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Original Article

Enhanced diagnostic approaches for malignant pleural effusions: an extensive biochemical and statistical analysis

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Abstract

Introduction: Malignant pleural effusions are a common and debilitating complication of advanced malignancies, affecting approximately one million patients annually. This condition leads to significant morbidity and a decline in quality of life. Accurate diagnosis and effective management are critical yet challenging due to the overlap in biochemical markers between malignant and benign pleural effusions.

Aim: This study evaluates an extended panel of biochemical parameters, including albumin gradient, total protein, cholesterol, pH, glucose, specific gravity, and lactate dehydrogenase (LDH), to enhance diagnostic precision.

Materials and methods: In order to achieve this, we conducted a cross-sectional, observational case-control study in order to analyze pleural fluid samples from 151 Bulgarian patients, including 79 with malignant effusions and 72 with benign effusions. Biochemical markers, such as albumin gradient, total protein, cholesterol, lactate dehydrogenase (LDH), pH, glucose, and specific gravity, were measured using advanced clinical chemistry analyzers.

Statistical analyses, including Mann-Whitney U tests, t-tests, and Spearman's rank correlations, were used to identify diagnostic markers.

Results: The key findings highlighted the diagnostic value of albumin gradient, total protein, and cholesterol levels, which are strongly associated with malignant effusions. LDH and specific gravity also demonstrated potential as supplementary markers, while pH and glucose measurements showed limited utility in differentiating malignancy.

Conclusion: Combining these biochemical parameters enhances the precision of pleural effusion analysis, offering a more robust framework for diagnosing and managing malignant pleural effusions effectively.

Keywords

albumin gradient, biochemical markers, diagnostic differentiation, malignant pleural effusion, pleural fluid analysis

Introduction

Malignant pleural effusions (MPE) are a major challenge in medical practice, putting a strain on healthcare systems due to their socioeconomic impact. MPEs affect nearly one million people each year and are a leading cause of severe shortness of breath and a lower quality of life. Pleural effusions are a common complication of cancer, affecting approximately 20% of cancer patients.[1]

The prognosis for patients with MPE is often poor, with median survival ranging from 4 to 9 months, depending on the cancer type and its stage.[2] These statistics highlight the importance of early detection and the creation of effective strategies for diagnosing and managing this condition and reducing its toll on patients.

Normal pleural fluid is a clear straw-yellow liquid. Several baseline indicators are used to investigate pleural pathologies:

pH of pleural fluid

The acidity of pleural fluid is a poorly studied parameter in modern literature. Only one study exists that addresses the normal pH of pleural fluid in humans.[3] It defines normal reference values for pleural fluid pH as ranging between 7.6 and 7.64. The pH of pleural effusions is almost always lower than that of normal pleural fluid and is much closer to the pH of blood. In cases of transudative pleural effusion, there is a significant increase in pleural pH (7.45–7.55) compared to exudative pleural effusions, where it typically ranges from 7.30 to 7.45.[4]

When pleural pH falls below 7.3, the condition is known as pleural acidosis.[5] Values below 7.20 are associated with severe forms of pneumonia, pleural carcinomatosis, esophageal rupture, tuberculosis, rheumatoid diseases, and are even considered an indication for urgent thoracentesis, as persistently low pH dramatically increases the risk of developing pleural empyema.[6] Several authors have studied the mechanism of pleural acidosis[7-10], linking it to active inflammatory processes, activation of immune cells (particularly leukocytes), and increased acid production.[11-13] Accumulation of carbon dioxide and lactate further deepens the acidosis.[14,15]

Carbon dioxide buildup is linked to both increased production and reduced transport across pleural membranes, as well as increased leukocyte glycolysis.[16,17] Lactate is formed as a byproduct of neutrophil glycolysis and pathogen metabolism in bacterial hydrothorax.[18]

Detailed literature analysis even reveals cases of pleural alkalosis, where pleural fluid pH reaches 7.8.[19] This is most commonly associated with the presence of a chylous effusion, also known as chylothorax.

In addition to distinguishing between exudates and transudates, one of the most practical uses of pleural pH measurement is its prognostic value in predicting the success of pleurodesis. Pleural pH remains the sole independent predictor for successful pleurodesis, with the likelihood of obliterating the pleural space progressively decreasing with lower pleural pH. When pH falls below 7.15, the positive predictive value drops to around 45.7%.[20]

Specific gravity

The specific gravity of pleural fluid is the most precise indirect method for measuring its viscosity. Tavana's comprehensive study[21] aimed to determine the sensitivity and specificity of specific gravity in differentiating exudative from transudative pleural effusions.

Samples were collected from 100 patients undergoing thoracentesis. Based on Light's criteria, punctates were classified, and specific gravity was measured using refractometry. Among the samples, 70% were classified as exudative effusions and 30% as transudative. In the exudative group, the mean specific gravity was 1033.6, while it was 1021.4 in the transudative group.

The optimal sensitivity and specificity were achieved at a threshold of 1024, with sensitivity, specificity, positive predictive value, and negative predictive value of 91.4%, 66.7%, 86.5%, and 76.9%, respectively.

