2) AC (Table 2), who were admitted to the thoracic oncology department of the N.N. Alexandrov Republican Scientific and Practical Center for OMR between January 1, 2022, and December 31, 2023. Inclusion criteria included a newly diagnosed stage IIIA or IIIB SCLC, while exclusion criteria were the presence of metachronous or secondary cancer, and patient refusal to participate in the study. No patient dropped out during the first year of observation. Patients with T1N2M0, T2N2M0, T3N1M0, and T3N2M0 underwent surgical tumor resection (surgical volume - R0) followed by 4 courses of adjuvant polychemotherapy, consisting of a combination of vinorelbine (V) at 25 – 30 mg/m^2 and cisplatin (C) at 80 mg/m^2. Meanwhile, in patients with T4N0M0, T4N1M0, and T4N2M0, two courses of neoadjuvant chemotherapy, consisting of a combination of V+C were administered, followed by surgical tumor resection and two additional courses of adjuvant polychemotherapy of V+C.

2.2. Ethical approval and consent

All patients provided written informed consent. The study was performed according to the ethical regulations in Belarus and approved by the Ethics Committee at Belarusian State Medical University, protocol №2 from April 10, 2021.

2.3. Sample collection and analysis

Blood samples were collected from patients of the "study group" before treatment (39 patients with SCLC and 40 patients with AC). Blood cell concentrations were determined on a Sysmex XE-5000 hematology analyzer (Sysmex Group, Japan). Albumin and CRP levels

Table 1. Characteristics of patients with SCLC

Parameter	Retrospective group (%)	Study group (%)	*P*-value
Number of patients, total	416	73	
Age, years (M±σ)	58±29	59±27	0.379
<40	17 (4.1)	2 (2.7)	
41 – 50	72 (17.3)	13 (17.8)	
51 – 60	197 (47.4)	33 (45.2)	
61 – 70	98 (23.6)	20 (27.64)	
>70	32 (7.7)	5 (6.8)	
Gender			0.435
Male	297 (71.4)	56 (76.7)	
Female	119 (28.6)	17 (23.3)	
Smoking status, male			0.286
Former	18 (6.1)	7 (12.5)	
Current	267 (89.9)	46 (82.1)	
Never	12 (4.0)	3 (5.4)	
Smoking status, female			0.315
Former	20 (16.8)	2 (11.8)	
Current	93 (78.2)	14 (82.4)	
Never	6 (5.0)	1 (5.9)	
Stage III (based on 8th edition of TNM staging of lung cancer)			0.421
T1N2M0	46 (11.1)	7 (9.6)	
T2N2M0	69 (16.6)	15 (20.5)	
T3N1M0	66 (15.9)	10 (13.7)	
T4N0M0	48 (11.5)	9 (12.3)	
T4N1M0	50 (12.0)	9 (12.3)	
T3N2M0	76 (18.3)	12 (16.4)	
T4N2M0	61 (14.7)	11 (15.1)	
Degree of tumor differentiation			0.127
G I	150 (36.1)	23 (31.5)	
G II	166 (39.9)	37 (50.71)	
G III	100 (24.0)	13 (17.8)	
Localization			0.519
Right lung	215 (51.7)	41 (56.2)	
Left lung	201 (48.3)	32 (43.8)	

Abbreviation: SCLC: Squamous cell lung cancer.

Table 2. Characteristics of patients with AC

Parameter	Retrospective group (%)	Study group (%)	*P*-value
Number of patients, total	451	77	
Age, years (M ± σ)	59±26	58±28	0.258
<40	14 (3.1)	3 (3.9)	
41 – 50	75 (16.6)	13 (16.9)	
51 – 60	201 (44.6)	38 (49.4)	
61 – 70	107 (23.7)	20 (26.0)	
>70	54 (12.0)	3 (3.9)	
Gender			0.315
Male	329 (72.9)	58 (75.3)	
Female	122 (27.1)	19 (24.7)	
Smoking status, male			0.193
Former	21 (6.4)	7 (12.5)	
Current	285 (86.6)	48 (85.7)	
Never	23 (7.0)	3 (5.4)	
Smoking status, female			0.143
Former	27 (22.1)	5 (26.3)	
Current	86 (70.5)	12 (63.2)	
Never	9 (7.4)	2 (10.5)	
Stage III (based on 8th edition of TNM staging of lung cancer)			0.329
T1N2M0	50 (11.1)	7 (9.1)	
T2N2M0	72 (16.0)	16 (20.8)	
T3N1M0	72 (16.0)	9 (11.7)	
T4N0M0	53 (11.8)	10 (13.0)	
T4N1M0	55 (12.2)	11 (14.3)	
T3N2M0	84 (18.6)	13 (16.9)	
T4N2M0	65 (14.4)	11 (14.3)	
Degree of tumor differentiation			0.724
G I	168 (37.3)	25 (32.5)	
G II	184 (40.8)	39 (50.6)	
G III	99 (21.9)	13 (16.9)	
Localization			0.357
Right lung	249 (55.2)	40 (51.9)	
Left lung	201 (44.8)	37 (48.1)	

Abbreviation: AC: Adenocarcinoma.

were determined using a biochemical analyzer AU680 (Beckman Coulter, USA) with original reagent kits. The SII was calculated using the equation P × N/L, where P, N, and L are platelets, neutrophils, and lymphocytes, respectively. The IPI was calculated as ([CRP] × N)/(L × [albumin]), while the systemic inflammatory response index (SIRI) was calculated using the equation N × M/L, where M represents monocytes.

