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EVALUATING THE DIAGNOSTIC VALUE OF EXTENDED BIOCHEMICAL ANALYSIS AND TUMOR MARKERS IN DIFFERENTIATING MALIGNANT PLEURAL EFFUSIONS

AUTHOR'S ABSTRACT

of the dissertation for the conferral of the academic and scientific degree of

"DOCTOR"

Medical specialty

Thoracic surgery

Plovdiv

2025

Contents

Abbreviations used:

MPI: Malignant Pleural Effusion

VATS: Video-Assisted Thoracoscopic Surgery

SEAG: Serum-Effusion Albumin Gradient

TG: Triglycerides

LDH: Lactate Dehydrogenase

Glu: Glucose

PSG: Pleural Specific Gravity

TP: Total Protein

CHOL: Cholesterol

TLC: Total Leucocyte Count

SEG: Segmented Neutrophils

MON: Monocytes

LYMPH: Lymphocytes

CT: Computed Tomography

CLIA: Chemiluminescence Immunoassay

ROC: Receiver Operating Characteristic

AUC: Area Under the Curve

INTRODUCTION

This dissertation is dedicated to one of the most common and clinically significant complications of oncological diseases — malignant pleural effusions (MPE). These represent a serious multidisciplinary issue with substantial impact on the healthcare system from both medical and socio-economic perspectives. MPE affects approximately 1 million patients annually worldwide and causes pronounced symptoms, particularly progressive dyspnea and severely reduced quality of life.

The study addresses the epidemiological characteristics, diagnostic challenges, and treatment opportunities for patients with MPE, emphasizing the need for a personalized diagnostic and therapeutic approach. Despite the widespread use of the classical Light's criteria for distinguishing pleural exudates from transudates, the diagnostic value of pleural fluid remains underutilized. In this context, the current research analyzes the potential application of extended panels of biochemical parameters and tumor markers in the differential diagnosis of MPE.

The role of pleural puncture as a basic and primary diagnostic tool is discussed, along with the need for in-depth analysis of the collected pleural fluid. Special attention is given to the potential of tumor markers in pleural fluid, which may contribute to the early and more accurate identification of malignant origin in pleural effusions. The indications and limitations of invasive methods are also discussed, particularly video-assisted thoracoscopic surgery (VATS), which remains the gold standard for histological confirmation but is associated with risks and high costs.

The study highlights the lack of systematized and locally adapted data on the topic within Bulgarian clinical practice. Therefore, the necessity of developing an effective diagnostic-therapeutic algorithm is presented — one that integrates accessible biochemical and tumor markers, tailored to the individual clinical context. The goal is to achieve early and reliable diagnosis, treatment optimization, and improved quality of life for patients with MPE.

OBJECTIVES AND TASKS OF THE STUDY

Main Objective of The Study

To analyze and define the diagnostic capabilities of extended biochemical analysis and evaluate the combined sensitivity and specificity of selected tumor markers in pleural fluid. The study aims to determine their significance in the context of individualized, patient-centered treatment strategies for malignant pleural effusions and to build an optimal diagnostic-therapeutic algorithm.

Specific Tasks

1. To develop a clinical and demographic profile of patients with confirmed malignant pleural effusions
2. To assess the diagnostic value of conventional biochemical analysis in distinguishing exudative from transudative effusions
3. To examine the reliability of cytological analysis of pleural fluid in identifying neoplastic processes in the pleural space
4. To analyze the diagnostic contribution of extended biochemical panels in patients with MPE
5. To determine the diagnostic relevance of selected tumor markers tested in pleural fluid in suspected pleural carcinomatosis
6. To establish the relationship between sensitivity and specificity of tumor markers applied to pleural fluid
7. To formulate hypothetical diagnostic threshold values for selected tumor markers for future inclusion in diagnostic panels for neoplastic pleural diseases

MATERIALS AND METHODS

1. Materials

1.1. Study Design

A cross-sectional, observational case-control study was conducted among a Bulgarian patient population with hydrothorax hospitalized at the Department of Thoracic Surgery, University Hospital "Kaspela" Ltd. Anamnestic, clinical-physical, and clinical-laboratory data were analyzed, including biochemical and immunological indicators, as well as chest imaging studies.

1.2. Selection of Clinical and Control Groups

The study included 151 patients — men and women hospitalized in the Department of Thoracic Surgery. The control group included 72 patients with benign diseases confirmed histologically — 38 with inflammatory and 34 with non-inflammatory pleural effusions. Malignant pleural involvement was confirmed in 79 patients. Both groups represent a representative sample of major types of pleural pathology.

All ethical standards were observed, including informed consent and personal data protection. All patients met predefined inclusion criteria.

1.3. Inclusion Criteria

- Signed informed consent
- Imaging evidence of pleural effusion
- Clinical indication for drainage, pleural biopsy, and pleurodesis

1.4. Exclusion Criteria

- Failure to meet inclusion criteria
- Previous pleurodesis on the affected side
- Traumatic or postoperative pleural effusion

1.5. Study Location

All stages — history, imaging, sample collection, and laboratory analysis — were conducted at University Hospital "Kaspela", Plovdiv.

2. Methods

2.1. Clinical Methods

2.1.1. Medical History

Detailed information was collected regarding symptoms, disease duration, history of infections, cancer, prior treatments, and previous thoracentesis.

2.1.2. Physical Examination

General and local chest examinations were performed, including visual inspection, percussion, and auscultation to determine the volume and location of the pleural effusion.

2.2. Imaging Diagnostics

2.2.1. X-ray

All patients were examined with a digital X-ray machine (SIMENS AXIOM Luminos dRF) in anteroposterior projection before surgery.

2.2.2. Computed Tomography (CT)

CT was performed after drainage using a Simmens SOMATOM go.UP device with high-resolution protocol and 3D reconstruction.

2.2.3. Ultrasound

Performed with a HITACHI ALOKA f37 ultrasound with convex transducer (3.0–5.0 MHz) to guide pleural puncture.

2.3. Interventional Methods

2.3.1. Ultrasound-guided Pleural Puncture

Patients were informed and positioned seated with their back to the operator. Puncture was performed in the 7th–8th intercostal space along the scapular line using a Veress needle and an infusion system. Samples were collected for cytology, microbiology, and biochemical analysis. Up to 1500 mL were evacuated, the needle was then removed, the site was dressed, and the patient was sent for CT.

2.3.2. VATS Biopsy and Pleurodesis

All patients underwent VATS biopsy and talc pleurodesis (3 g) under general anesthesia with single-lung ventilation. One-port access was used in the 4th/5th intercostal space. Suspicious areas were biopsied or random parietal pleura samples if none were visible. Pleurodesis was performed on a collapsed lung. A suction drain was placed and removed after 48–72 hours.

2.4. Histological Analysis

Performed at the Pathoanatomical Laboratory of University Hospital "Kaspela".

2.4.1. Classic Technique

5 μm paraffin sections stained with hematoxylin-eosin and mounted in Canadian balsam.

2.4.2. Immunohistochemistry

4 μm sections processed using standardized protocols with antibodies from Biocare Medical. Antigen retrieval and visualization performed with Novolink Polymer Detection System. A reaction was considered positive with clear membrane, cytoplasmic, or nuclear staining.

Antibodies Used:

- TTF1 (8G7G3/1)
- p63 (4A4)
- CD56 (BC56C04)
- Ki67 (SP6)
- CK7 (BC1)
- CK20 (Ks20.8)
- PAX8
- CDX2 (CDX2-88)

2.5. Clinical Laboratory Methods

All clinical laboratory tests were conducted at the Clinical Laboratory of the Department of General and Clinical Pathology, University Hospital “Kaspela” Ltd., Plovdiv. Pleural fluid was obtained through a closed system for biological material, collected either during thoracentesis or intraoperatively.

A portion of the pleural fluid was used to determine clinical-biochemical parameters, while the remainder was used for quantitative testing of tumor markers and cytological analysis.

2.5.1. Clinical Biochemical Parameters

All analyses were performed using the Beckman Coulter AU480 clinical chemistry analyzer with original manufacturer protocols.

Glucose

- Method: Enzymatic UV/hexokinase
- Reagents: Beckman Coulter, Cat. No. OSR6121
- Calibrator: BE-Beckman
- Reliability:
- CV: 1.12–1.36% (within series); 1.86–2.04% (over time)
- Inaccuracy: −d from 1.27 to 0.36%

pH

- Method: Test strips (SDTS Standard Diagnostics – Urocolor)
- Accuracy: ±0.1 to ±0.5 pH units
- Precision: ±0.2 to ±0.3 pH units

Total Cholesterol

- Method: Enzymatic (CHOD-PAP)
- Reagents: Beckman Coulter, Cat. OSR6116
- Repeatability: 0.4–0.8%

Albumin

- Method: Bromocresol green (BCG)
- Reagents: Beckman Coulter, Cat. OSR6102
- Repeatability: 0.31–0.99%

Total Protein

- Method: Biuret
- Reagents: Beckman Coulter, Cat. OSR6132
- Repeatability: 0.26–0.84%

Lactate Dehydrogenase (LDH)

- Method: IFCC, 37°C
- Reagents: Beckman Coulter, Cat. OSR6128
- Repeatability: 0.76–1.54%

Triglycerides

- Method: Enzymatic (GPO-PAP)
- Reagents: Beckman Coulter, Cat. OSR60118
- Repeatability: 0.5–1.1%

Specific Gravity

- Method: Test strips (SDTS Standard Diagnostics – Urocolor)

Cytogram

- Method: Microscopic examination
- Equipment: Laboratory Medical Teaching Microscope, model XSZ-207

2.5.2. Tumor Markers

Measurements were performed using the Beckman Coulter Access 2 and Snibe Maglumi 4000 Plus analyzers.

- **CA125**
  - Method: CLIA (sandwich test)
  - Range: 0.7–5,000 U/mL (up to 100,000 U/mL with automatic dilution)
  - Repeatability: up to 4.0% CV

- **CA72-4**
  - Method: CLIA (Snibe Maglumi 4000 Plus)
  - Range: 0.2–500 U/mL (up to 10,000 U/mL)
  - Repeatability: up to 8.25% CV

- **PIVKA-II**
  - Method: CLIA (Snibe Maglumi 4000 Plus)
  - Range: 5–30,000 mAU/mL (up to 300,000 mAU/mL)
  - Repeatability: up to 6.57% CV

- **CA15-3**
  - Method: CLIA (Beckman Coulter Access 2)
  - Range: 0.5–1,000 U/mL (up to 10,000 U/mL)
  - Repeatability: up to 2.6% CV

- **CEA (Carcinoembryonic Antigen)**
  - Method: CLIA (Beckman Coulter Access 2)
  - Range: 0.1–1,000 ng/mL (up to 10,000 ng/mL)

- Repeatability: up to 3.97% CV

- **CA19-9**
  - Method: CLIA (Beckman Coulter Access 2)
  - Range: 0.8–2,000 U/mL (up to 20,000 U/mL)
  - Repeatability: up to 8.9% CV

2.6. Statistical Methods

Data processing and analysis were performed using IBM SPSS Statistics, and graphical visualization with MS Office 365. The statistical methods were selected based on the objectives of the dissertation, data type, and established practices in clinical studies in thoracic surgery.