Glucose

Quantitative determination of glucose in pleural fluid is an important step in the biochemical analysis of pleural effusions. Values can vary significantly, ranging from 0.00 to 29.36 mmol/L, with a threshold value of 3.30 mmol/L considered significant.[22]

Several studies have examined the correlation between glucose and pleural fluid acidity.[22-26] Many authors adopt the hypothesis that there is a direct proportional relationship, where a decrease in pH corresponds to glucose depletion. However, an in-depth study by Fitzgerald et al.[27], involving data from multiple centers, demonstrates high variability in predictive intervals, suggesting the two parameters should be interpreted independently.

For example, while linear dependence is observed in many non-infectious effusions, tuberculous effusions often exhibit drastically low glucose levels unrelated to pleural fluid pH.[28] Glucose reduction is attributed to the presence of bacteria, polymorphonuclear cells, and malignant cells.

Protein profile

Normal pleural fluid is relatively low in proteins. Protein presence and composition are critical for distinguishing effusion types, as emphasized in Light's criteria.

Samanta et al.[29] conducted a case-control study, identifying elevated serum albumin and pleural fluid albumin levels in tuberculosis-associated effusions compared to malignant effusions. Tuberculosis effusions demonstrated significantly higher serum effusion albumin gradient (SEAG) than malignant effusions. The critical threshold for total protein in pleural fluid is 6.2 g/dL, providing 92% sensitivity and 100% specificity in distinguishing tuberculosis

effusions from malignancies.

Lactate dehydrogenase (LDH)

LDH is a cytoplasmic enzyme found in all organ cells that plays an essential role in anaerobic metabolism. It is an indicator of inflammation or cellular damage, such as ischemia, dehydration, or bacterial and chemical injury.

In pleural effusions, LDH values above 1000 U/L are significant, often pointing to infectious causes, rheumatoid serositis, tuberculosis, or malignancy.[30] LDH levels are also incorporated into Light's criteria for distinguishing exudative from transudative effusions.

Cholesterol and triglycerides

Cholesterol, a key sterol in the human body, enters pleural fluid through increased vascular permeability or as a product of degenerating cells. A clinical-laboratory threshold of >45 mg/dL is used for differentiating exudative from transudative effusions.[31] In chylous effusions, cholesterol levels are <5.18 mmol/L (<200 mg/dL), while pleural triglycerides are >1.24 mmol/L (>110 mg/dL).[32]

Materials and methods

In order to achieve the established objectives, a cross-sectional observational case-control study was conducted on a Bulgarian population of patients diagnosed with pleural effusions.

A total of 151 patients participated in the analysis. In the control group of 72 patients, a benign disease was diagnosed and confirmed through subsequent biopsy. Of these, 38 cases were identified as inflammatory, while 34 were verified as pleural effusions of non-inflammatory origin. Malignant pleural involvement was confirmed in 79 patients. These two groups are representative of the main types of pleural pathology.

Pleural fluid was obtained using a closed container for biological material. The biological material was collected during thoracentesis or intraoperatively during VATS. A portion of the collected pleural fluid was used to determine biochemistry parameters, while the remaining fluid was set aside for the analysis of tumor markers and cytological examination. All analyses were carried out using the clinical chemistry analyzer Beckman Coulter, model AU480, in accordance with the original programs.

The choice of statistical methods was made according to the objectives of the study, the type of variables, and established practices in scientific research in the field of thoracic surgery. The systematization, processing, and analysis of primary data in the form of quantitative and qualitative variables were carried out using the statistical software package IBM SPSS Statistics. The analysis and conclusions from the study were drawn after a summarized presentation of the empirical results in tabular form and were illustrated with the corresponding graphs. The graphical analysis was performed using MS Office 365. To objectify the results of the analyses conducted, the following statistical and mathematical methods were used:

- Mann-Whitney Wilcoxon Test: A non-parametric statistical analysis used to compare two independent groups. Its purpose is to determine whether the distribution of the two parameters differs significantly from each other.
- Kolmogorov-Smirnov's One-Sample Test: A non-parametric test used to check whether a given sample follows a specific distribution. It compares the empirical distribution of data with the theoretical distribution.
- Independent Samples t-test: A parametric test used to compare the means of two independent groups.
- Levene's Test for Equality of Variances: A statistical test used to check for equality of variances among two or more groups.
- Correlation Analysis: A statistical method used to assess the relationship between two or more variables. It helps to understand whether changes in one independent variable are associated with changes in another dependent variable. It does not establish a causal relationship but measures the degree of association between the variables.

Results

To analyze whether different variables from the full panel of biochemical markers follow a normal distribution, we first applied Kolmogorov-Smirnov's One-Sample test. The obtained results are shown in **Table 1**.

It is evident that all included variables significantly deviate from a normal distribution, as supported by the asymptotic significance, which is below the standard significance level of $p<0.05$ in all cases. Therefore, these variables are not assumed to follow a normal distribution.

This hypothesis is further supported by the application of the Independent Samples t-test, which allows us to check whether there is a statistically significant difference between the mean values of the necessary variables in the two groups of patients. The Levene's test for Equality of Variances checks whether the variance of the two groups is statistically significant and whether both groups demonstrate homogeneity of variances. The t-test for Equality of Means seeks to find a difference between the means of the two groups and tests whether this difference is statistically significant. The results presented in **Table 2** demonstrate that a statistically significant difference exists for the 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, $p=0.002$. Notably, 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, $p=0.011$ show equal variances but p-values are close to significant.