The concentrations of CYFRA 21-1 and SCC were determined using an automatic Cobas e411 analyzer (Rosche Diagnostics GmbH, Germany), based on the principle of electrochemiluminescence.[11]

The concentrations of CXCL5, CXCL8, TPA, HIF -1α, TuM2 PK, and hyaluronic acid were measured on a Brio automatic ELISA analyzer (Seac, Italy) with ELISA kits (FineTest, China).[12]

Blood cell receptors CXCR1, CXCR2, and CD44v6 were determined using a Navios flow cytometer (Beckman Coulter, USA).[13]

2.4. Statistical analysis

The dependence of the relapse-free period duration on the observation time was assessed using Kaplan-Meier graphs. Single and multivariate Cox proportional hazard models were used to analyze the relationship between the determined laboratory parameters and survival. Comparison of groups with different risks of NSCLC relapse was performed using the Log-Rank test. The data analysis included non-parametric statistics methods (MedCalc Software, Belgium). Differences in the values of the determined parameters between groups of patients were evaluated using Mann–Whitney U-test. The overall prognostic value of the laboratory tests was assessed by constructing receiver operating characteristic (ROC) curves, followed by calculating the area under the ROC curve (AUC).[34] Statistical significance was defined as $P < 0.05$.

3. Results

Kaplan-Meier graphs show that the relapse-free survival of patients with tumor descriptors T4N1M0 and T4N2M0 in SCLC (Figure 1A) and AC (Figure 1C) differs significantly from patients with other TNM descriptors (T1N2M0, T3N1M0, T2N2M0, T4N0M0, and T3N2M0). In patients with T4N1M0 (stage IIIA AC), the relapse-free survival after treatment is lower than in patients with other T and N variants of Stage IIIA. In addition, patients with T3N2M0 (stage IIIB) experience slower disease relapses than the other T and N variants within the same stage. According to the Kaplan-Meier graph (Figure 1A and C), the relapse-free survival of patients 1 year after treatment can be divided into two groups. One is characterized by a relatively high survival rate, includes patients with T1N2M0, T3N1M0, T2N2M0, T4N0M0, and T3N2M0. The other group typically with a shorter relapse-free period includes patients with T4N1M0 and T4N2M0. The difference in survival between these groups is statistically significant, as indicated by the results of the Log-Rank test (Figure 1B and D). The χ^2 value for differences in relapse-free survival between risk groups based on TNM stratification for patients with operable Stage III SCLC is 10.25 ($P = 0.017$), and for patients with AC, it is 10.31 ($P = 0.025$).

We compared several blood laboratory parameters between the two risk groups of relapse-free survival for Stage III SCLC patients (Table 3). Only the proportion of lymphocytes with the CXCR1 receptor, monocytes with the CXCR2 receptor, and the CYFRA 21-1 level showed

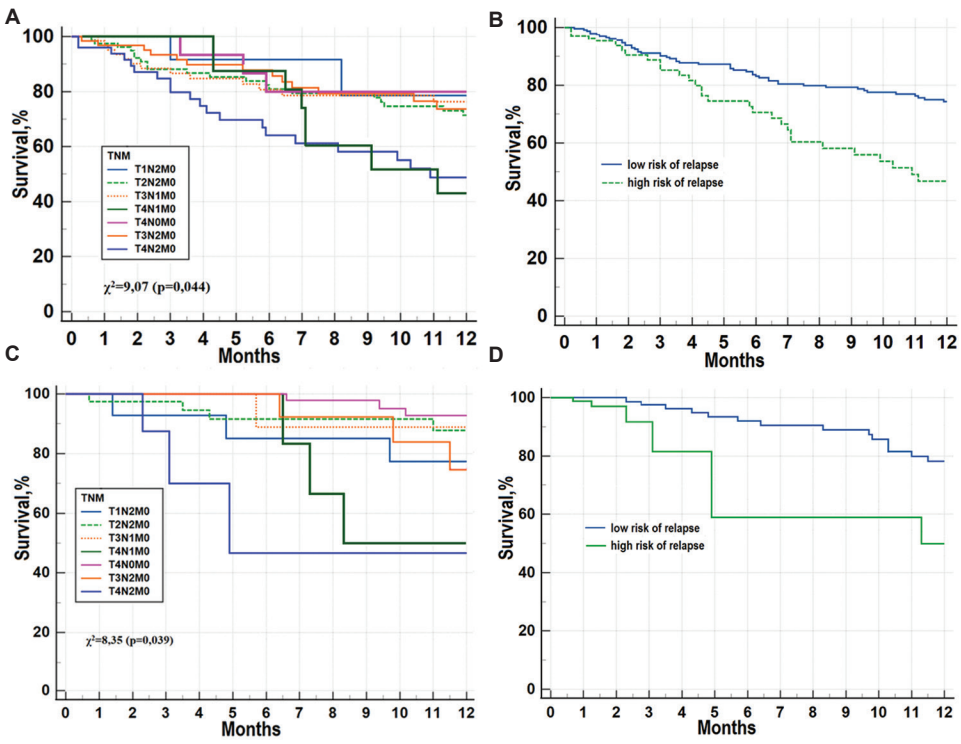

Figure 1. Relapse-free survival of patients with Stage III squamous cell lung cancer (A and B) and adenocarcinoma (C and D), stratified by the TNM classifications over the course of 12 months after the treatment.