Statistical Tests Used

2.6.1. Pearson's Chi-Square Test

A non-parametric test used to assess differences between observed and expected frequencies in categorical data.

2.6.2. Mann-Whitney Wilcoxon Test

A non-parametric test comparing two independent groups in terms of value distribution.

2.6.3. Kolmogorov-Smirnov One-Sample Test

A test to verify the match between empirical and theoretical data distribution.

2.6.4. Independent Samples t-test

A parametric test comparing mean values between two independent groups.

2.6.5. Levene's Test for Equality of Variances

A test used to assess the equality of variances across two or more groups.

2.6.6. Correlation Analysis

A statistical method to evaluate the relationship between two or more variables.

2.6.7. Receiver Operating Characteristic (ROC) Curve

A graphical method used to evaluate the performance of binary classifiers.

2.6.8. Youden Index

A statistical measure combining sensitivity and specificity to assess the overall performance of a diagnostic test in distinguishing between positive and negative cases.

RESULTS AND DISCUSSION

Results from Task №1

Developing of a Clinical and Demographic Profile of Patients with Malignant Pleural Effusions

Between March 2023 and April 2024, a total of 151 patients with pleural effusions were studied. For the purpose of the study, they were divided into two main groups:

- **Group 1** – 79 patients with histologically verified malignant pleural effusion
- **Group 2** – 72 patients with confirmed non-malignant hydrothorax, further subdivided into:
    - 38 patients with inflammatory pleural effusion
    - 34 patients with non-inflammatory pleural effusion

Gender distribution among all included patients showed a predominance of men – 91 men (60.3%) and 60 women (39.7%) (**Figure 1**).

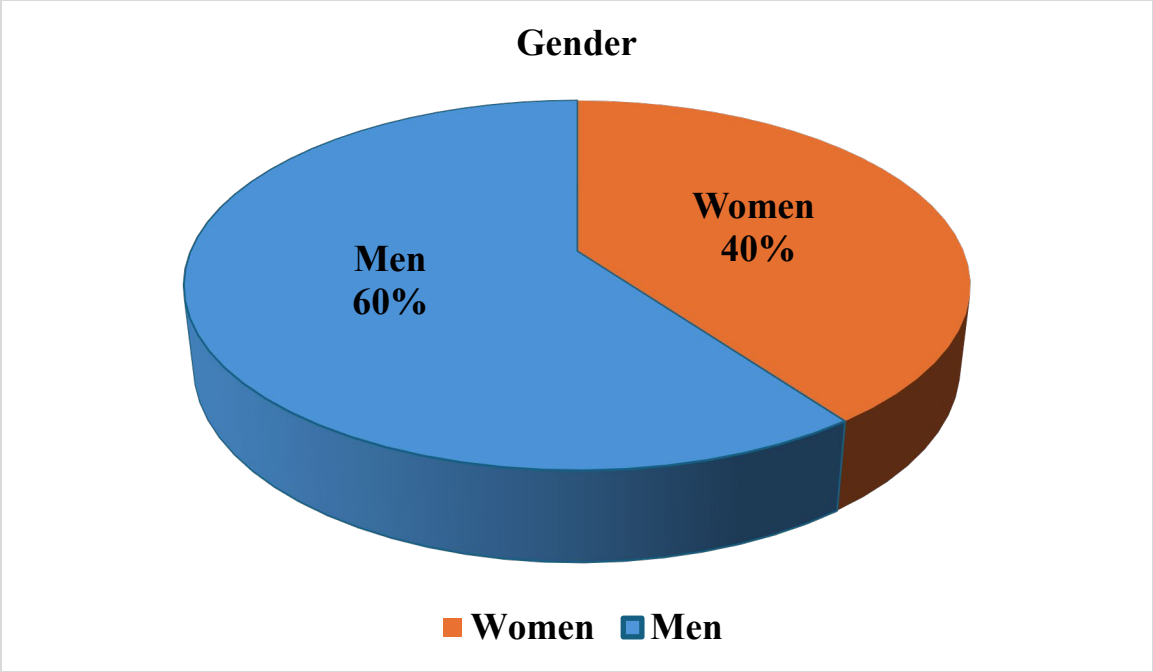

Figure 1: *Distribution of the groups by gender*

In the malignant group, women were slightly more prevalent – 51.8% (n=41) versus 48.2% (n=38) men. In contrast, in the non-malignant group, the distribution was markedly different – 73.1% (n=53) men versus 26.39% (n=19) women. A Pearson's χ^2-test revealed a statistically significant difference between the two groups regarding sex (p=0.001; χ^2=10.237) (**Table 1**).

Table 1: *Pearson's Chi-Square Test applied for gender*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	10.237	1	.001		
Continuity Correction	9.199	1	.002		
Likelihood Ratio	10.417	1	.001		
Fisher's Exact Test				.002	.001
Linear-by-Linear Association	10.169	1	.001		
N of Valid Cases	151				

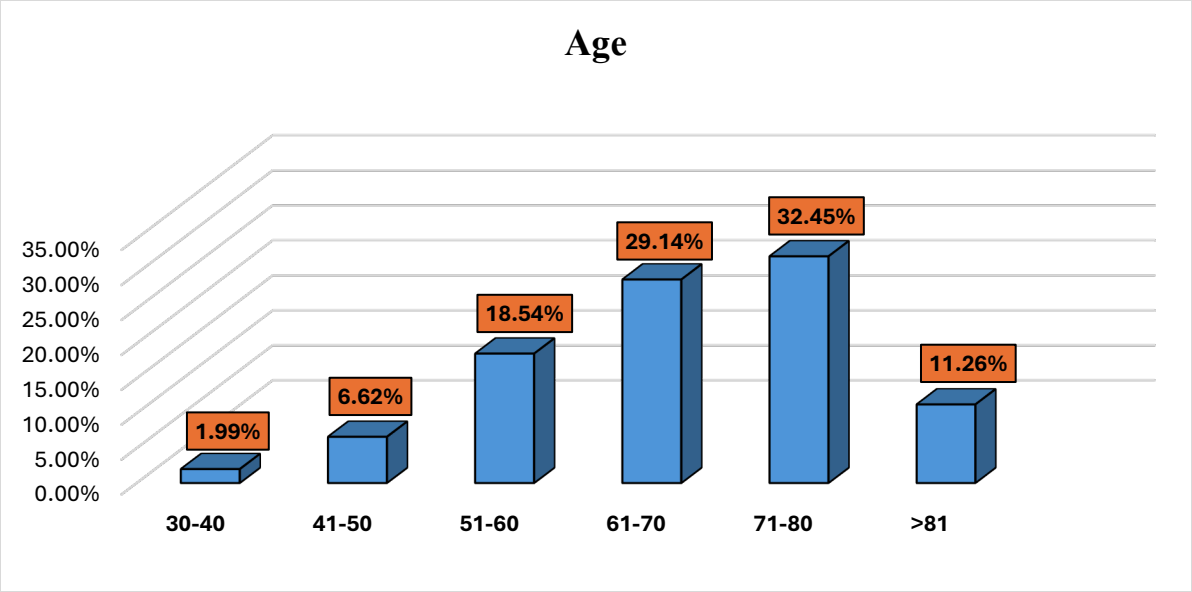

Figure 2: *Distribution of the groups by age*

The average age of all patients was 67.5 years. For patients with malignant effusion, the mean age was 70.5 years, whereas for those with non-malignant effusion, it was 82 years (**Figure 2**). The youngest patient in the study was 30 years old, and the oldest was 88 years old. The most commonly affected age group was 71–80 years. According to analysis using the Mann-Whitney test, no statistically significant difference in age distribution between groups was found (p=0.094) (**Table 2**).

Table 2: *The Mann-Whitney U test applied to the variable age*

Test Statistics

	възраст
Mann-Whitney U	2402.500
Wilcoxon W	5562.500
Z	-1.646
Asymp. Sig. (2-tailed)	.100

Out of all 151 patients, 72 (47.7%) had pleural effusion not associated with a malignant disease. In 45 cases (29.8%), there was a history of a known oncological disease, while in 34 patients (22.5%), the malignant pleural effusion was the first manifestation of a previously undiagnosed malignancy (**Figure 3)**.

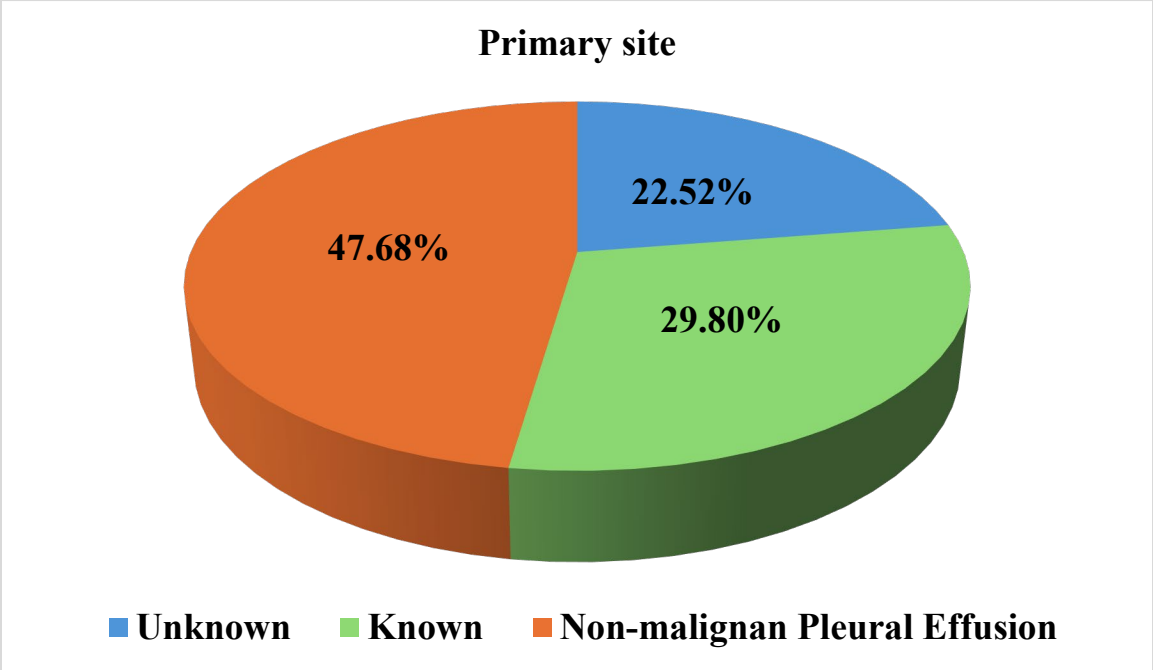

Figure 3: *Distribution of the groups according to the known primary site*

Side involvement analysis showed that the left hemithorax was affected in 53.64% (n=81) of cases, the right hemithorax in 31.79% (n=48), and bilateral involvement in 14.57% (n=22). In the non-malignant group, the distribution was (**Figure 4**):