The benign group shows higher specific gravity values, while cholesterol levels are elevated in the malignant group. Commenting on the cytological examination results, the

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values for monocytes show equal variances, and the difference in p is close to significant but does not reach it: Levene's Test (Sig.=0.562); t(149)=−1.768, p=0.079.

If we apply the Mann-Whitney U test, we obtain the results presented in **Table 3**.

Focusing on the presented data, we note that the

Table 1. The Kolmogorov-Smirnov test applied for all parameters

	Normal parameters			Most extreme differences			Statistical test	Asymp. sig. (2-tailed)
	N	Mean	Std. deviation	Absolute	Positive	Negative		
SEAG	151	16.0397	5.36269	0.085	0.085	−0.051	0.035	0.009
TG	151	0.4865	1.74971	0.410	0.395	−0.410	0.410	<0.001
pH	151	7.2748	0.42304	0.331	0.331	−0.231	0.331	<0.001
PSG	151	1012.8146	4.22596	0.300	0.230	−0.300	0.300	<0.001
GLU	151	5.6530	3.67905	0.173	0.173	−0.167	0.173	<0.001
TP	151	35.1987	10.73873	0.083	0.053	−0.083	0.033	0.013
LDH	151	431.4503	622.20932	0.269	0.269	−0.261	0.269	<0.001
CHOL	151	1.7025	0.81446	0.085	0.085	−0.054	0.035	0.009
TLC	151	119.77	175.413	0.264	0.264	−0.253	0.264	<0.001
SEG	151	15.88	17.672	0.238	0.238	−0.203	0.238	<0.001
MON	151	5.91	3.001	0.137	0.137	−0.073	0.137	<0.001
LYMPH	151	78.12	19.168	0.214	0.162	−0.214	0.214	<0.001

Table 2. The t-test for Independent Samples applied to our biochemical parameters

		Levene's test for Equality of Variances		t-test for Equality of Means					95% Confidence interval of the difference	
		F	Sig.	t	dr	Sig. (2 tailed)	Mean difference	Sid. error difference	Lower	Upper
SEAG	Equal variances assumed	7.443	0.007	4.786	149	<0.001	3.90612	0.8162	2.29330	5.51893
	Equal variances not assumed			4.703	122.8	<0.001	3.90612	0.8306	2.26197	5.55027
TG	Equal variances assumed	1.832	0.178	−1.007	149	0.315	−0.28717	0.2851	−0.85047	0.27614
	Equal variances not assumed			−1.054	79.814	0.295	−0.28717	0.2723	−0.82915	0.25481
pH	Equal variances assumed	1.054	0.306	−1.661	149	0.099	0.11384	0.0685	−0.24924	0.02157
	Equal variances not assumed			−1.674	148.2	0.096	0.11384	0.0680	−0.24822	0.02054
PSG	Equal variances assumed	0.741	0.391	2.855	149	0.005	1.92071	0.6727	0.59145	3.24997
	Equal variances not assumed			2.843	144.2	0.005	1.92071	0.6755	0.58550	3.25592
GLU	Equal variances assumed	0.091	0.763	0.158	149	0.874	0.09518	0.6014	−1.0932	1.28355
	Equal variances not assumed			0.155	120.0	0.877	0.09518	0.6129	−1.1183	1.30866

TP	Equal variances assumed	11.829	<0.001	−3.227	149	0.002	−5.47679	1.697	−8.8306	−2.1230
	Equal variances not assumed			−3.175	125.4	0.002	−5.47679	1.725	−8.8905	−2.0631
LDH	Equal variances assumed	0.064	0.801	0.947	149	0.345	−96.08527	101.4	−296.48	104.308
	Equal variances not assumed			−0.957	146.7	0.340	−96.08527	100.4	−294.48	102.307
CHOL	Equal variances assumed	1.943	0.165	−2.559	149	0.011	−0.33346	0.1303	−0.59097	−0.07596
	Equal variances not assumed			−2.525	130.6	0.013	−0.33346	0.1321	−0.59472	−0.07220
TLC	Equal variances assumed	0.874	0.351	−1.050	149	0.296	−29.993	28.57	−86.449	26.464
	Equal variances not assumed			−1.071	133.3	0.286	−29.993	26.01	−85.385	25.399
SEG	Equal variances assumed	0.549	0.460	−0.261	149	0.794	−0.754	2.888	−6.462	4.953
	Equal variances not assumed			−0.259	140.5	0.796	−0.754	2.909	−6.506	4.997
MON	Equal variances assumed	0.338	0.562	−1.768	149	0.079	−0.858	0.486	−1.817	0.101
	Equal variances not assumed			−1.757	141.9	0.081	−0.858	0.488	−1.824	0.108
LYMPH	Equal variances assumed	0.867	0.353	0.580	149	0.563	1.816	3.130	−4.369	8.001
	Equal variances not assumed			0.575	138.2	0.566	1.816	3.157	−4.427	8.060