Table 3. Level of laboratory parameters in patients (stage III SCLC, study group) with low and high risk of relapse

Indicator	Low risk	High risk	*P*-value
Neutrophilic leukocytes, $\times10^9$/L	5.13 (3.66; 6.57)	5.17 (3.81; 7.14)	0.844
Monocytes, $\times10^9$/L	0.87 (0.68; 1.03)	1.03 (0.86; 1.23)	0.121
Lymphocytes, $\times10^9$/L	2.38 (2.13; 3.07)	2.48 (2.09; 3.29)	0.728
Basophilic leukocytes, $\times10^9$/L	0.03 (0.02; 0.04)	0.04 (0.03; 0.07)	0.260
Eosinophilic leukocytes, $\times10^9$/L	0.14 (0.08; 0.23)	0.22 (0.17; 0.38)	0.298
Platelets, $\times10^9$/L	307.3 (257.8; 387.3)	313.8 (247.3; 391.5)	0.954
Neutrophilic leukocytes/Lymphocytes	1.99 (1.48; 3.28)	1.98 (1.35; 2.81)	0.789
Platelets/Lymphocytes	128.4 (84.5; 201.3)	120.3 (101.2; 137.2)	0.678
Lymphocytes/Monocytes	2.81 (2.01; 3.81)	2.69 (2.17; 3.12)	0.465
Eosinophilic leukocytes/Monocytes	0.177 (0.092; 0.263)	0.291 (0.137; 0.414)	0.297
CXCR1, granulocytes, %	94.6 (92.5; 97.5)	94.6 (93.7; 96.9)	0.825
CXCR1, granulocytes, MFI	32.0 (28.5; 51.5)	32.9 (28.5; 53.0)	0.645
CXCR1, lymphocytes, %	0.90 (0.65; 1.65)	3.55 (2.85; 3.70)	*0.001
CXCR1, lymphocytes, MFI	12.84 (3.50; 15.85)	12.85 (7.06; 14.85)	0.872
CXCR1, monocytes, %	0.61 (0.45; 19.65)	0.85 (0.40; 17.40)	0.316
CXCR1, monocytes, MFI	31.7 (3.6; 41.8)	33.5 (17.9; 35.5)	0.591
CXCR2, granulocytes, %	91.8 (63.1; 95.2)	92.5 (72.9; 95.5)	0.993
CXCR2, granulocytes, MFI	66.4 (68.5; 98.7)	65.5 (51.6; 79.5)	*0.029
CXCR2, lymphocytes, %	15.2 (10.5; 21.5)	15.0 (13.6; 18.5)	1.005
CXCR2, lymphocytes, MFI	12.9 (11.3; 15.5)	13.5 (11.5; 14.5)	0.933
CXCR2, monocytes, %	1.42 (0.60; 2.35)	2.55 (1.90; 3.70)	0.044
CXCR2, monocytes, MFI	62.3 (27.5; 75.5)	63.9 (37.9; 70.5)	0.581
CD44v6, granulocytes, %	2.22 (1.65; 4.15)	2.60 (1.75; 5.05)	0.745
CD44v6, granulocytes, MFI	2.70 (2.45; 3.50)	2.65 (2.10; 6.15)	0.849
CD44v6, lymphocytes, %	0.90 (0.25; 1.45)	1.05 (0.35; 1.75)	0.462
CD44v6, lymphocytes, MFI	3.85 (2.05; 6.50)	4.15 (1.85; 6.50)	0.323
CD44v6, monocytes, %	1.45 (0.25; 3.15)	1.40 (0.15; 1.85)	0.444
CD44v6, monocytes, MFI	3.95 (3.60; 35.45)	4.30 (2.45; 37.20)	0.483
Albumin, g/L	41.3 (38.9; 45.5)	42.5 (41.1; 44.75)	0.518
CRP, mg/dL	1.42 (0.39; 6.05)	1.67 (1.24; 4.85)	0.784
C-RB/Albumin	0.032 (0.01; 0.17)	0.036 (0.03; 0.13)	0.770
CXCL5, pg/mL	914.5 (679.8; 1542.7)	983.6 (753.9; 1713.8)	0.856
CXCL8, pg/mL	111.8 (101.1; 220.5)	118.1 (75.8; 267.3)	0.540
Hyaluronic acid, ng/mL	22.7 (20.8; 35.9)	24.5 (21.8; 25.2)	0.531
HIF-1a, pg/mL	3.17 (2.75; 3.95)	3.47 (2.71; 4.74)	0.922
SCC, ng/mL	2.76 (1.64; 5.37)	2.99 (2.04; 6.05)	0.591
TPA, pg/mL	953.0 (767.9; 1108.7)	1027.3 (805.0; 1229.3)	0.652
TuM2-PK, pg/mL	1830.0 (1493.7; 2173.3)	1829.0 (1428.5; 2329.6)	0.962
CYFRA 21-1, ng/mL	5.06 (3.22; 5.987)	7.53 (5.37; 18.15)	*0.020
IPI	0.057 (0.012; 0.207)	0.092 (0.031; 0.229)	0.679
SII	673.3 (362.6; 1221.3)	597.6 (366.2; 1017.7)	0.641
SIRI	1.57 (1.04; 3.08)	1.84 (1.45; 3.15)	0.554

Note: * $P<0.05$ indicates the value is statistically significant.
Abbreviations: MFI: Mean fluorescence intensity; CXCR1: C-X-C motif chemokine receptor 1; CD44v6: CD44 variant isoform v6; CXCR2: C-X-C motif chemokine receptor 2; CRP: C-reactive protein; CXCL5: C-X-C motif chemokine ligand 5; CXCL8: C-X-C motif chemokine ligand 8; HIF-1a: Hypoxia-inducible factor 1-alpha; SCC: Squamous cell carcinoma antigen; TPA: Tissue polypeptide antigen; TuM2-PK: Tumor type M2 pyruvate kinase; CYFRA 21-1: Cytokeratin 19 fragment antigen 21-1; IPI: Inflammatory prognostic index; SII: Systemic immune-inflammatory index; SIRI: systemic inflammatory response index.

significant differences between patients with a high and low risk of relapse-free survival.