- 38.9% left
- 38.9% right
- 22.2% bilateral

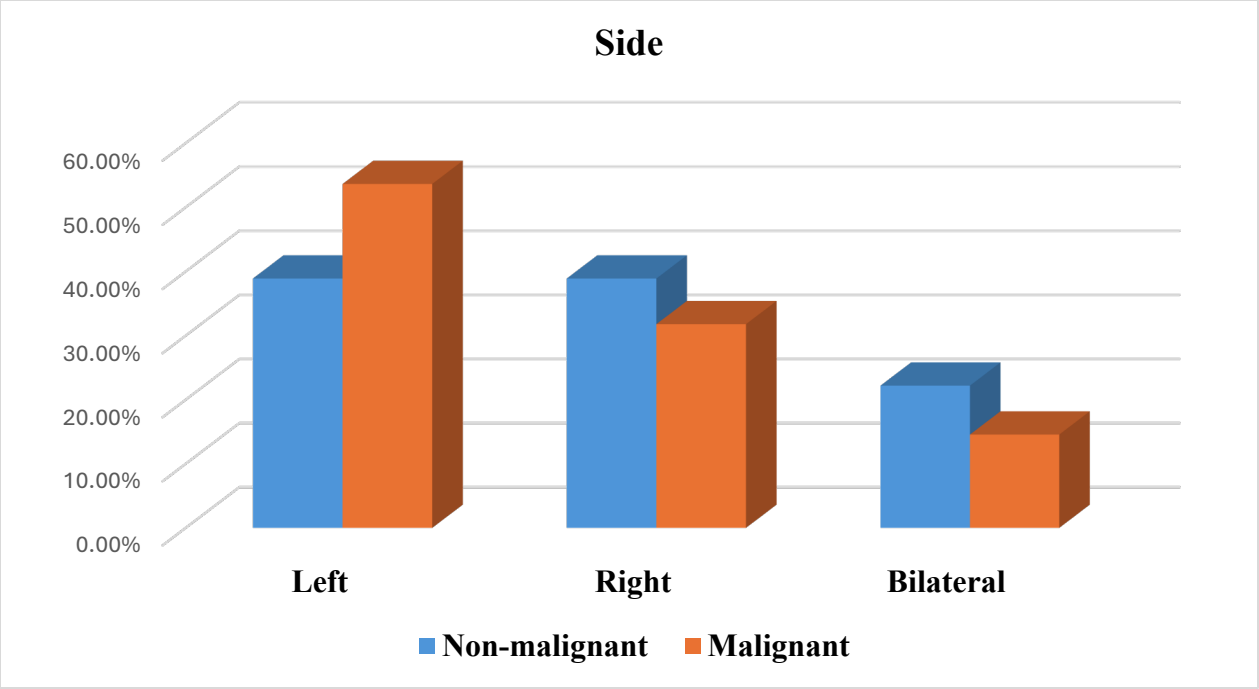

Figure 4: *Relationship between pleural involvement and side*

In malignant pleural effusions, however, left-sided involvement predominated – 67.1% (n=53), compared to 25.3% (n=20) on the right and 7.6% (n=6) bilateral. The difference was statistically significant (p=0.001; χ^2=13.299a) – (**Table 3**).

Table 3: *Pearson's Chi-Square Test applied to the variable side*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	13.299	2	.001
Likelihood Ratio	13.575	2	.001
Linear-by-Linear Association	12.963	1	<.001
N of Valid Cases	151		

Symptomatology (**Figure 5**):

- 63.3% (n=50) of patients with malignant pleural effusion presented with clinical symptoms;

- Among patients with inflammatory non-malignant effusions, symptoms were present in 78.8% (n=30);
- Among non-inflammatory non-malignant effusions, 64.7% (n=22) had symptoms.

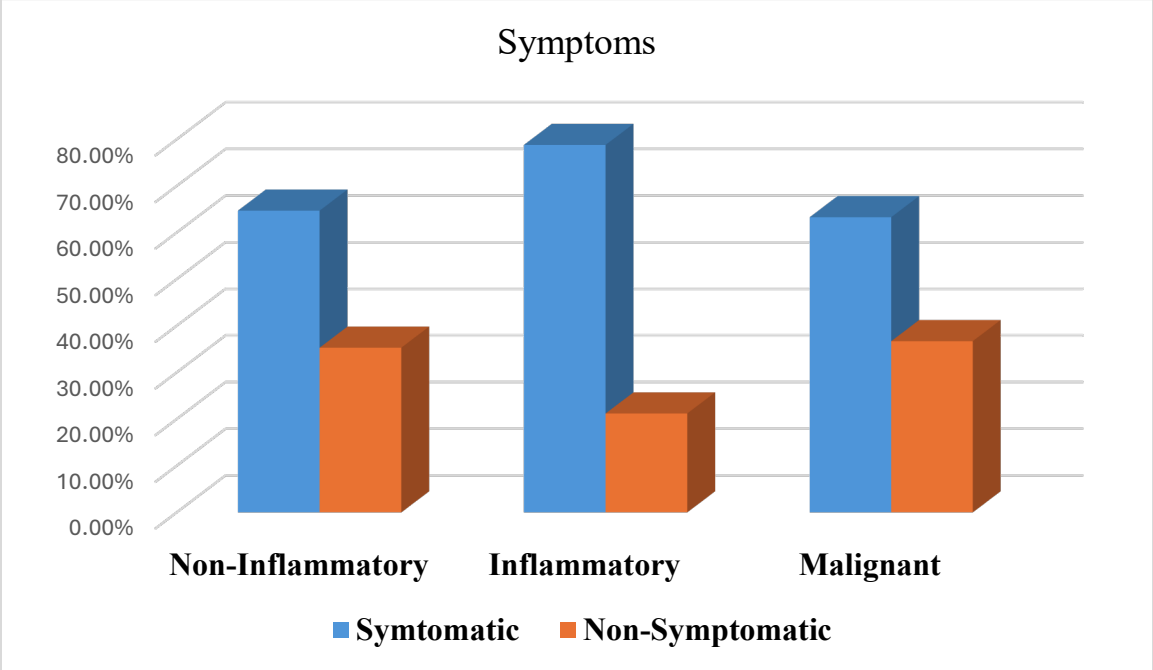

Figure 5: *Relationship between pleural involvement and symptomatology*

Despite these data, χ^2-analysis showed no statistically significant difference between the groups regarding the presence of symptoms (p=0.242; χ^2=1.371a) (**Table 4**).

Table 4: *Pearson's Chi-Square Test applied to the variable symptomatology*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.371	1	.242		
Continuity Correction	.994	1	.319		
Likelihood Ratio	1.377	1	.241		
Fisher's Exact Test				.297	.159
Linear-by-Linear Association	1.362	1	.243		
N of Valid Cases	151				

Histological Analysis

In the examination of 79 patients with histologically verified pleural carcinomatosis, the distribution of primary malignant diseases was as follows (**Figure 6**):

- Lung carcinoma – 31 cases
- Breast carcinoma – 17 cases
- Clear cell renal carcinoma – 6 cases
- Endometrial and ovarian carcinoma – 5 cases each
- Colorectal carcinoma – 4 cases
- Gastric and pancreatic carcinoma – 2 cases each
- Mesothelioma – 2 cases
- Single cases of:
    - Urothelial carcinoma
    - Testicular tumor
    - Sarcoma
    - Chronic lymphocytic leukemia

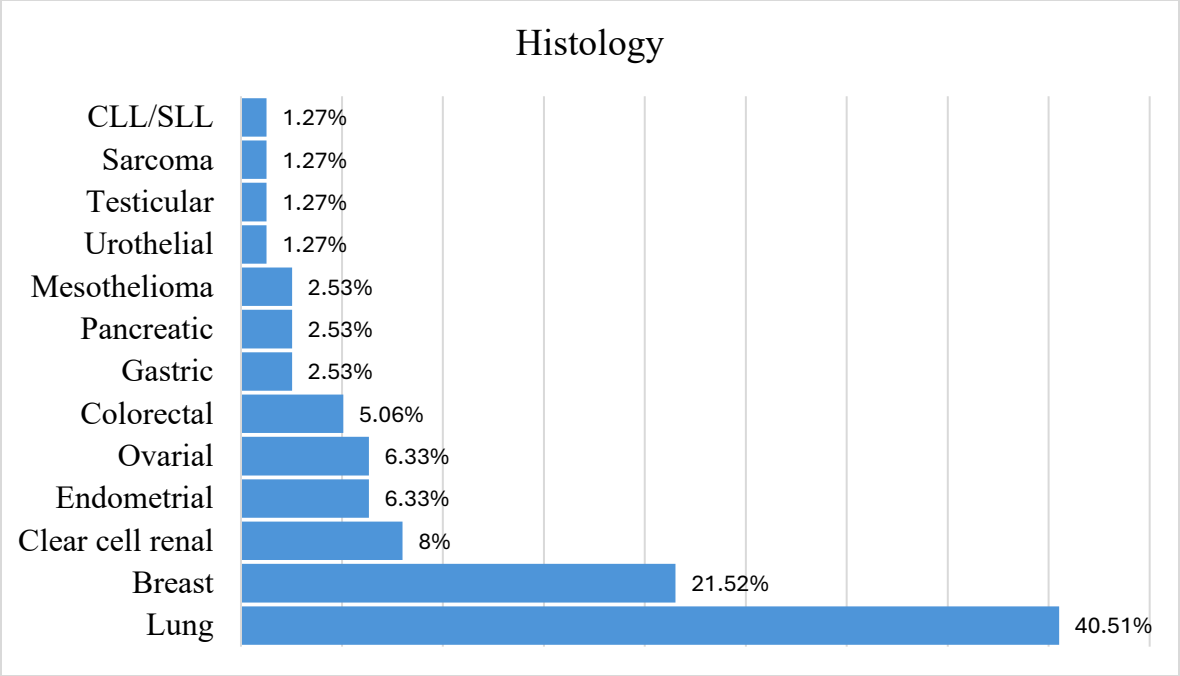

Figure 6: *Distribution of the groups according to histological type*

Discussing the Results from Task №1

a) Gender

The data show that malignant pleural effusions are more common in women (51.8%), while non-malignant effusions are more frequent in men (73.1%), which is statistically significant. This finding is supported by a study by Foote, who analyzed 686 patients with malignant effusions and found a higher risk in women (59%), with 351 of them having non-gynecologic tumors.

b) Age

The most affected age group is 71–80 years, consistent with the results of Warnisher et al., who found that individuals around the age of 70 are most commonly affected. No statistically significant difference between the groups was observed.

c) Oncological history

In 29.8% of patients with malignant effusion, there was a known history of cancer, while in 22.5%, the pleural effusion was the first sign of a neoplasm. This corresponds with data from Fashoyin-Aje, who reported that in 30% of patients, effusion symptoms were the first manifestation of a tumor.

d) Side of involvement

Malignant pleural effusions more frequently affect the left hemithorax (67.1%), while non-malignant effusions are evenly distributed or bilateral. This may be due to anatomical differences (lymphatic drainage, mediastinum, heart and diaphragm position) and the mechanism of development of malignant vs. benign effusions.

e) Symptomatology

A large proportion of malignant effusions are asymptomatic (36.7%) and are often detected during routine imaging. Non-malignant effusions, especially inflammatory, more commonly cause symptoms. However, there is no statistically significant difference, meaning symptomatology cannot be used as a reliable differentiating criterion.

f) Histology

The main histological type among malignant effusions is lung carcinoma (40.5%), followed by breast carcinoma (21.5%). This aligns with international data, including a study by Migliore, which found the primary tumors to be:

- Lung cancer – 40%
- Breast carcinoma – 25%
- Lymphoma – 10%
- Gynecological and gastrointestinal tumors – ~5% each

Conclusions from Task №1

1. **Gender difference** – Malignant pleural effusions show a predilection for women (51.8%), while non-malignant effusions are more common in men (73.1%). The difference is statistically significant (p=0.001).
2. **Age** – The most commonly affected are patients between 71 and 80 years. The average age is 67.5 years, with no statistically significant difference between groups (p=0.094).
3. **Oncological history** – In almost one-third of patients with malignant effusion, it was the first clinical manifestation of neoplasia (22.5%).
4. **Side of involvement** – Malignant pleural effusions more often affect the left hemithorax (67.1%) – a statistically significant difference (p=0.001).
5. **Symptomatology** – Malignant effusions are more often asymptomatic, but the difference is not statistically significant.