Table 3. The Mann-Whitney U test carried out for our chosen markers

	Mann-Whitney U	**Wilcoxon W**	**Z**	**Asymp. sig. (2-tailed)**
SEAG	1722.000	4882.000	−4.190	<0.001
TG	2176.000	4804.000	−2.490	0.013
pH	2467.500	5095.500	−1.569	0.117
PSG	2134.500	5294.500	−2.903	0.004
GLU	2800.500	5960.500	−0.162	0.871
TP	2027.500	4655.500	−3.044	0.002
LDH	2054.000	4682.000	−2.943	0.003
CHOL	1902.000	4530.000	−3.512	<0.001
TLC	2313.000	4941.000	−1.979	0.048
SEG	2578.500	5206.500	−0.993	0.321
MON	2207.500	4835.500	−2.387	0.017
LYMPH	2366.000	5526.000	−1.783	0.075

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Table 4. The established correlations across the biochemical panel

			SEAG	TG	pH	PSG	GLU	TP	LDH	CHOL	TLC	SEG	MON	LYMPH
Spearman's rho	SEAG	Correlation coef.	1.000	−0.322	−0.180	0.081	0.030	−0.531	−0.446	−0.405	−0.080	−0.035	−0.049	0.072
		Sig. (2-tailed)		<0.001	0.027	0.322	0.716	<0.001	<0.001	<0.001	0.326	0.666	0.547	0.379
		N	151	151	151	151	151	151	151	151	151	151	151	151
	TG	Correlation coef.	−0.322	1.000	0.117	−0.102	−0.048	0.397	0.450	0.457	0.195	0.161	0.132	−0.191
		Sig. (2-tailed)	<0.001		0.151	0.212	0.562	<0.001	<0.001	<0.001	0.016	0.048	0.106	0.019
		N	151	151	151	151	151	151	151	151	151	151	151	151
	pH	Correlation coef.	−0.180	−0.117	1.000	−0.340	−0.118	0.120	0.059	0.178	0.145	0.157	0.027	−0.141
		Sig. (2-tailed)	0.027	0.151		<0.001	0.148	0.143	0.473	0.029	0.076	0.055	0.738	0.084
		N	151	151	151	151	151	151	151	151	151	151	151	151
	PSG	Correlation coef.	0.081	−0.102	−0.340	1.000	−0.029	−0.241	<0.000	−0.089	−0.107	0.010	0.163	−0.058
		Sig. (2-tailed)	0.322	0.212	<0.001		0.722	0.003	0.996	0.275	190	0.906	0.045	0.482
		N	151	151	151	151	151	151	151	151	151	151	151	151
	GLU	Correlation coef.	0.030	−0.048	−0.118	−0.029	1.000	−0.009	−0.310	−0.077	−0.196	−0.228	−0.035	0.216
		Sig. (2-tailed)	0.716	0.562	0.148	0.722		0.916	<0.001	0.350	0.018	0.005	0.668	0.008
		N	151	151	151	151	151	151	151	151	151	151	151	151
	TP	Correlation coef.	−0.531	0.397	120	−0.241	−0.009	1.000	0.460	0.586	0.357	0.207	0.104	−0.253
		Sig. (2-tailed)	0.001	<0.001	143	0.003	0.916		<0.001	<0.001	<0.001	0.011	20.04	0.002
		N	151	151	151	151	151	151	151	151	151	151	151	151
	LDH	Correlation coef.	−0.446	0.450	0.059	0.000	−0.310	0.460	1.000	0.397	0.352	0.409	0.168	−0.426
		Sig. (2-tailed)	<0.001	<0.001	0.473	0.996	<0.001	<0.001		<0.001	<0.001	<0.001	0.039	<0.001
		N	151	151	151	151	151	151	151	151	151	151	151	151

	CHOL	Correlation coef.	0.405	0.457	0.178	−0.089	−0.077	0.586	0.397	1.000	0.287	0.210	0.101	−0.248
		Sig. (2-tailed)	<0.001	<0.001	0.029	0.275	0.350	<0.001	<0.001		<0.001	0.010	0.216	0.002
		N	151	151	151	151	151	151	151	151	151	151	151	151
	TLC	Correlation coef.	−0.080	0.195	0.145	−0.107	−0.196	0.357	0.352	0.287	1.000	0.435	0.308	−0.488
		Sig. (2-tailed)	0.326	0.016	0.076	190	0.016	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001
		N	151	151	151	151	151	151	151	151	151	151	151	151
	SEG	Correlation coef.	−0.035	0.161	0.157	0.010	−0.228	0.207	0.409	0.210	0.435	1.000	0.367	0.947
		Sig. (2-tailed)	0.666	0.048	0.055	0.906	0.005	0.011	<0.001	0.010	<0.001		<0.001	<0.001
		N	151	151	151	151	151	151	151	151	151	151	151	151
	MON	Correlation coef.	−0.049	0.132	0.027	0.163	−0.035	0.104	0.168	0.101	0.308	0.367	1.000	0.561
		Sig. (2-tailed)	0.547	0.106	0.738	0.045	0.668	0.204	0.039	0.216	<0.001	<0.001		<0.001
		N	151	151	151	ISI	151	151	151	151	151	151	151	151
	LYMPH	Correlation coef.	0.072	0.191	−0.141	−0.058	0.216	−0.253	−0.426	−0.248	−0.488	−0.947	−0.561	1.000
		Sig. (2-tailed)	0.379	0.019	0.084	0.482	0.008	0.002	<0.001	0.002	<0.001	<0.001	<0.001	
		N	151	151	151	151	151	151	151	151	151	151	151	151

albumin gradient, triglyceride levels, specific gravity, total protein, LDH levels, cholesterol levels, and the number of monocytes show a statistically significant difference between the malignant and benign groups. Others, such as pH, glucose levels, and the number of segmented neutrophils, do not show a significant difference. The total leukocyte count and the number of lymphocytes is at the borderline of significance.