The results of the Cox proportional hazards model analysis confirm the relationship of all 3 parameters with relapse-free survival in both the univariate and multivariate models ($P < 0.05$, Table 4).

In the high-risk group of AC recurrence, only the absolute values of the concentration of monocytes, eosinophilic leukocytes, the ratio between them, the proportion of lymphocytes with the CXCR1 receptor and the CYFRA 21-1 level were significantly higher than in the low-risk group (Table 5). The remaining parameters (SII, IPI, SIRI indices, HIF-1α, CXCL5, CXCL8, TuM2 PK, CXCR1, etc.) did not demonstrate any significant differences between the high- and low-risk groups of patients. Only these five parameters were included in the Cox proportional hazards models, where they were found to significantly affect patient survival (Table 6).

The results of ROC analysis show the prognostic characteristics of the selected indicators for the duration of relapse-free survival in Stage III SCLC (Table 7). The proportion of blood lymphocytes expressing the CXCR1 receptor demonstrated the highest prognostic efficiency (76.7%). The prognostic efficiency was 71.2% and 74.0% for CXCR2-positive monocytes and CYFRA 21-1, respectively. To improve the accuracy of the results, the values of these parameters were subjected to logistic regression analysis. The resulting Equation I includes a combination of these indicators. The prognostic accuracy for the calculated threshold value (> 0.417) was 87.7% (Table 7).

$$Y = \frac{\exp(-5.315 + 0.116 * [CYFRA] + 1.901 * [CXCR1] + 0.279 * [CXCR2])}{1 + \exp(-5.315 + 0.116 * [CYFRA] + 1.901 * [CXCR1] + 0.279 * [CXCR2])} \quad (I)$$

Logistic regression equation for predicting relapse-free survival in patients with Stage III SCLC.

Note: [CYFRA] – the concentration (ng/ml) of the CYFRA 21-1 antigen in the blood serum; [CXCR1] – the relative amount (percentage) of the CXCR1 receptor in lymphocytes; [CXCR2] – the relative amount (percentage) of the CXCR2 receptor in monocytes; "Y" is the result of the regression equation.

According to the AUC expert scale, the prognostic model is classified as "very good" quality with an AUC of 0.831.[14] The optimal threshold value for distinguishing low- and high-risk groups for tumor recurrence is 0.417, with a sensitivity of 84.9%, and specificity of 89.0% (Table 7). Specifically, if the Y value is >0.417, the probability that the patient has a high risk of tumor recurrence is 90.4%. Conversely, if the Y value is ≤0.417, the probability that the patient has a low risk of tumor recurrence is 84.9%.

The performance of the proposed regression model is demonstrated by the Kaplan-Meier graph, which shows relapse-free survival in patients with Stage III SCLC (Figure 2).

The 1-year follow-up shows the distribution of high and low relapse-free survival of patients with Stage III SCLC according to the results of the regression equation Y (Figure 2), which corresponds to TNM stratification (Figure 1B). By the end of the 1st year, the survival rate for the low-risk group was 74% according to TNM stratification, and 76% according to the regression equation of blood parameters. For patients with a high risk, survival after treatment was 47% and 45%, according to TNM stratification and regression equation of blood parameters, respectively. Besides, there is a clear difference between high and low survival of patients based on the regression equation as early as the first month after the treatment, with this difference increasing over time. In contrast, the curve difference in high and low relapse-free survival based on TNM stratification becomes visible only two months after the treatment.

According to the results of ROC analysis for selected parameters in Stage III AC, the highest specificity (84.4%) was found for the relative number of lymphocytes expressing the CXCR1, while its diagnostic sensitivity did not exceed 66.2% (Table 8). The values of other selected indicators showed comparable figures, with sensitivity

Table 4. Cox proportional hazards models for SCLC patients

Indicator	Univariate model			Multivariate model		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
CXCR1, lymphocytes, %	1.122	1.003 – 1.241	*0.007	1.091	1.001 – 1.181	*0.021
CXCR2, monocytes, %	1.023	1.002 – 1.044	*0.023	1.013	1.001 – 1.025	*0.043
CYFRA 21-1, ng/ml	1.102	1.009 – 1.195	*0.027	1.073	1.009 – 1.137	*0.022

Note: *: $P<0.05$ indicates the value is statistically significant.
Abbreviations: HR: Hazards ratio; 95% CI: 95% 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.