6. **Histology** – The most common primary tumor is lung carcinoma, followed by breast carcinoma, consistent with global literature data.

Results from Task №2

Study on the Diagnostic Accuracy of Conventional Biochemical Analysis in Differentiating Exudative from Transudative Pleural Effusions

To further differentiate the pleural effusions included in the study, the Light's criteria classification was applied, according to which effusions are divided into exudative and transudative. Out of the total number of pleural fluid samples analyzed, 72.85% (n=110) were classified as exudative, and 27.15% (n=41) as transudative (**Figure 7**).

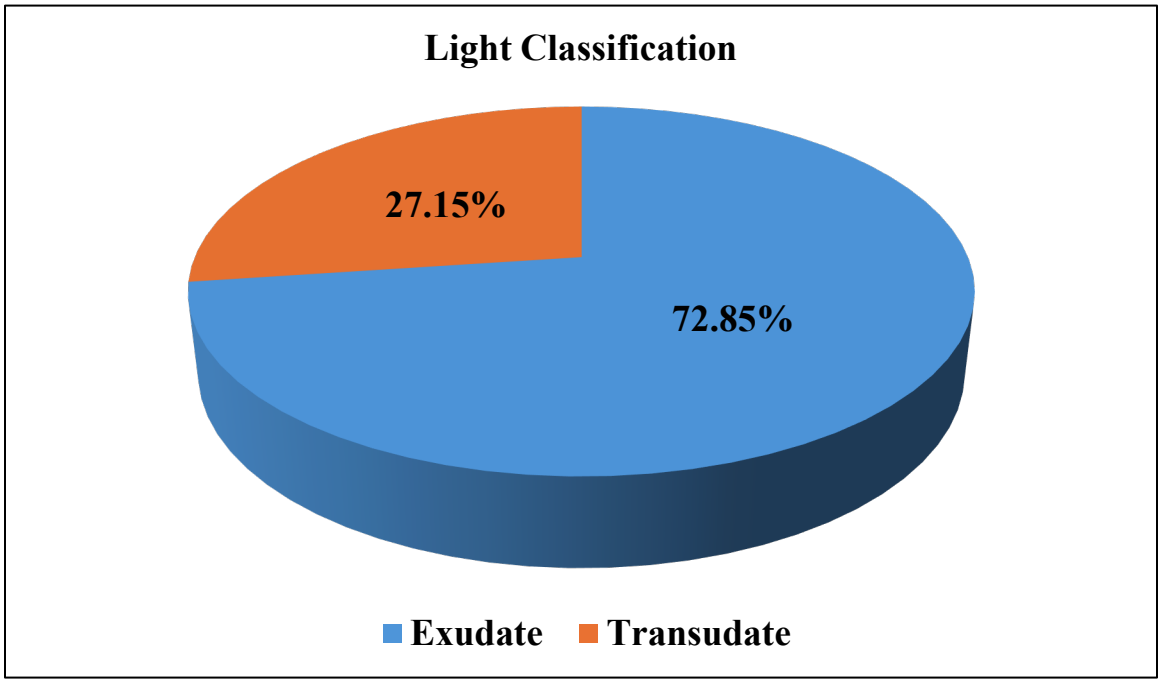

Figure 7: *Distribution of the groups according to Light's criteria*

From the exudative group, 38.2% (n=42) were identified in patients with non-malignant histology, which represents 58.3% of all patients with non-malignant histology. The remaining 61.8% (n=68) had histologically confirmed pleural carcinomatosis, corresponding to 86% of all cases with malignant histology.

Among patients with transudative effusions, 73.2% (n=30) had non-malignant histology, representing 13.9% of the total non-malignant group. In total, 26.8% (n=11) of patients with malignant pleural effusion fell into the transudative group, which corresponds to 27.2% of the malignant group.

Application of the Pearson's Chi-Square Test showed a statistically significant difference with $p<0.001$ and $X^2 = 14.66a$ (**Table 5**), demonstrating the reliability of Light's criteria in the classification of pleural effusions.

Table 5: *Pearsons Chi-Square Test за Критериите на Лайт*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	14.657	1	<.001		
Continuity Correction	13.288	1	<.001		
Likelihood Ratio	15.030	1	<.001		
Fisher's Exact Test				<.001	<.001
Linear-by-Linear Association	14.560	1	<.001		
N of Valid Cases	151				

Discussing the Results from Task №2

By applying Light's criteria, we were able to evaluate their diagnostic accuracy, particularly in the context of malignant pleural effusions. We found that 26.8% of patients with pleural carcinomatosis were classified as having transudative effusions, which represents a potential for false-negative interpretation. This could be due to low protein content in the early phases of malignant hydrothorax development. As the disease progresses and protein accumulates, the transudative effusion transforms into an exudative one.

Other potential causes for the transudative nature of malignant effusions include:

- Endobronchial obstruction
- Hypoalbuminemia
- Various comorbid conditions

While exudates can evolve from transudates with disease progression, the reverse process (exudate to transudate) is extremely rare.

Light's criteria demonstrated high sensitivity in differentiating exudative effusions. In 86.1% of malignant effusion cases, the effusion was classified as exudative.

Conversely, transudative effusions were predominant among patients with non-malignant histology (73.2%).

Conclusions from Task №2

1. Light's criteria demonstrate high diagnostic accuracy in differentiating exudative from transudative effusions, with a statistically significant difference ($p<0.001$) underscoring their reliability.

2. The majority of malignant effusions are classified as exudative (86%), while the transudative nature predominates in non-malignant conditions (73.2%).

3. The presence of transudative effusions in malignant cases (26.8%) suggests a potential for false-negative results, especially in the early stages of the disease.

4. In patients with malignant diseases, a transudative effusion can progress into an exudative one, whereas the reverse transformation is extremely rare.

5. Concomitant pathological processes, such as obstruction, hypoalbuminemia, and cardiovascular complications, can result in a transudative or mixed nature of the effusion in the context of malignancy.

Results from Task №3

Assessment of the Reliability of Cytological Analysis of Pleural Fluid for Detection of Neoplastic Pleural Involvement

Based on the results of the cytopathological examination, 84.8% (n=128) of all examined patients had negative cytology, while 15.2% (n=23) showed the presence of tumor cells in the pleural fluid (**Figure 8**).

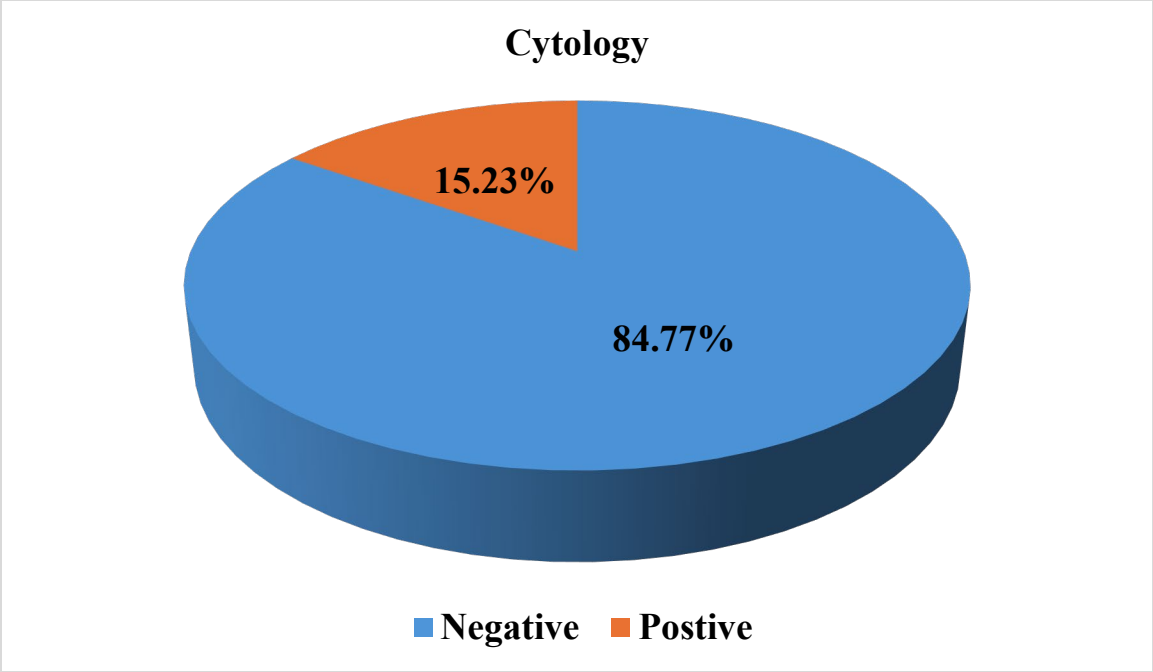

Figure 8: *Group distribution according to cytology*

Application of Pearson's Chi-Square Test established statistical significance with $p < 0.001$; $X^2 = 20.42$ (**Table 6**).

Table 6: *Pearson's Chi-Square Test applied for cytology*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	20.425	1	<.001		
Continuity Correction	18.427	1	<.001		
Likelihood Ratio	24.868	1	<.001		
Fisher's Exact Test				<.001	<.001
Linear-by-Linear Association	20.289	1	<.001		
N of Valid Cases	151				

Of the patients with negative cytology,

- 55.47% (n=77) had non-malignant histology – comprising 98.6% of the entire non-malignant group.

- Patients with malignant histology and negative cytology numbered 44.53% (n=57), which equals 72.2% of all cases with confirmed pleural carcinomatosis.

Among the patients with positive cytology,

- 95.7% (n=22) had malignant histology,
- And only 4.3% (n=1) had non-malignant histology.

Discussing the Results from Task №3

The cytopathological examination of pleural fluid demonstrated high specificity, but limited sensitivity. Only 27.8% of all malignant effusions were detected by cytology.

We observed:

- A false-positive rate of 4.3%, and
- A false-negative rate of 72.2%.

The false-negative results are due to technical and interpretative errors, such as:

- Low cellularity,
- Delayed transportation of the sample, or
- Misinterpretation between reactive mesothelial changes and tumor cells.