To delve into the relationship between the examined indicators, it is necessary to search for a correlation between them. For this, we will use Spearman's rank correlation coefficient, which evaluates the degree of dependence between two variables without assuming normal distribution of the data. The values range from -1 to 1. To confirm a direct relationship, the value should be positive, while negative values indicate an inverse relationship. For convenience, **Table 4** highlights the established correlations and the type of relationship with the respective coefficients.

Discussion

Albumin gradient and total protein

A good amount of evidence suggests a correlation between total protein and albumin, as a major component in its composition. This justifies considering both parameters together.

High total protein content in pleural effusion increases the likelihood that it is malignant. This was proven by Hsieh et al.[33], when the fluid from 27 patients, 14 of whom were cytologically verified as having malignant hydrothorax, was subjected to two-dimensional gel electrophoresis. Using ELISA and Western blot methods, the protein profiles of each effusion were identified.

Their results clearly showed that total protein content was significantly elevated in the malignant group.

The albumin gradient, as a function of albumin content in pleural fluid, provides much more accurate information compared to isolated albumin testing. It can effectively differentiate exudative from transudative pleural effusions. This concept is supported by recent observations from a study conducted by Benin Ceyhan, who recommends using an upper limit of the albumin gradient at 1.2 g/dl, which provides 76% sensitivity and 100% specificity for detecting exudative pleural effusions.[34] Additionally, Roth et al.[35] demonstrated that a low albumin gradient successfully predicts the malignant etiology of pleural effusion.

To reinforce the benefits of studying the albumin gradient (serum-effusion albumin gradient), we will present a prospective observational study conducted by Sandeesha et al.[36], where pleural punctates from 66 patients were examined and then classified into two groups based on the etiology of the pleural effusions. The data show that of the 22 transudative effusions, 20 were correctly classified using the albumin gradient. Of the 44 exudative effusions, 41 fell into the correct group. When compared to Light's criteria, it was found that testing the albumin gradient provided diagnostic value for distinguishing exudative from transudative pleural effusions at 92.42%, compared to 87.87% for Light's criteria.

By conducting a comparative analysis between the two groups in our study, we found the following. Using Kolmogorov-Smirnov test values, total protein (p=0.013) and albumin gradient (p=0.009) show non-normal distribution. When applying the Mann-Whitney U test, with p=0.002 and p<0.001 for total protein and albumin gradient respectively, we see that these values show promising levels of statistical significance in distinguishing malignant from benign pleural effusions. The analysis of the results also leads us to the hypothesis that elevated protein content is associated with the severity of pleural damage, whether malignant or inflammatory.

Triglycerides

The relationship between triglyceride levels and secondary malignant diseases of the pleura remains controversial. Research on triglycerides in pleural fluid does not provide significant advantages for diagnosing malignant pleural effusions based on the sources used. There is a noticeable lack of adequate studies examining their values in secondary malignant diseases of the pleura. According to the results we obtained from the Kolmogorov-Smirnov test, the triglyceride values in the examined patients with pleural effusions do not follow a normal distribution with p<0.001. On the other hand, Independent Samples tests show conflicting results, with the Equality of Variances test giving p=0.178, and the Equality of Means test showing p=0.315.

Additionally, when analyzing the Mann-Whitney U data, triglycerides show good statistical significance with p=0.013. Therefore, triglyceride values in pleural punctates should be interpreted cautiously and in the context of the overall analysis of the expanded biochemical panel.

Cholesterol

An extensive review of available literature shows that increased levels of cholesterol in pleural fluid are associated with an exudative nature of hydrothorax. In a study conducted by Shen et al.[37], pleural cholesterol was linked to high sensitivity (88%) and specificity (96%) in detecting malignant pleural effusions. On the other hand, Leers et al.[38] reports sensitivity, specificity, PPV, and NPV values for the method as 75.7%, 98%, 99.1%, and 59.2%, respectively. In an effort to determine reference values for cholesterol in pleural fluid, Guleria et al.[39] suggests an upper limit of 60 mg/dl, which, according to their study, provides a sensitivity of 88.2% and specificity of 100%. For comparison, in the same study, using Light's criteria, sensitivity of 98% and specificity of 80% were achieved.[39]

In addition, Hamm et al. demonstrates that malignant pleural effusions register significantly elevated levels of pleural cholesterol, independent of serum cholesterol with

an average of 94 mg/dl, followed by inflammatory pleural effusions (76 mg/dl) and non-malignant effusions (30 mg/dl). He also suggests an upper limit of 60 mg/dl, which differentiates exudative from transudative pleural effusions with only a 5% error.[40]

Comparing these data with our results, we can observe several intersections. From the Kolmogorov-Smirnov test, with p=0.009, we can conclude that the values do not follow a normal distribution. From the Equality of Variances test, there is an equal variation in the data as p=0.165. However, the Equality of Means test gives a difference between the means with p=0.011.

Given our results and the available information, we can conclude that pleural cholesterol is one of the best diagnostic tools to aid the diagnosis of malignant pleural effusions.