Table 5. Level of laboratory parameters in patients (stage III AC, study group) with low and high risk of relapse

Indicator	Low risk	High risk	*P*-value
Neutrophilic leukocytes, $\times10^9$/L	4.95 (3.56; 6.38)	5.01 (3.71; 6.92)	0.816
Monocytes, $\times10^9$/L	0.62 (0.51; 0.72)	0.93 (0.68; 1.19)	0.032
Lymphocytes, $\times10^9$/L	2.32 (2.08; 2.99)	2.42 (2.02; 3.18)	0.705
Basophilic leukocytes, $\times10^9$/L	0.03 (0.02; 0.04)	0.04 (0.03; 0.06)	0.251
Eosinophilic leukocytes, $\times10^9$/L	0.12 (0.06; 0.21)	0.36 (0.30; 0.45)	0.006
Platelets, $\times10^9$/L	298.1 (248.1; 379.5)	303.7 (244.7; 382.7)	0.923
Neutrophilic leukocytes/Lymphocytes	1.93 (1.43; 3.19)	1.93 (1.34; 2.74)	0.764
Platelets/Lymphocytes	120.6 (82.2; 198.3)	114.9 (99.1; 140.8)	0.656
Lymphocytes/Monocytes	2.75 (1.97; 3.69)	2.57 (2.11; 2.92)	0.449
Eosinophilic leukocytes/Monocytes	0.158 (0.088; 0.287)	0.453 (0.288; 0.682)	*0.022
CXCR1, granulocytes, %	91.6 (89.8; 94.1)	91.7 (89.9; 94.0)	0.798
CXCR1, granulocytes, MFI	31.1 (27.1; 49.6)	30.8 (26.6; 51.4)	0.624
CXCR1, lymphocytes, %	1.60 (0.70; 2.30)	3.56 (3.40; 4.80)	*0.016
CXCR1, lymphocytes, MFI	12.22 (3.44; 15.33)	12.39 (6.85; 14.36)	0.845
CXCR1, monocytes, %	0.59 (0.41; 19.03)	0.79 (0.39; 16.93)	0.306
CXCR1, monocytes, MFI	30.45 (3.51; 39.60)	32.78 (17.39; 33.95)	0.572
CXCR2, granulocytes, %	88.7 (62.2; 93.3)	84.3 (70.7; 92.3)	0.961
CXCR2, granulocytes, MFI	64.5 (66.6; 95.7)	62.8 (50.1; 76.9)	*0.028
CXCR2, lymphocytes, %	14.71 (10.19; 21.02)	15.12 (13.14; 18.09)	0.973
CXCR2, lymphocytes, MFI	12.53 (10.93; 14.52)	12.63 (11.16; 14.16)	0.903
CXCR2, monocytes, %	1.38 (0.58; 2.25)	2.51 (1.84; 3.64)	*0.043
CXCR2, monocytes, MFI	60.5 (26.8; 73.4)	61.7 (36.8; 68.7)	0.562
CD44v6, granulocytes, %	2.12 (1.60; 3.98)	2.49 (1.70; 4.85)	0.720
CD44v6, granulocytes, MFI	2.61 (2.33; 3.44)	2.39 (2.04; 5.92)	0.821
CD44v6, lymphocytes, %	0.88 (0.19; 1.44)	1.03 (0.34; 1.70)	0.448
CD44v6, lymphocytes, MFI	3.67 (1.94; 6.26)	3.93 (1.79; 6.35)	0.313
CD44v6, monocytes, %	1.35 (0.22; 3.07)	1.42 (0.15; 1.75)	0.430
CD44v6, monocytes, MFI	3.79 (3.49; 34.41)	4.25 (2.38; 36.13)	0.468
Albumin, g/L	40.7 (37.7; 43.9)	41.7 (39.8; 43.4)	0.501
CRP, mg/dL	1.37 (0.38; 5.90)	1.62 (1.20; 4.74)	0.758
C-RB/Albumin	0.032 (0.010; 0.146)	0.036 (0.029; 0.116)	0.746
CXCL5, pg/mL	883.4 (655.5; 1506.1)	937.0 (731.2; 1704.1)	0.828
CXCL8, pg/mL	105.6 (99.0; 212.9)	114.6 (73.4; 270.2)	0.522
Hyaluronic acid, ng/mL	23.7 (20.1; 33.9)	23.9 (21.1; 25.4)	0.515
HIF-1a, pg/mL	3.26 (2.68; 3.80)	3.39 (2.63; 4.58)	0.892
SCC, ng/mL	2.67 (1.64; 5.22)	2.89 (1.98; 5.85)	0.572
TPA, pg/mL	941.0 (747.7; 1069.7)	1017.2 (781.8; 1194.5)	0.631
TuM2-PK, pg/mL	1781.3 (1451.8; 2112.3)	1783.7 (1453.6; 2284.0)	0.931
CYFRA 21-1, ng/mL	3.97 (2.22; 4.24)	7.39 (3.60; 12.31)	*0.032
IPI	0.05 (0.01; 0.20)	0.09 (0.03; 0.22)	0.657
SII	669.73 (352.71; 1180.76)	583.49 (367.80; 991.07)	0.620
SIRI	1.54 (1.01; 2.98)	1.77 (1.41; 3.02)	0.536

Note: *: $P<0.05$ indicates the value is statistically significant.
Abbreviations: MFI: Mean fluorescence intensity; CXCR1: C-X-C motif chemokine receptor 1; CD44v6: CD44 variant isoform v6; CXCR2: C-X-C motif chemokine receptor 2; CRP: C-reactive protein; CXCL5: C-X-C motif chemokine ligand 5; CXCL8: C-X-C motif chemokine ligand 8; HIF-1a: Hypoxia-inducible factor 1-alpha; SCC: Squamous cell carcinoma antigen; TPA: Tissue polypeptide antigen; TuM2-PK: Tumor type M2 pyruvate kinase; CYFRA 21-1: Cytokeratin 19 fragment antigen 21-1; IPI: Inflammatory prognostic index; SII: Systemic immune-inflammatory index; SIRI: systemic inflammatory response index.