According to literature, false-negative results can reach up to 30%. The sensitivity of the method is highest in adenocarcinoma (89.9%) and lowest in mesothelioma (33.3%). The most common tumor type among cases with positive cytology was breast carcinoma (91.3%).

The morphology of the pleural effusion also plays a role:

- A cloudy, golden-colored appearance is associated with higher test sensitivity.
- In contrast, pleural thickening seen on imaging reduces the method’s accuracy.

Conclusions from Task №3

1. Positive cytology is a strong predictor of malignant involvement – in 95.7% of cases, it correlates with pleural carcinomatosis.
2. False-positive cases (1.4% of the non-malignant group) are likely due to reactive changes and mesothelial hyperplasia, but the percentage is low, confirming the high specificity of the method.
3. False-negative results are a significant limitation – in 72.2% of malignant effusions, cytology failed to detect tumor cells.
4. Technical errors during sampling, storage, and analysis have a significant impact on the outcome. Critical factors include:
    - Low cellular content (cellularity),
    - Rapid cellular degradation, and
    - Errors in morphological evaluation.
5. Morphological characteristics of the effusion (e.g., cloudiness, high protein content) increase the likelihood of detecting tumor cells, whereas pleural thickening reduces this probability.

Results from Task №4

Detailed Analysis of the Diagnostic Value of Extended Biochemical Testing in Patients with Malignant Pleural Effusions

Analysis of the Normality of the Distribution of Biochemical Markers

To assess whether the variables from the full panel of biochemical indicators follow a normal distribution, we initially applied the Kolmogorov-Smirnov One-Sample Test. The results are presented in **Table 7**.

Table 7: *The Kolmogorov-Smirnov test, applied to the studied biochemical markers*

One-Sample Kolmogorov-Smirnov Test

	N	Normal Parameters		Most Extreme Differences			Test Statistic	Asymp. Sig. (2-tailed)
		Mean	Std. Deviation	Absolute	Positive	Negative		
SEAG	151	16.0397	5.36269	.085	.085	-.051	.085	.009
TG	151	.4865	1.74971	.410	.395	-.410	.410	<.001
pH	151	7.2748	.42304	.331	.331	-.231	.331	<.001
PSG	151	1012.8146	4.22596	.300	.230	-.300	.300	<.001
GLU	151	5.6530	3.67905	.173	.173	-.167	.173	<.001
TP	151	35.1987	10.73873	.083	.053	-.083	.083	.013
LDH	151	431.4503	622.20932	.269	.269	-.261	.269	<.001
CHOL	151	1.7025	.81446	.085	.085	-.054	.085	.009
TLC	151	119.77	175.413	.264	.264	-.253	.264	<.001
SEG	151	15.88	17.672	.238	.238	-.203	.238	<.001
MON	151	5.91	3.001	.137	.137	-.073	.137	<.001
LYMPH	151	78.12	19.168	.214	.162	-.214	.214	<.001

The column *N* shows the number of observations for each variable, *Mean* – the average value, *Standard Deviation* – the standard deviation, and *Absolute* – the largest absolute deviation between the empirical and normal curve. *Positive* and *Negative* indicate the direction of the greatest deviation relative to the normal curve. It is evident that all included variables significantly deviate from the normal distribution, as supported by the asymptotic significance ($p < 0.05$ in all cases). Therefore, these variables do not follow a normal distribution.

Test for Differences Between Groups: Independent Samples t-test

To further confirm the findings, we used the Independent Samples t-test to check for statistically significant differences in the means of selected variables between the two patient groups.

Table 8: *Independent samples t-test, applied to the biochemical parameters*

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
SEAG	Equal variances assumed	7.443	.007	4.786	149	<.001	3.90612	.8162	2.29330	5.51893
	Equal variances not assumed			4.703	122.8	<.001	3.90612	.8306	2.26197	5.55027
TG	Equal variances assumed	1.832	.178	-1.007	149	.315	-.28717	.2851	-.85047	.27614
	Equal variances not assumed			-1.054	79.814	.295	-.28717	.2723	-.82915	.25481
pH	Equal variances assumed	1.054	.306	-1.661	149	.099	-.11384	.0685	-.24924	.02157
	Equal variances not assumed			-1.674	148.2	.096	-.11384	.0680	-.24822	.02054
PSG	Equal variances assumed	.741	.391	2.855	149	.005	1.92071	.6727	.59145	3.24997
	Equal variances not assumed			2.843	144.2	.005	1.92071	.6755	.58550	3.25592
GLU	Equal variances assumed	.091	.763	.158	149	.874	.09518	.6014	-1.0932	1.28355
	Equal variances not assumed			.155	120.0	.877	.09518	.6129	-1.1183	1.30866
TP	Equal variances assumed	11.829	<.001	-3.227	149	.002	-5.47679	1.697	-8.8306	-2.1230
	Equal variances not assumed			-3.175	125.4	.002	-5.47679	1.725	-8.8905	-2.0631
LDH	Equal variances assumed	.064	.801	-.947	149	.345	-96.08527	101.4	-296.48	104.308
	Equal variances not assumed			-.957	146.7	.340	-96.08527	100.4	-294.48	102.307
CHOL	Equal variances assumed	1.943	.165	-2.559	149	.011	-.33346	.1303	-.59097	-.07596
	Equal variances not assumed			-2.525	130.6	.013	-.33346	.1321	-.59472	-.07220
TLC	Equal variances assumed	.874	.351	-1.050	149	.296	-29.993	28.57	-86.449	26.464
	Equal variances not assumed			-1.071	133.3	.286	-29.993	28.01	-85.385	25.399
SEG	Equal variances assumed	.549	.460	-.261	149	.794	-.754	2.888	-6.462	4.953
	Equal variances not assumed			-.259	140.5	.796	-.754	2.909	-6.506	4.997
MON	Equal variances assumed	.338	.562	-1.768	149	.079	-.858	.486	-1.817	.101
	Equal variances not assumed			-1.757	141.9	.081	-.858	.488	-1.824	.108
LYMPH	Equal variances assumed	.867	.353	.580	149	.563	1.816	3.130	-4.369	8.001
	Equal variances not assumed			.575	138.2	.566	1.816	3.157	-4.427	8.060

Levene's Test for Equality of Variances determines whether there is a statistically significant difference in variance between groups and whether they demonstrate homogeneity. The t-test for equality of means assesses the presence of a statistically significant difference in means. The result columns include:

- *t* – t-test statistic
- *df* – degrees of freedom
- *Sig. (2-tailed)* – the p-value showing statistical significance
- *Mean Difference* – the difference between means
- *95% Confidence Interval* – the interval within which the true difference lies with 95% confidence

Table 8 shows statistically significant differences in albumin gradient values (Levene's Test: Sig. = 0.007; $t(122.835) = 4.703$; $p < 0.001$) and total protein (Levene's Test: Sig. = 0.001; $t(125.389) = -3.175$; $p = 0.002$).

Particular attention should be paid to the results for specific gravity (Levene's Test: Sig. = 0.391; $t(149) = 2.855$; $p = 0.005$) and cholesterol (Levene's Test: Sig. = 0.165; $t(149) = -2.559$; $p = 0.011$), where variances are similar but p-values are close to significance. The non-malignant group shows higher specific gravity, while cholesterol is elevated in malignant cases.

Regarding cytological examination, monocyte values show equal variation, and the p-value is close but not statistically significant (Levene's Test: Sig. = 0.562; $t(149) = -1.768$; $p = 0.079$).

Analysis Using the Mann-Whitney U Test

To better understand rank differences in biochemical markers between patients with malignant and non-malignant pleural effusions, we used the Mann-Whitney U Test. The results are shown in **Table 9**, where:

- *N* is the number of units in each group
- *Mean Rank* indicates the average rank (a higher rank means higher values for that marker)
- *Sum of Ranks* is used in calculating the statistic for non-normal distributions

***Table 9**: Rank statistics for the studied biochemical parameters*

Ranks

	malig_hist	N	Mean Rank	Sum of Ranks
SEAG	немалигнена хистология	72	91.58	6594.00
	малигнена хистология	79	61.80	4882.00
	Total	151		
TG	немалигнена хистология	72	66.72	4804.00
	малигнена хистология	79	84.46	6672.00
	Total	151		
pH	немалигнена хистология	72	70.77	5095.50
	малигнена хистология	79	80.77	6380.50
	Total	151		
PSG	немалигнена хистология	72	85.85	6181.50
	малигнена хистология	79	67.02	5294.50
	Total	151		
GLU	немалигнена хистология	72	76.60	5515.50
	малигнена хистология	79	75.45	5960.50
	Total	151		
TP	немалигнена хистология	72	64.66	4655.50
	малигнена хистология	79	86.34	6820.50
	Total	151		
LDH	немалигнена хистология	72	65.03	4682.00
	малигнена хистология	79	86.00	6794.00
	Total	151		
CHOL	немалигнена хистология	72	62.92	4530.00
	малигнена хистология	79	87.92	6946.00
	Total	151		
TLC	немалигнена хистология	72	68.63	4941.00
	малигнена хистология	79	82.72	6535.00
	Total	151		
SEG	немалигнена хистология	72	72.31	5206.50
	малигнена хистология	79	79.36	6269.50
	Total	151		
MON	немалигнена хистология	72	67.16	4835.50
	малигнена хистология	79	84.06	6640.50
	Total	151		
LYMPH	немалигнена хистология	72	82.64	5950.00
	малигнена хистология	79	69.95	5526.00
	Total	151		

Conclusion from the Analysis

With the exception of the albumin gradient, specific gravity, and lymphocyte count, all other parameters are elevated in malignant pleural effusions.

Additional Results from the Mann-Whitney U Test

Table 10 presents the results, where:

- *Mann-Whitney U* shows the number of cases where the lower-ranked group "wins" over the higher-ranked one
- *Wilcoxon W* is the sum of ranks for the smaller group
- *Z* – the value showing the difference between the groups
- *Asymp. Sig. (2-tailed)* is the p-value indicating statistical significance

Table 10: *Mann–Whitney U test applied to the biochemical parameters*

Mann-Whitney U Test

	Mann-Whitney U	Wilcoxon W	Z	Asymp. Sig. (2-tailed)
SEAG	1722.000	4882.000	-4.190	<.001
TG	2176.000	4804.000	-2.490	.013
pH	2467.500	5095.500	-1.569	.117
PSG	2134.500	5294.500	-2.903	.004
GLU	2800.500	5960.500	-.162	.871
TP	2027.500	4655.500	-3.044	.002
LDH	2054.000	4682.000	-2.943	.003
CHOL	1902.000	4530.000	-3.512	<.001
TLC	2313.000	4941.000	-1.979	.048
SEG	2578.500	5206.500	-.993	.321
MON	2207.500	4835.500	-2.387	.017
LYMPH	2366.000	5526.000	-1.783	.075

Markers such as albumin gradient, triglycerides, specific gravity, total protein, LDH, cholesterol, and monocyte count show statistically significant differences between malignant and non-malignant groups. Markers like pH,

glucose, and segmented neutrophil count do not demonstrate significant differences. The total leukocyte count, and lymphocyte count are on the borderline of statistical significance.