Glucose and pH

Several studies link about one-third of malignant pleural effusions with a pleural fluid pH<7.30 at the initial manifestation of hydrothorax.[41,42] This lowered pH is associated with glucose levels in the pleural effusion <60 mg/dL.[43] These results are connected to the degree of tumor invasion in the pleural space. Malignant pleural effusions with low pH and glucose are more often positive for tumor cells and are diagnosed already in the cytopathological examination.[44,45]

According to our data, the pH of the punctate has a non-normal distribution of the data in the Kolmogorov-Smirnov test with p<0.001. In the Independent Samples tests, particularly the Equality of Variances test, we get p=0.306, while the Equality of Means test gives p=−1.007.

Additionally, the Mann-Whitney U test shows that there is no statistically significant difference between the malignant and benign groups with p=0.117. Therefore, the acidity of pleural effusion in malignant pleural diseases is not as informative as, for example, in inflammatory diseases.

Regarding glucose values, we see that according to our results, there is a non-normal distribution of the obtained values, p<0.001 in the Kolmogorov-Smirnov test. Expanding this analysis, in the Independent Samples tests, the Equality of Variances test gives p=0.763, and the Equality of Means test gives p=0.874. The statistical significance assessment from the Mann-Whitney U test confirms the hypothesis that glucose does not have statistical significance in differentiating between malignant and benign pleural effusions with p=0.871.

Specific gravity

The study of specific gravity in pleural fluid to differentiate malignant from benign pleural effusions is sparsely studied in the available literature. However, there are studies that examine its potential use in distinguishing between exudative and transudative pleural effusions. A study by Abdollahi and Nozarian[46] shows that using an upper limit of 1022, measuring specific gravity can achieve sensitivity, specificity, PPV, and NPV values of 92.1%, 68.1%, 88.1%, and 78.3%, respectively, in differentiating exudates from transudates. They conducted a prospective cross-sectional study for two years, collecting 268 samples, 125 of which were pleural punctates and 143 peritoneal fluids. From the pleural group, based on Light's criteria, 61 cases were classified as exudates and 64 as transudates. Analyzing the sensitivity and specificity of relative weight in the two groups, he concluded that measuring specific gravity through refractometry is an acceptable method for differentiation between exudative and transudative pleural effusions.

According to our data, the values of relative weight in pleural punctates from the examined patients show a non-normal distribution according to the Kolmogorov-Smirnov test (p<0.001). This is also true in the Independent Samples t-test. Although the parameters show equal variation in the data in the Equality of Variances test p=0.391, the Equality of Means test gives p=0.005. When applying the Mann-Whitney U test, we see that p=0.004, indicating a statistically significant difference between the values in the two groups.

Lactate dehydrogenase (LDH)

Several studies highlight the importance of lactate dehydrogenase in the diagnosis of malignant pleural effusions and its significance in differentiating between exudative and transudative pleural effusions.[47]

According to Vergnon et al.[48], the increased amount of LDH isoenzyme LDH-5 is a good marker for detecting malignant pleural effusions, specifically hydrothorax caused by primary small-cell lung carcinoma and lymphoma. They also note that this isoenzyme and its values in pleural punctates are dependable for monitoring such patients. LDH values are also related to the prognosis for survival in patients with malignant pleural effusions. This is best studied in patients with secondary pleural involvement from lung adenocarcinoma, as shown by a retrospective cohort study by Verma et al.[49]

From the data obtained from the Kolmogorov-Smirnov test, we see that LDH does not demonstrate a normal distribution of the data with p<0.001. When applying the Independent Samples tests, it is evident that the parameters show equal variation in the data in the equality of variances test with p=0.801. In the Equality of Means test, p=0.345. The Mann-Whitney U test reveals a statistically significant difference for LDH between the malignant and benign groups with p=0.003.

As with the total protein levels in the punctate, we hypothesize that LDH levels are indicative of the severity of pleural damage in both inflammatory and malignant pleural effusions, with malignant effusions possibly being a predictor of the degree of pleural carcinomatosis and a poor prognostic factor.[49]

From all examined parameters of the extended panel of biochemical markers, the tests performed, and the correlations drawn, the most dependable remain the examination

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of total protein and albumin gradient, lactate dehydrogenase levels. However, the diagnostics can be enhanced by strengthening their application and including cholesterol levels, as well as investigating the total leukocyte count and their subpopulations.

Conclusions

The extended biochemical analysis of pleural punctate reveals that several parameters, including the albumin gradient, total protein, and cholesterol levels, are valuable for differentiating between malignant and benign pleural effusions. Among these, the albumin gradient and total protein stand out as key diagnostic markers, with high total protein levels associated with malignant effusions and the albumin gradient being useful for distinguishing exudative from transudative effusions.

Pleural cholesterol has shown high sensitivity and specificity in diagnosing malignant effusions and is recommended as an important diagnostic parameter. On the other hand, pH and glucose levels, although they correlate with other conditions, are less informative in diagnosing malignant pleural effusions.

While the specific gravity parameter remains underexplored, it holds potential for further use in distinguishing different types of effusions. Combining these biomarkers can enhance diagnostic accuracy, providing a more comprehensive approach to identifying pleural effusions.