Table 6. Cox proportional hazards models for selected laboratory parameters in III-stage AC patients

Indicator	Univariate model			Multivariate model		
	HR	95% CI	*P*-value	HR	95% CI	*p*-value
CXCR1, lymphocytes, %	1.137	1.005 – 1.275	0.012*	1.114	1.003 – 1.222	0.017*
CYFRA 21-1, ng/mL	1.215	1.009 – 1.419	0.016*	1.182	1.007 – 1.413	0.016*
Monocytes, $\times 10^9$/L	1.189	1.093 – 1.289	0.022*	1.162	1.074 – 1.254	0.024*
Eosinophilic leukocytes, $\times 10^9$/L	11.337	1.205 – 14.248	0.027*	10.121	1.181 – 13.325	0.031*
Eosinophilic leukocytes/Monocytes	12.153	1.511 – 22.799	0.019*	11.371	1.409 – 21.335	0.021*

Note: $P<0.05$ indicates the value is statistically significant.
Abbreviations: CXCR1: HR: Hazards ratio; 95% CI: 95% Confidence interval; CXCR1: C-X-C motif chemokine receptor 1; CYFRA 21-1: Cytokeratin 19 fragment antigen 21-1.

Table 7. Significance of determining laboratory parameters in blood for predicting low and high risk of recurrence in patients with stage III SCLC (ROC analysis data)

Indicator	TV	SE	SP	PPV	NPV	AUC	ACC
CXCR1, lymphocytes, %	>2.25	75.3	79.5	79.5	76.3	0.729	76.7
CXCR2, monocytes, %	>2.05	69.9	74.0	72.6	71.2	0.687	71.2
CYFRA 21-1, ng/mL	>6.02	80.8	68.5	72.6	76.7	0.711	74.0
Y	>0.417	84.9	89.0	90.4	84.9	0.831	87.7

Abbreviations: TV: Threshold value; SE: Sensitivity; SP: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under ROC-curve; ACC: Accuracy; 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.

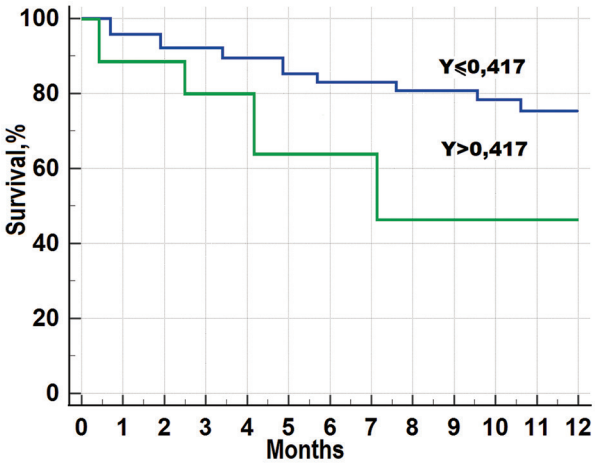

Figure 2. Relapse-free survival of patients with Stage III squamous cell lung cancer according to the results of the regression equation Y.

ranging from 62.3% to 76.6%. As a result, the diagnostic efficiency for predicting relapse-free survival was the lowest for monocyte concentration (64.9%), and the highest for CXCR1-positive lymphocytes (75.3%).

CYFRA 21-1, CXCR1-positive lymphocytes, and eosinophilic leukocytes/monocytes (E/M) ratio were included in the regression analysis. The reliability of the regression equation (2), which uses a combination of these markers to predict the risk of tumor recurrence, is also evidenced by the results of the ROC analysis. The AUC of 0.841 indicates a "very good" quality of the prognostic model.[14] The optimal TV for distinguishing the low- and high-risk groups of tumor recurrence is 0.597 (Table 8). Specifically, if the value of Z > 0.597, the probability that the patient has a high risk of tumor recurrence is 89.6%. Conversely, if the value of Z ≤ 0.597, the probability that the patient has a low risk of tumor recurrence is 84.4%.

$$Z = \frac{\exp(-14.022 + 0.539 * [CYFRA] + 1.294 * [CXCR1] + 12.035 * [E/M])}{1 + \exp(-14.022 + 0.539 * [CYFRA] + 1.294 * [CXCR1] + 12.035 * [E/M])} \quad \text{(II)}$$

Logistic regression equation for predicting of relapse-free survival in patients with Stage III AC.

Note: [CYFRA] – the concentration (ng/ml) of the CYFRA 21-1 antigen in blood serum; [CXCR1] – the relative amount (percentage) of the CXCR1 receptor in lymphocytes; [E/M] – Eosinophilic leukocytes to monocytes ratio; "Z" is the result of the regression equation.