Correlation Analysis

To clarify relationships between the examined markers, we used Spearman's rank correlation coefficient, which assesses the degree of association between two variables without assuming a normal distribution. Values range from -1 to 1, where a positive value indicates a direct relationship and a negative value – an inverse one. **Table 11** marks the identified correlations and their type with the corresponding coefficients.

Table 11: *The identified correlation relationships among the biochemical parameters examined in pleural fluid*

			SEAG	TG	pH	PSG	GLU	TP	LDH	CHOL	TLC	SEG	MON	LYMPH
Spearman's rho	SEAG	Correlation Coefficient	1.000	-.322	-.180	.081	.030	-.531	-.446	-.405	-.080	-.035	-.049	.072
		Sig. (2-tailed)	.	<.001	.027	.322	.716	<.001	<.001	<.001	.326	.666	.547	.379
		N	151	151	151	151	151	151	151	151	151	151	151	151
	TG	Correlation Coefficient	-.322	1.000	.117	-.102	-.048	.397	.450	.457	.195	.161	.132	-.191
		Sig. (2-tailed)	<.001	.	.151	.212	.562	<.001	<.001	<.001	.016	.048	.106	.019
		N	151	151	151	151	151	151	151	151	151	151	151	151
	pH	Correlation Coefficient	-.180	.117	1.000	-.340	-.118	.120	.059	.178	.145	.157	.027	-.141
		Sig. (2-tailed)	.027	.151	.	<.001	.148	.143	.473	.029	.076	.055	.738	.084
		N	151	151	151	151	151	151	151	151	151	151	151	151
	PSG	Correlation Coefficient	.081	-.102	-.340	1.000	-.029	-.241	.000	-.089	-.107	.010	.163	-.058
		Sig. (2-tailed)	.322	.212	<.001	.	.722	.003	.996	.275	.190	.906	.045	.482
		N	151	151	151	151	151	151	151	151	151	151	151	151
	GLU	Correlation Coefficient	.030	-.048	-.118	-.029	1.000	-.009	-.310	-.077	-.196	-.228	-.035	.216
		Sig. (2-tailed)	.716	.562	.148	.722	.	.916	<.001	.350	.016	.005	.668	.008
		N	151	151	151	151	151	151	151	151	151	151	151	151
	TP	Correlation Coefficient	-.531	.397	.120	-.241	-.009	1.000	.460	.586	.357	.207	.104	-.253
		Sig. (2-tailed)	<.001	<.001	.143	.003	.916	.	<.001	<.001	<.001	.011	.204	.002
		N	151	151	151	151	151	151	151	151	151	151	151	151
	LDH	Correlation Coefficient	-.446	.450	.059	.000	-.310	.460	1.000	.397	.352	.409	.168	-.426
		Sig. (2-tailed)	<.001	<.001	.473	.996	<.001	<.001	.	<.001	<.001	<.001	.039	<.001
		N	151	151	151	151	151	151	151	151	151	151	151	151
	CHOL	Correlation Coefficient	-.405	.457	.178	-.089	-.077	.586	.397	1.000	.287	.210	.101	-.248
		Sig. (2-tailed)	<.001	<.001	.029	.275	.350	<.001	<.001	.	<.001	.010	.216	.002
		N	151	151	151	151	151	151	151	151	151	151	151	151
	TLC	Correlation Coefficient	-.080	.195	.145	-.107	-.196	.357	.352	.287	1.000	.435	.308	-.488
		Sig. (2-tailed)	.326	.016	.076	.190	.016	<.001	<.001	<.001	.	<.001	<.001	<.001
		N	151	151	151	151	151	151	151	151	151	151	151	151
	SEG	Correlation Coefficient	-.035	.161	.157	.010	-.228	.207	.409	.210	.435	1.000	.367	-.947
		Sig. (2-tailed)	.666	.048	.055	.906	.005	.011	<.001	.010	<.001	.	<.001	<.001
		N	151	151	151	151	151	151	151	151	151	151	151	151
	MON	Correlation Coefficient	-.049	.132	.027	.163	-.035	.104	.168	.101	.308	.367	1.000	-.561
		Sig. (2-tailed)	.547	.106	.738	.045	.668	.204	.039	.216	<.001	<.001	.	<.001
		N	151	151	151	151	151	151	151	151	151	151	151	151
	LYMPH	Correlation Coefficient	.072	-.191	-.141	-.058	.216	-.253	-.426	-.248	-.488	-.947	-.561	1.000
		Sig. (2-tailed)	.379	.019	.084	.482	.008	.002	<.001	.002	<.001	<.001	<.001	.
		N	151	151	151	151	151	151	151	151	151	151	151	151

Discussing the Results from Task №4

a) Albumin Gradient and Total Protein

Existing evidence supports a link between total protein and albumin – its main component – warranting their combined investigation. High protein content in pleural effusion increases the likelihood of malignancy. This was confirmed by Hsieh and colleagues, who used two-dimensional gel electrophoresis to identify protein profiles in 27 patients, 14 of whom had cytologically confirmed malignant effusion. Their analysis showed significantly elevated protein levels in the malignant group.

The albumin gradient provides more precise information compared to examining albumin alone and effectively distinguishes exudative from transudative effusions. This is supported by Benin Ceyhan's study, recommending an upper albumin gradient threshold of 1.2 g/dl, offering 76% sensitivity and 100% specificity for exudative effusions. Roth additionally proved that a low albumin gradient successfully predicts malignant etiology.

A prospective study by Sandeesha involving 66 patients showed a high classification accuracy via albumin gradient – 92.42%, compared to 87.87% with Light's criteria.

In our analysis, the Kolmogorov-Smirnov test indicated non-normal distribution for total protein ($p=0.013$) and albumin gradient ($p=0.009$). The Mann-Whitney U test showed statistical significance ($p=0.002$ for protein and $p<0.001$ for albumin gradient). Albumin gradient values were higher in malignant effusions (mean rank 86.18 vs. 65.80).

b) Cholesterol

Significantly elevated cholesterol levels in malignant effusions confirm Porcel JM's findings, who reported that values above 45 mg/dl had 87% sensitivity and 92% specificity for malignant pleural effusions.

Our analysis confirmed a similar trend via Mann-Whitney U Test ($p=0.011$), with the malignant group having a higher mean rank (82.67 vs. 70.41).

c) Specific Gravity and Protein

Increased specific gravity in malignant pleural effusions aligns with Antoniou's study, which showed statistical significance ($p=0.002$). Our analysis also confirms this ($p=0.005$).

d) Lymphocytes and Monocytes

A high lymphocyte count in pleural fluid is a classic indicator of malignancy, though other causes (e.g., tuberculosis) are possible.

Our results showed a near-significant difference in lymphocyte count ($p=0.054$), while monocyte count was statistically significant ($p=0.012$), also supporting the hypothesis of an inflammatory response in malignant effusions.

Conclusions from Task №4

1. Extended biochemical analysis of pleural fluid provides valuable information for differentiating malignant from non-malignant effusions, with albumin gradient, total protein, and cholesterol being especially important markers.

2. High total protein content is associated with malignant effusions, while the albumin gradient is key in distinguishing exudative from transudative effusions.

3. Pleural cholesterol has high diagnostic value, demonstrating good sensitivity and specificity for identifying malignant pleural effusions.

4. Parameters like pH and glucose, though useful in other conditions, are less informative for diagnosing malignant effusions.

5. Specific gravity, though less studied, has potential as an additional diagnostic marker and warrants further investigation.

Results from Task №5

Assessment of the Role of Tumor Markers in Pleural Effusion

To evaluate the significance of the studied tumor markers in pleural fluid, we applied the previously described methods to assess the normality of data distribution. Results from the Kolmogorov-Smirnov test indicate that the variables do not follow a normal distribution. The greatest deviations from normality were observed with the markers CA19-9 ($p = 0.453$) and PIVKA ($p = 0.447$). The results are summarized in **Table 12**.

***Table 12**: Kolmogorov–Smirnov test applied to tumor marker values*

One-Sample Kolmogorov-Smirnov Test

		CEA	CA19-9	CA72-4	CA125	CA15-3	PIVKA
N		151	151	151	151	151	151
Normal Parameters	Mean	109.3696	138.6150	120.4977	1303.5977	77.5986	210.0407
	Std. Deviation	266.88958	461.52682	201.02610	1477.59573	189.80190	852.71540
Most Extreme Differences	Absolute	.373	.453	.370	.202	.353	.447
	Positive	.373	.453	.370	.202	.353	.447
	Negative	-.341	-.382	-.274	-.189	-.342	-.403
Test Statistic		.373	.453	.370	.202	.353	.447
Asymp. Sig. (2-tailed)		<.001	<.001	<.001	<.001	<.001	<.001

Next, we used the Independent Samples Test to compare the mean values between the two groups, as well as Levene's Test to assess equality of variances. The resulting data are presented in **Table 13**.

Table 13: *The t-test applied to the values of the tumor markers*

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
CEA	Equal variances assumed	38.843	<.001	-3.576	149	<.001	-149.71940	41.87124	-232.45753	-66.98127
	Equal variances not assumed			-3.691	108.609	<.001	-149.71940	40.55984	-230.11094	-69.32787
CA19-9	Equal variances assumed	50.137	<.001	-3.502	149	<.001	-253.98417	72.52402	-397.29259	-110.67574
	Equal variances not assumed			-3.669	78.084	<.001	-253.98417	69.21901	-391.78629	-116.18204
CA72-4	Equal variances assumed	162.418	<.001	-6.388	149	<.001	-186.00299	29.11723	-243.53902	-128.46695
	Equal variances not assumed			-6.592	109.344	<.001	-186.00299	28.21470	-241.92164	-130.08434
CA125	Equal variances assumed	22.504	<.001	-4.448	149	<.001	-1009.40429	226.95989	-1457.88001	-560.92856
	Equal variances not assumed			-4.556	125.234	<.001	-1009.40429	221.54990	-1447.87104	-570.93754
CA15-3	Equal variances assumed	8.444	.004	-2.394	149	.018	-72.89599	30.44846	-133.06255	-12.72943
	Equal variances not assumed			-2.427	142.865	.016	-72.89599	30.02958	-132.25571	-13.53627
PIVKApun	Equal variances assumed	1.459	.229	.559	149	.577	77.90151	139.25464	-197.26749	353.07052
	Equal variances not assumed			.548	117.904	.585	77.90151	142.07094	-203.44000	359.24303

Among all analyzed markers, only PIVKA did not show a statistically significant difference between the malignant and non-malignant groups. The remaining markers demonstrated notable differences, most prominently CEA and CA19-9.

Application of the Mann-Whitney U and Wilcoxon W tests, which assess the significance of parameters, confirmed that all tumor markers—except PIVKA—are statistically significant with $p < 0.005$. This suggests that these markers are associated with the malignant nature of pleural effusion. Further details are found in **Table 14**.