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Competing Interests

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References

1. Piggott LM, Hayes C, Greene J, et al. Malignant pleural disease. Breathe (Sheff) 2023; 19(4):230145. doi: 10.1183/20734735.0145-2023
2. Gonnelli F, Hassan W, Bonifazi M, et al. Malignant pleural effusion: current understanding and therapeutic approach. Respir Res 2024; 25(1):47. doi: 10.1186/s12931-024-02684-7
3. Yamada SV. [On the serous fluid in the pleural cavity of healthy people]. Z Ges Exp Med 1933; 90:342–8 [German].
4. Sahn S. The value of pleural fluid analysis. Am J Med Sci 2008; 335:7–15
5. Light R, MacGregor M, Luschinger P, et al. Pleural effusion: the diagnostic separation of transudates and exudates. Ann Intern Med 1972; 77:507–13
6. Ewig S, Höffken G, Ker WV, et al. [S3 Guideline treatment of adult patients with outpatient pneumonia and prevention]. S3 Leitlinie Behandlung von erwachsenen Patienten mit ambulanter Pneumonie und Prävention. Update. 2016; 151–200 [German].
7. Mercer RM, Corcoran JP, Porcel JM, et al. Interpreting pleural fluid results. Clin Med (Lond) 2019; 19(3):213–7. doi: 10.7861/clinmedicine.19-3-213
8. Light RW, MacGregor MI, Ball WC Jr, et al. Diagnostic significance of pleural fluid pH and PCOz. Chest 1973; 64:591–6.
9. Potts DE, Willcox MA, Good JT Jr, et al. The acidosis of low glucose pleural effusions. Am Rev Respir Dis 1978; 117:665–71.
10. Moline J, Lavandfer M, Baudouin J, et al. [Inter & diagnostic of the measurement of PO* and PCOz and pH in pleurisy and ascites]. Inter & diagnostique de la mesure des PO* and PCOz et du pH darts les pleuresies et les ascites. Presse Med (Paris) 1970; 78:1512–3 [French].
11. Sahn SA, Potts DE. Turpentine pleurisy in rabbits: a model of pleural fluid acidosis. Am Rev Respir Dis 1977; 115:242–6.
12. Sahn SA, Taryle DA, Good JT. Experiment empyema: time course and pathogenesis of pleural fluid acidosis and low pleural fluid glucose. Am Rev Respir Dis 1979; 120:355–61
13. Sahn SA, Reller LB, Taryle DA, et al. The contribution of leukocytes and bacteria to the low pH of empyema fluid. Am Rev Respir Dis 1983; 128:811–5.
14. Falchuk KH, Goetzl EJ, Kulka JP. Respiratory gases of synovial fluids: an approach to synovial tissue circulatory-metabolic imbalance in rheumatoid arthritis. Am J Med 1970; 49(2):223–31.
15. Light RW, Luchsinger PC. Metabolic activity of pleural fluid. J Appl Physlol 1973; 34:97–101.
16. Momenin N, Colletti PM, Kaptein EM. Low pleural fluid-to-serum glucose gradient indicates pleuroperitoneal communication in peritoneal dialysis patients: presentation of two cases and a review of the literature. Nephrol Dial Transplant 2012; 27(3):1212–9. doi: 10.1093/ndt/gfr393
17. Karnovsky ML. Metabolic basis of phagocytic activity. Physiol Rev 1962; 42:143–68.
18. Houston MC. Pleural fluid pH: diagnostic, therapeutic, and prognostic value. Am J Surg 1987; 154(3):333–7. doi: 10.1016/0002-9610(89)90623-5
19. Rudrappa M, Paul M. Chylothorax. [Updated 2023 Feb 21]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459206/.
20. Heffner JE, Nietert PJ, Barbieri C. Pleural fluid pH as a predictor of pleurodesis failure: analysis of primary data. Chest 2000; 117(1):87–95. doi: 10.1378/chest.117.1.87
21. Tavana S, Tavakoli H, Hashemzadeh M, et al. Specific gravity of pleural fluid determined by refractometer to discriminate exudates and transudates. Res J Med Sci 3 2009: 3(3):91–4.
22. Sobhey KE, Naglaa BA. Diagnostic significance of pleural fluid pH and pCO2. Egypt J Chest Dis Tuberc 2015; 64(4):877–85. doi: 10.1016/j.ejcdt.2015.05.004
23. Good JT Jr, Taryle DA, Maulitz RM, et al. The diagnostic value of pleural fluid pH. Chest 1980; 78:55–9. doi: 10.1378/chest.78.1.55
24. Rodriguez-Panadero F, Lopez MJ. Low glucose and pH levels in malignant pleural effusions. Diagnostic significance and prognostic value in respect to pleurodesis. Am Rev Respir Dis 1989; 139:663–7.