The diagnostic efficiency of predicting the probability of low or high risk of tumor recurrence using the results of the logistic equation increased significantly, reaching 89.0% (sensitivity: 85.7%, specificity: 94.8%) (Table 6). The performance of the proposed regression model, based on

Table 8. Diagnostic significance of determining low and high risk of recurrence of AC (ROC analysis data)

Indicator	TV	SE	SP	PPV	NPV	AUC	ACC
CXCR1, lymphocytes, %	>2.55	66.2	84.4	81.8	70.1	0.715	75.3
CYFRA 21-1, ng/mL	>4.16	71.4	74.0	75.3	70.1	0.709	72.7
Monocytes, $\times 10^9$/L	>0.77	66.2	63.6	66.2	63.6	0.627	64.9
Eosinophilic leukocytes, $\times 10^9$/L	>0.24	62.3	74.0	72.7	63.6	0.651	67.5
Eosinophilic leukocytes/Monocytes	>0.313	76.6	63.6	70.1	70.1	0.673	70.1
Z	>0.597	85.7	94.8	89.6	84.4	0.841	89.0

Abbreviations: TV: Threshold value; SE: Sensitivity; SP: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; AUC: Area under ROC-curve; ACC: Accuracy; CXCR1: C-X-C motif chemokine receptor 1; CYFRA 21-1: Cytokeratin 19 fragment antigen 21-1.

the obtained threshold value Z = 0.597, is demonstrated by the Kaplan-Meier graph of the survival of patients with Stage III AC before progression (Figure 3).

The analysis of the quality of the logistic regression equation (2) shows that all the selected indicators make a significant contribution. This is demonstrated by the significant decrease in the negative doubled value of the logarithm of the likelihood function ($\Delta = 35.1$, $P < 0.05$), indicating the good quality of the proposed model. This is also evidenced by the Hosmer–Lemeshev goodness-of-fit criterion, which was calculated to be 12.1 ($P = 0.158$). The $P > 0.05$ confirms the consistency of the regression equation in classifying patients as having low or high risks of tumor occurrence in AC.

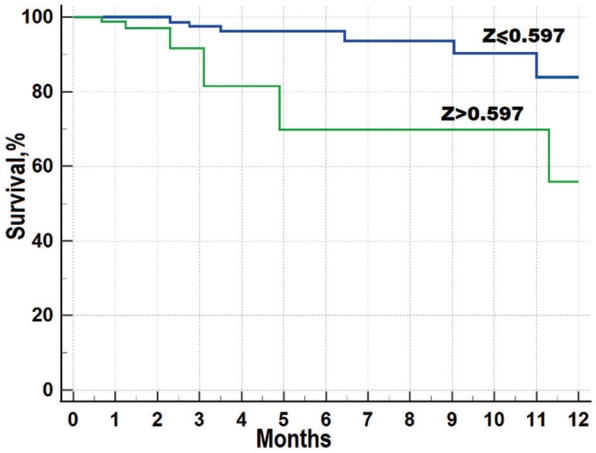

Figure 3. Relapse-free survival of patients with Stage III adenocarcinoma according to the results of the regression equation Z.

4. Discussion

The prognosis for patients with Stage III (T1-4N0-2) NSCLC remains poor, with their overall five-year survival after treatment varying and the median not exceeding 20 months.[1-3] It is vital to anticipate disease progression, not only to save time but also to reduce treatment expenditures by switching to alternative therapeutic strategies.[2,15] One of the approaches to optimizing the effectiveness of treatment in this category of patients is the ability to predict those at high risk of relapse. Despite the relevance and motivation of researchers, prognostic markers of NSCLC remain controversial. As has been demonstrated, the impacts of these prognostic markers are influenced by the histological type of the tumor, the disease stage, and the treatment regimen employed.[17-19,22] However, there is no information regarding the use of prognostic markers for patients with Stage III NSCLC, specifically for the two main histological types: AC and SCLC.

Nevertheless, most researchers have observed a correlation between the concentration of CYFRA 21-1 in blood serum and both relapse-free and overall survival in patients with advanced stages of NSCLC. However, these studies analyzed a mixed cohorts in terms of the disease stage, including patients at early stages of the disease.[3,9,10] It is evident that to obtain such information, cohort studies focusing on patients with relatively homogeneous characteristics and individual histological subtypes of NSCLC are required. The scarcity of information on biomarkers prompted us to conduct our own retrospective and prospective study involving patients with Stage III (T1-4N0-2) SCLC and AC. Based on the pathogenesis of these tumors, we decided to investigate blood cells parameters and proteins, including the CXCR1 and CXCR2 receptors, as well as their ligands, the proinflammatory cytokines CXCL5 and CXCL8.

First, Kaplan-Meier survival analysis of the results from one year of retrospective observation showed a significant difference in relapse-free survival between patients with tumor descriptors T4N1M0 and T4N2M0 in SCLC and AC (high risk of recurrence) and those with other combination of TNM descriptors (T1N2M0, T3N1M0, T2N2M0, T4N0M0, and T3N2M0) (low risk of recurrence).

Then, the results of the TNM stratification were used in the study group to compare the levels of 42 detectable laboratory parameters between patients with high and low

risk of relapse. It has been previously shown that cellular ratios such as N/L, P/L, and L/M, as well as the level of CRP, were known as indicators of the inflammatory reaction associated with tumor development.[15] At the same time, elevated levels of CRP, N/L, and P/L ratios, along with a decreased L/M ratio, have been associated with a poor prognosis in NSCLC. The prognostic value of the N/L ratio was higher compared to P/L ratio.[16] Conversely, findings from other studies suggest that the prognosis of NSCLC is associated with the cellular ratio of P/L rather than the N/L ratio.[17,18] Similar inconsistency of results has also been found in the assessment of the prognostic value of CRP.[19]

The findings of our investigation demonstrated that in Stage III SCLC, only the proportion of lymphocytes expressing the CXCR1 receptor, monocytes expressing the CXCR2 receptor, and the CYFRA 21-1 level showed significant differences between patients with high and low risk of relapse-free survival. For the high-risk group of AC recurrence, the absolute concentrations of monocytes, eosinophilic leukocytes, eosinophilic-to-monocyte ratio, the proportion of lymphocytes expressing the CXCR1 receptor, and the CYFRA 21-1 level, are significantly higher than in the low-risk group. In contrast, other parameters, including SII, IPI, and SIRI indices, the levels of HIF-1α, CXCL5, CXCL8, TuM2 PK, and CXCR1, did not demonstrate significant differences between the high- and low-risk groups of patients.