Table 14: *Mann–Whitney U and Wilcoxon tests applied to assess distribution characteristics*

Mann-Whitney U

	CEA	CA19-9	CA72-4	CA125	CA15-3	PIVKA
Mann-Whitney U	1443.500	2023.000	879.500	1574.500	1291.500	2440.000
Wilcoxon W	4071.500	4651.000	3507.500	4202.500	3919.500	5068.000
Z	-5.218	-3.066	-7.353	-4.730	-5.784	-1.505
Asymp. Sig. (2-tailed)	<.001	.002	<.001	<.001	<.001	.132

Discussing the Results from Task №5

Combining extended biochemical analysis with evaluation of tumor markers in pleural fluid offers a promising and minimally invasive approach to diagnosing malignant pleural effusions. The classical classification of pleural effusions as transudative or exudative, based on Light's criteria, has proven to be quite accurate, especially for exudative effusions. However, some transudative effusions may be incorrectly classified as exudative, leading to unnecessary further investigations.

Tumor markers play a key role in confirming or ruling out malignancy in pleural diseases. Despite the large number of studies, they do not yield definitive results, but it is well-established that CEA, CA15-3, and CYFRA 21-1 are elevated in malignant pleural effusions. This is supported by our Mann-Whitney U test, which showed a significant difference between the malignant and non-malignant groups, except for PIVKA.

A study by Porcel involving 1,575 patients also found elevated levels of CEA and CA15-3 in malignant cases, proposing threshold values above which these markers are significantly increased. Similar results were confirmed by Khalaf and Antonangelo, who found high diagnostic value in the combined use of CEA and CA15-3.

Other large studies, such as SIMPLE and DIAPHRAGM, have validated the role of tumor markers in the diagnostic process of malignant pleural effusions.

Conclusions from Task №5

1. All tumor markers, except for PIVKA, show statistically significant differences between the malignant and non-malignant groups, making them useful for distinguishing malignant pleural effusions.

2. High levels of CEA and CA15-3 are associated with malignancy in pleural effusions and enhance diagnostic sensitivity, especially when combined with cytopathology.

3. Studies by Porcel and Khalaf confirm the diagnostic significance of these markers in pleural effusions.

4. Correlation analysis shows that tumor markers are interrelated, which supports more accurate diagnosis.

5. Including tumor markers in the diagnostic algorithm improves accuracy and reduces the need for unnecessary testing

Results from Task №6

Relationship Between Sensitivity and Specificity of Tumor Markers in Pleural Fluid

To evaluate the relationship between sensitivity and specificity of the studied tumor markers and to determine example cut-off values, we used ROC (Receiver Operating Characteristic) analysis.

Figure 9 illustrates the ROC curve for the CEA marker, where the X-axis represents specificity (False Positive Rate), and the Y-axis represents sensitivity (True Positive Rate). The green diagonal line represents random classification ($p = 0.5$), while the blue curve significantly exceeds it, confirming the strong diagnostic ability of the model.

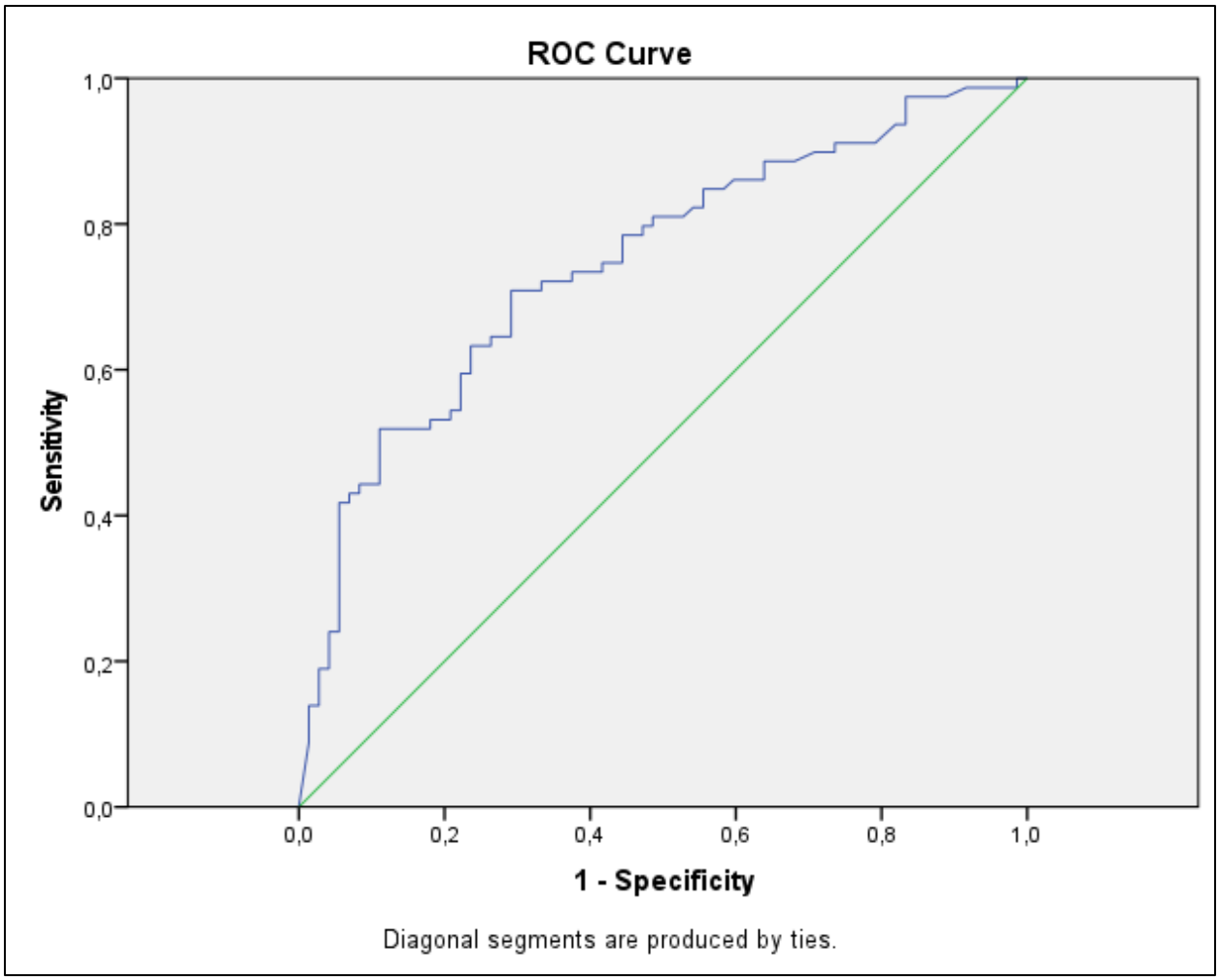

Figure 9: *Receiver Operating Characteristic (ROC) curve for the tumor marker CEA*

Table 15 presents the results of the AUC (Area Under the Curve) analysis for CEA, showing good classification ability with a result of 0.746, corresponding to 74.6% correct classification of malignant pleural effusions. The low standard error and statistically significant p-value further confirm the reliability of the results.

Table 15: *Distribution of the area under the curve (AUC) for the tumor marker CEA.*

Area Under the Curve

Test Result Variable(s):CAEpun

Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
,746	,040	,000	,668	,824

A similar analysis was conducted for the remaining markers, with their ROC curves compiled in **Figure 10**, and the results summarized in **Table 16**.

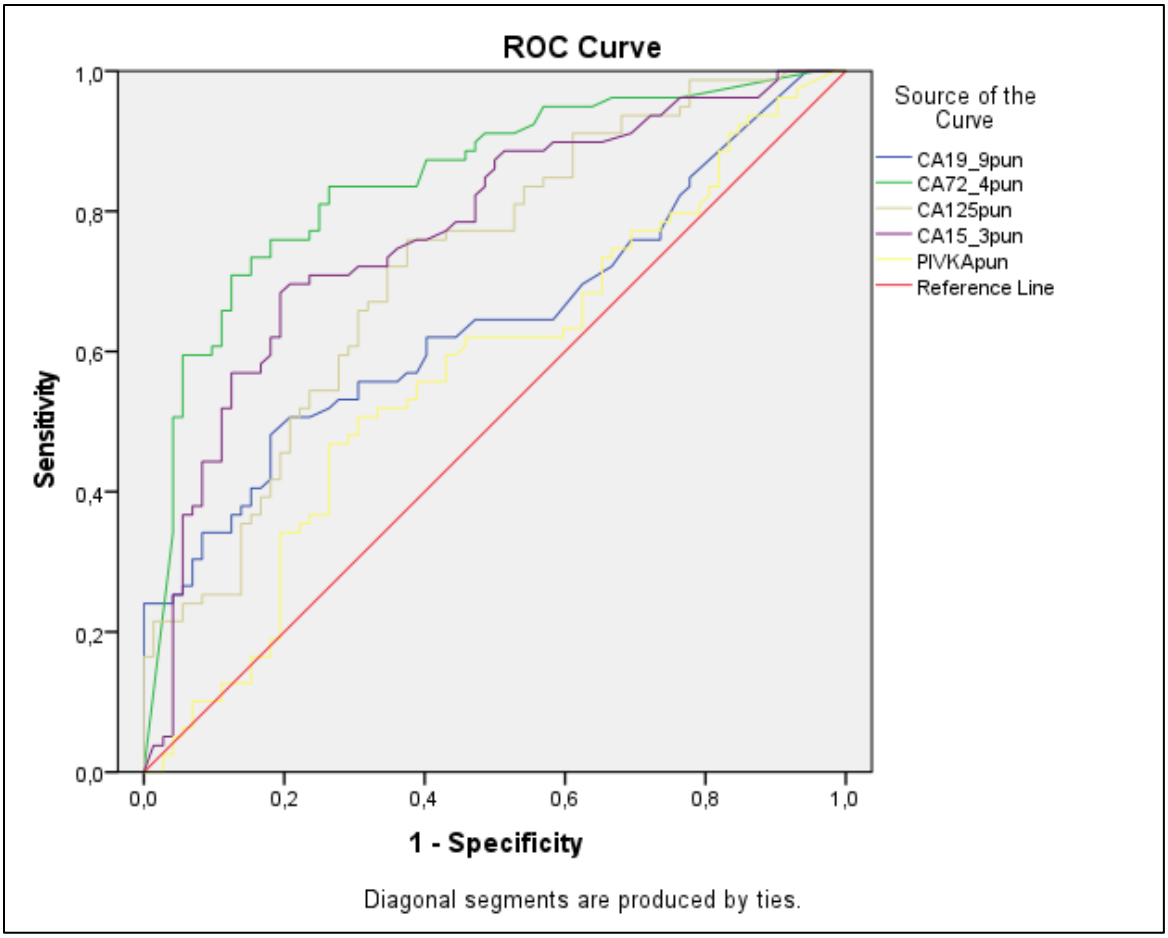

Figure 10: *Receiver Operating Characteristic (ROC) curves for the remaining tumor markers*

Table 16: *Distribution of the area under the curves (AUC) for the remaining tumor markers*

Area Under the Curve

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
CA19_9pun	,644	,045	,002	,556	,732
CA72_4pun	,845	,032	,000	,782	,909
CA125pun	,723	,041	,000	,643	,804
CA15_3pun	,773	,039	,000	,697	,848
PIVKApun	,571	,047	,132	,479	,663

Discussing the Results from Task №6

To support our findings, we reference a study by Trape, who analyzed 402 patients, including 122 with malignant pleural effusions, and proposed threshold values for each marker, achieving a combined sensitivity of 63.9%. Comparable reference values are noted in other studies as well.