doi: 10.1164/ajrccm/139.3.663
25. Potts DE, Levin DC, Sahn SA. Pleural fluid pH in parapneumonic effusions. Chest 1976; 70:328–31. doi: 10.1378/chest.70.3.328
26. Kouritas VK, Hatzoglou C, Foroulis CN, et al. Low glucose level and low pH alter the electrochemical function of human parietal pleura. Eur Respir J 2007; 30(2):354–7. doi: 10.1183/09031936.00047106
27. Fitzgerald DB, Leong SL, Budgeon CA, et al. Relationship of pleural fluid pH and glucose: a multi-center study of 2,971 cases. J Thorac Dis 2019; 11(1):123–30. doi: 10.21037/jtd.2018.12.101. Erratum in: J Thorac Dis. 2023 May 30;15(5):2885-2886. PMID: 30863580; PMCID: PMC6384369.
28. Barber LM, Mazzadi L, Deakins DD, et al. Glucose level in pleural fluid as a diagnostic aid. Dis Chest 1957; 31:680–7. doi: 10.1378/chest.31.6.680
29. Samanta S, Sharma A, Das B, et al. Significance of total protein, albumin, globulin, serum effusion albumin gradient and LDH in the differential diagnosis of pleural effusion secondary to tuberculosis and cancer. J Clin Diagn Res 2016; 10(8):BC14–8. doi: 10.7860/JCDR/2016/20652.8379
30. Drent M, Cobben NA, Henderson RF, et al. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J 1996; 9:1736–42. doi: 10.1183/09031936.96.09081736
31. Hamal AB, Yogi KN, Bam N, et al. Pleural fluid cholesterol in differentiating exudative and transudative pleural effusion. Pulm Med 2013; 2013:135036. doi: 10.1155/2013/135036
32. Ur Rehman K, Sivakumar P. Non-traumatic chylothorax: diagnostic and therapeutic strategies. Breathe (Sheff) 2022; 18(2):210163. doi: 10.1183/20734735.0163-2021
33. Hsieh WY, Chen MW, Ho HT, et al. Identification of differentially expressed proteins in human malignant pleural effusions. Eur Respir J 2006; 28(6):1178–85. doi: 10.1183/09031936.06.00135405
34. Ceyhan B, Celikel T. Comment on: Serum-effusion albumin gradient in separation of transudative and exudative pleural effusions. Chest 1994; 105(3):974–5.
35. Roth BJ, O'Meara TF, Cragun WH. The serum-effusion albumin gradient in the evaluation of pleural effusions. Chest 1990; 98(3):546–9. doi: 10.1378/chest.98.3.546
36. Sandeesha V, Ravi Kiran CV, Ushakiran P, et al. A comparative study of serum effusion albumin gradient and Light's criteria to differentiate exudative and transudative pleural effusion. J Family Med Prim Care 2020; 9(9):4847–52. doi: 10.4103/jfmpc.jfmpc_321_20
37. Shen Y, Zhu H, Wan C, et al. Can cholesterol be used to distinguish pleural exudates from transudates? evidence from a bivariate meta-analysis. BMC Pulm Med 2014; 14:61. doi: 10.1186/1471-2466-14-61
38. Leers MPG, Kleinveld HA, Scharnhorst V. Differentiating transudative from exudative pleural effusion: should we measure effusion cholesterol dehydrogenase? Clin Chem Lab Med 2007; 45(10):1332–8 doi: 10.1515/CCLM.2007.285
39. Guleria R, Agarwal SR, Sinha S, et al. Role of pleural fluid cholesterol in differentiating transudative from exudative pleural effusion. Natl Med J India 2003; 16(2):64-69.
40. Hamm H, Brohan U, Bohmer R, et al. Cholesterol in pleural effusions. A diagnostic aid. Chest 1987; 92(2):296–302. doi: 10.1378/chest.92.2.296
41. Sahn SA, Good JT Jr. Pleural fluid pH in malignant effusions. Diagnostic, prognostic, and therapeutic implications. Ann Intern Med 1988; 108(3):345–9. doi: 10.7326/0003-4819-108-3-345
42. Momenin N, Colletti PM, Kaptein EM. Low pleural fluid-to-serum glucose gradient indicates pleuroperitoneal communication in peritoneal dialysis patients: presentation of two cases and a review of the literature. Nephrol Dial Transplant 2012; 27(3):121.
43. Rodríguez-Panadero F, López Mejías J. Low glucose and pH levels in malignant pleural effusions. Diagnostic significance and prognostic value in respect to pleurodesis. Am Rev Respir Dis 1989; 139(3):663–7. doi: 10.1164/ajrccm/139.3.663
44. Sanchez-Armengol A, Rodriguez-Panadero F. Survival and talc pleurodesis in metastatic pleural carcinoma, revisited. Report of 125 cases. Chest 1993; 104(5):1482–5. doi: 10.1378/chest.104.5.1482
45. Roy B, Shak HJ, Lee YCG. Pleural fluid investigations for pleural infections. J Lab Precis Med 2021;6:12.
46. Abdollahi A, Nozarian Z. Diagnostic value of measurement specific gravity by refractometric and dipstick method in differentiation between transudate and exudate in pleural and peritoneal fluid. Iran J Pathol 2016; 11(4):363–9. PMID: 28855928.
47. Miloslav M, Statny B, Melinova L, et al. Diagnosis of pleural effusions: experience with clinical studies, 1986–1990. Chest 1995; 107:1598–603.
48. Vergnon JM, Guidollet J, Gateau O, et al. Lactic dehydrogenase isoenzyme electrophoretic patterns in the diagnosis of pleural effusion. Cancer 1984; 54:507–511.
49. Verma A, Phua CK, Sim WY, et al. Pleural LDH as a prognostic marker in adenocarcinoma lung with malignant pleural effusion. Medicine (Baltimore). 2016;95(26):e3996.