Therefore, only different parameters were used in the Cox proportional hazards models, which show their equal significance for patient survival. Besides, the results of the multivariate analysis confirmed the results of the univariate analysis, establishing a connection between the selected set of parameters and relapse-free survival. This approach validated the identification of the selected parameters as prognostic markers and assessed their influence on prognosis in terms of odds ratios.

However, the generally accepted criteria for the diagnostic and prognostic value of a specific marker include threshold value, diagnostic sensitivity, specificity, efficiency, etc.[34] The results of the ROC analysis for the selected indicators showed that the proportion of blood lymphocytes expressing the CXCR1 receptor demonstrated the highest prognostic efficiency (75.3%) for predicting relapse-free survival in patients with stage III SCLC. For Stage III AC, the highest specificity (84.4%) was observed for the relative proportion of lymphocytes expressing the CXCR1, while the diagnostic sensitivity of this indicator did not exceed 66.2%. The diagnostic sensitivity values of other selected indicators were in the range of 62.3% to 76.6%. As a result, the diagnostic efficiency in predicting relapse-free survival was the lowest for the concentration of monocytes (64.9%), and the highest for lymphocytes expressing CXCR1 receptor (75.3%).

Three parameters in patients with SCLC (proportions of lymphocytes expressing the CXCR1 receptor, monocytes expressing the CXCR2 receptor, and CYFRA 21-1 level) and three parameters in patients with AC (CYFRA 21-1 level, lymphocytes expressing the CXCR1 receptor, and the E/M ratio) were included in the regression analysis to construct the equation. The resulting equations were expected to exhibit higher sensitivity and specificity in stratifying patients with Stage III AC based on the duration of relapse-free survival. Utilizing a combination of parameters or markers is a common technique for improving prognostic accuracy.[25-30] The convenience of the regression equation lies in its ability to combine several markers into a single numerical value, streamlining prognostic analysis.

All the prognostic characteristics (sensitivity, specificity etc.) were much higher than when using each indicator separately for a similar prognostic purpose. For Stage III SCLC, the prognostic accuracy in predicting relapse-free survival ($Y > 0.417$) was 87.7%.

For patients with Stage III AC, the optimal threshold value for distinguishing between low- and high-risk groups of tumor recurrence was 0.597. Specifically, if the value of $Z > 0.597$, the patient has an 89.6% probability of high tumor recurrence, while for value of $Z \leq 0.597$, 84.4% of patients are correctly predicted to have a low risk of tumor recurrence. The use of the logistic equation significantly improved the probability in accurately predicting a low or high risk of tumor recurrence, reaching an overall accuracy of 89.0% (with sensitivity of 85.7%, and specificity of 94.8%). As illustrated, all these indicators were notably higher than those achieved when using individual parameters for the same prognostic purpose.

The distribution of relapse-free survival into relatively high and low, according to the results of logistic equation, aligns to the results of TNM stratification. In patients with Stage III SCLC, the relapse-free survival at the end of the first year for those at low risk of tumor recurrence is 79% according to TNM stratification and 77% when selected blood parameters are included in the regression equation. For patients with a high risk of tumor recurrence, survival at the end of the 1st year after treatment is 49% and 48%, respectively. Another notable observation is that a clear difference between the high and low survival curves occurs as early as the 1st month after the treatment, with this difference subsequently increasing. In contrast, the differences in the high and low relapse-free survival curves constructed based on TNM appear only two months after the treatment. In subsequent studies, further verification of the proposed prognostic model is needed, not only to

predict the development of the tumor recurrence but also to evaluate the effectiveness of the therapy.

5. Conclusion

A regression equation has been developed to predict the probability of tumor recurrence in patients with Stage III SCLC based on measurements of CYFRA 21-1 concentration, the proportion of lymphocytes expressing the CXCR1 receptor, and blood monocytes expressing the CXCR2 receptor in the pre-operative period. If the result of the equation exceeds 0.417, the risk of relapse is high, and additional treatment measures are required to reduce it. The model's PPV is 90.4%, NPV is 84.9%, sensitivity is 84.9%, and specificity is 89.0%. Meanwhile, for the patients with Stage III AC, a different set of laboratory parameters (CYFRA 21-1 level, lymphocytes expressing the CXCR1 receptor, and the E/M ratio) can be incorporated into the logistic equation to improve the prognosis of relapse-free survival. If the result exceeds 0.597, the risk of relapse after the treatment is high. The model's PPV is 89.6%, NPV is 84.4%, sensitivity is 85.7%, and specificity is 94.8%.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All patients gave written voluntary consent to participate in the study. The study was approved by the decision of the Biomedical Ethics Committee of the educational institution "Belarusian State Medical University" (protocol of the Committee meeting №2 dated 10/04/2021).

Consent for publication

Not applicable.

Availability of data

Data used in this work can be made available to the readers by contacting the corresponding author.

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