Our results indicate that CEA is the most reliable marker with AUC = 0.746 and statistical significance ($p < 0.001$). CA19-9 shows moderate accuracy with AUC = 0.644 ($p = 0.002$), while CA72-4 demonstrates the best performance with AUC = 0.845 ($p < 0.001$), and is considered an excellent indicator of malignancy. CA15-3 also shows good diagnostic value (AUC = 0.773, $p < 0.001$), while the only weak marker is PIVKA, with AUC = 0.571 and non-significant statistics ($p = 0.132$).

Despite the relatively low sensitivity of some markers, their high specificity and combined use greatly assist in clinical decision-making, particularly when considering the need for more invasive procedures.

Conclusions from Task №6

1. CEA stands out with good diagnostic ability and statistical significance.
2. CA19-9 has moderate diagnostic accuracy and is suitable as a supplementary marker.
3. CA72-4 is the most accurate among the tested markers.
4. CA15-3 also demonstrates high diagnostic value.
5. PIVKA is a weak-performing marker and is not suitable for differential diagnosis.
6. Combining markers increases accuracy, even when individual markers have limited sensitivity.

Results from Task №7

Establishing Hypothetical Cut-Off Values with Potential Diagnostic Value in Malignant Pleural Diseases

By analyzing AUC (Area Under the ROC Curve) values, we can assess the ability of individual tumor markers to distinguish between malignant and non-malignant pleural effusions. Based on this, the tumor markers can be categorized into four levels of diagnostic effectiveness:

- Very Good: CA72-4
- Good: CA125 and CA15-3
- Moderate: CA19-9
- Weak: PIVKA

Among them, CA72-4 stands out with the highest discriminatory ability, while PIVKA does not show statistically significant value in differentiating between the two conditions.

For the purposes of differential diagnosis, it is necessary to determine a cut-off value— a threshold that separates positive from negative results. This value should offer an optimal balance between sensitivity and specificity, and is determined using Youden's Index:

$$J = \text{Sensitivity} + (1 - \text{Specificity}) - 1$$

Table 17 presents the values at which the best compromise between these two parameters is achieved, based on the analyzed ROC curves.

Table 17: *Proposed threshold values based on the respective Youden index.*

Tumor marker	Cut-off value	Youden index
CEA	>1.8	0.417
CA19-9	>3.75	0.3
CA72-4	>4.52	0.584
CA125	>544.43	0.384
CA-15-3	>9.65	0.489

Discussing the Results from Task №7

There are different perspectives on the appropriate cut-off values for tumor markers in pleural fluid. A study by Zhang, involving 619 patients with pleural effusion (274 with lung cancer and 74 with non-malignant conditions), reported sensitivity of 89.8% and specificity of 98.6% for CEA at a cut-off of 5.23 ng/ml (AUC = 0.978). For CYFRA 21-1, the same study reported sensitivity of 67.9% and specificity of 90.5% at a threshold of 31.39 ng/ml (AUC = 0.853).

Another study by Fan, examining elevated levels of markers such as CA125, CEA, CA15-3, CA19-9, etc., identified CEA as the most informative, with a cut-off of 3.7 ng/ml (AUC = 0.890). When combined with CYFRA 21-1, diagnostic accuracy increased significantly, reaching sensitivity of 79.9% and specificity of 95.7%.

A similar approach is supported by an analysis from Yang, who summarized data from 20 independent studies, reporting the following results for combined markers (sensitivity/specificity):

- CEA + CA125: 0.65 / 0.98
- CEA + CA15-3: 0.64 / 0.98
- CEA + CA19-9: 0.58 / 0.98
- CEA + CYFRA21-1: 0.82 / 0.92
- CA15-3 + CYFRA21-1: 0.88 / 0.94

The conclusion is that in unclear pleural effusions, combined analysis of CEA, CA15-3, and CA19-9 is recommended.

Feng also conducted a comparative study of 81 patients with lung adenocarcinoma and 96 with non-malignant effusions, examining CEA, CYFRA21-1, and CA19-9. The optimal cut-off values were 0.93, 0.85, and 0.81, respectively, and their combined use raised sensitivity to 95.06% (AUC = 0.95).

Our own analyses showed the following results:

- CEA: 75.9% sensitivity / 55.6% specificity
- CA19-9: 48.1% / 18.1%

- CA72-4: 70.9% / 12.5%
- CA125: 75.9% / 37.5%
- CA15-3: 68.4% / 80.6%

Sensitivity reflects how well the test detects the disease in truly affected patients, while specificity indicates how accurately it identifies healthy individuals.

The obtained values in our study largely align with international data. Nevertheless, they should be regarded as guidelines, not absolute reference values—especially in cases with borderline or atypical results.

Conclusions from Task №7

1. ROC analysis and application of Youden's Index allow for defining threshold values that provide optimal separation between malignant and non-malignant conditions.

2. Combining CEA with other tumor markers (such as CYFRA21-1, CA15-3, and CA19-9) significantly improves diagnostic accuracy.

3. The extracted cut-off values should be used as guiding indicators. The best results are achieved through combined application, contributing to more precise management of patients with uncertain pleural effusions.

SUMMARIZED CONCLUSIONS

1. **Gender and Age Distribution**

   Malignant pleural effusions are observed more frequently in women, likely due to the higher incidence of pleural metastases from gynecological tumors. On the other hand, non-malignant effusions are more common in men. In both groups, the highest incidence is recorded in the age range of 71–80 years, highlighting the link between age and the occurrence of pleural effusions.

2. **Clinical Presentation and Localization**

   In a significant number of patients with malignant effusions, these are the initial manifestation of a previously undiagnosed oncological disease. The left hemithorax is more often affected, likely due to anatomical and physiological characteristics of the organs on the left side of the thoracic cavity. Although symptoms are similar across different types of effusions, malignant effusions often present without clearly expressed symptoms, making early diagnosis based solely on clinical signs more challenging.

3. **Histological Types and Diagnostic Approaches**

   The most common histological forms in malignant effusions are metastatic lung carcinoma and breast carcinoma—findings consistent with global literature. Light's criteria remain a reliable tool for distinguishing between exudative and transudative effusions. Malignant effusions are typically exudates, although a small percentage can present as transudates, especially in the early stages of the disease, posing a risk for misdiagnosis. Cytological analysis of pleural fluid shows high specificity, but is not immune to diagnostic errors due to morphological similarities between reactive and neoplastic cells.

4. **Biochemical and Cellular Indicators**

   Parameters such as total protein, albumin gradient, and cholesterol in pleural fluid are useful for differentiating malignant from non-malignant effusions. High protein and cholesterol levels correlate with the presence of a malignant process. Lactate dehydrogenase (LDH) remains a key marker in differentiating both transudates vs. exudates and malignant vs. non-malignant effusions. Although pH and glucose testing lacks specificity for diagnosing malignant effusions, it can provide valuable information when comorbidities are present. Elevated leukocyte counts, especially lymphocytes and monocytes, often accompany malignant effusions.

5. **Tumor Markers and Diagnostic Value**

   Tumor markers such as CEA, CA15-3, and CA72-4 show high diagnostic effectiveness in distinguishing malignant from non-malignant pleural effusions. Their combined use significantly increases diagnostic accuracy. Particularly high diagnostic value is seen with CEA and CA72-4, while markers like PIVKA show limited usefulness. The determination of optimal cut-off values through ROC analysis and Youden's Index supports the differential diagnostic process.

6. **Limitations and the Need for a Combined Approach**

   The possibility of false-negative results in early stages of the disease underscores the need for a multimodal approach. Relying on a single diagnostic tool is insufficient—effective diagnosis requires integrated use of clinical, laboratory, and imaging methods.

SUMMARY

Our study demonstrates that tumor markers, particularly CEA, CA15-3, and CA72-4, play a key role in the diagnosis and prognosis of malignant pleural effusions. Using these markers in combination with classical biochemical tests of pleural fluid improves diagnostic accuracy and provides valuable prognostic information.

Therefore, we recommend integrating tumor markers into standard diagnostic algorithms when malignant pleural effusion is suspected, which will aid in early detection and optimize therapeutic strategies.

Further studies with larger populations and standardized methodologies are necessary to validate and establish their practical significance.

AUTHOR'S CONTRIBUTIONS

Scientific Contributions

1. Objective evaluation of the diagnostic value of tumor markers in pleural fluid—such as CA72-4, CEA, CA15-3, CA19-9, CA125, and PIVKA-II—and their ability to differentiate between malignant and non-malignant pleural effusions.
2. Determination of diagnostic cut-off values for tumor markers using ROC analysis and Youden's index, with CA72-4 demonstrating the highest diagnostic value.
3. Examination of an expanded biochemical panel in pleural fluid—identifying significant differences in the levels of albumin gradient, total protein, cholesterol, specific gravity, and monocytes between malignant and non-malignant effusions.
4. Emphasis on the limited sensitivity of conventional cytology, which yields false-negative results in over 70% of malignant cases, despite its high specificity.
5. Demonstration that Light's classical criteria, though useful, can misclassify up to 26.8% of malignant effusions as transudates—particularly in the early stages of the disease.

Practical Contributions

6. Identification of CA72-4 as the best standalone tumor marker with an AUC = 0.845 for distinguishing malignant from non-malignant pleural effusions.
7. Proposal for the combined use of CEA, CA15-3, and CA19-9, aiming to increase diagnostic accuracy and reduce the need for invasive procedures.
8. Development of locally applicable diagnostic reference values, tailored to Bulgarian clinical practice and the available medical equipment.
9. Support for early diagnosis of pleural neoplasms through the use of accessible and routinely performed laboratory tests.

PUBLICATIONS RELATED TO THE DISSERTATION WORK

1. Aleksiev V, Yavorov B, Markov D et al. LEUKOCYTE PROFILING IN MALIGNANT PLEURAL EFFUSIONS: A CYTOLOGICAL PERSPECTIVE ON DIAGNOSTIC ADVANCES №98/2025 Znanstvena misel journal DOI: 10.5281/zenodo.14721914
2. Aleksiev V, Markov D, Chapkanov A. INVESTIGATING CHOLESTEROL LEVELS IN PLEURAL FLUID: IMPLICATIONS FOR DIAGNOSIS OF MALIGNANT PLEURAL EFFUSIONS №95/2024 Znanstvena misel journal DOI: 10.5281/zenodo.13999071
3. V. Aleksiev, B. Yavorov, D. Markov. COMPREHENSIVE CLINICO-DEMOGRAPHIC ANALYSIS OF MALIGNANT PLEURAL EFFUSIONS IN THE BULGARIAN POPULATION: INSIGHTS FROM AN OBSERVATIONAL CASE-CONTROL STUDY 10.2478/AMB-2025-0043

PARTICIPATION IN CONGRESSES AND TRAININGS

1. 10th Jubilee National Congress of Thoracic, Cardiac and Vascular Surgery and 7th National Congress of the Bulgarian Society of Cardiac Surgery