ORIGINAL RESEARCH

Assessment of diagnostic efficacy: High fluorescent cells combined with CEA, NSE, and Cyfra21-1 in Malignant Pleural Effusion Identification

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Abstract

Objective

To determine routine and biochemical parameters, as well as tumor markers, that are significantly different between malignant pleural effusion (MPE) and benign pleural effusion (BPE), and to evaluate the diagnostic efficacy of the combination of routine and biochemical parameters, along with carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), and cytokeratin 19 fragment (Cyfra21-1) measurements in pleural effusion for identifying MPE.

Methods

1,238 patients with pleural effusion from the First Affiliated Hospital of Xi'an Jiaotong University were recruited and categorized into two groups: MPE (n = 397) and BPE (n = 841). Biomarker levels were compared, and receiver operating characteristic (ROC) curves were performed on the statistically significant indicators to assess the diagnostic efficacy for MPE.

Results

High-Fluorescent cells (HFC), CEA, NSE, and Cyfra21-1 were significantly elevated in MPE ($P < 0.001$) and positively correlated with its presence ($P < 0.001$). The area under the curve (AUC), cutoff value, sensitivity, and specificity were: [0.726 (95% CI: 0.696-0.756), 17.5, 8.5%, 57.4%]; [0.894 (95% CI: 0.873-0.914), 5.78, 80.7%, 88.6%]; [0.703(95% CI: 0.672-0.735), 10.97, 59.3%, 71.9%] and [0.774 (95% CI: 0.746-0.802), 34.61, 74.7%, 67.9%], respectively. When focusing on multi-biomarker strategy, the combination of HFC and CEA offered the highest diagnostic efficiency (AUC: 0.868; 95% CI: 0.847-0.889), with a sensitivity of 88.4% and a specificity of 70.7%.

Conclusion

HFC, CEA, NSE, and Cyfra21-1 are valuable diagnostic markers for MPE, with optimal cutoff values of 17.5×10^6/L, 5.78 ng/mL, 10.97 ng/mL, and 34.61 ng/mL, respectively. The HFC+CEA combinations enhanced diagnostic sensitivity and clinical utility.

Keywords: Malignant pleural effusion,High-fluorescent cells, CEA, NSE, Cyfra21-1

Introduction

Malignant pleural effusion (MPE) represents a pathological condition characterized by abnormal accumulation of fluid in the pleural cavity. According to the British Thoracic Society, approximately 70 individuals per 100,000 globally suffer from MPE[1]. The onset of MPE is closely associated with pleural metastasis of various malignancies such as lung cancer, breast cancer, and lymphoma[2]. The presence of MPE often indicates advanced disease progression, significantly impacting patients' quality of life and prognosis[3]. Therefore, rapid and accurate diagnosis of MPE is crucial for formulating effective treatment plans and assessing outcomes.

In clinical practice, routine and biochemical analyses of pleural fluid are essential for evaluating the nature of pleural effusions. MPE typically exhibits elevated levels of total protein (TP) and lactate dehydrogenase (LDH), along with low glucose (GLU) levels, which are associated with increased pleural permeability and the high metabolic activity of tumor cells[4]. Additionally, cell classification counts are significant for determining the etiology of pleural effusions. Effusions dominated by neutrophils may suggest parapneumonic effusions, while lymphocyte-dominant effusions may be linked to cancer or tuberculosis[5]. High-Fluorescent cell counts (HFC) have shown high efficacy in the diagnosis of MPE[6,7].

Image-Guided Pleural Biopsy (IGPB) and Image-Guided Thoracoscopy (IGT), such as those guided by Computed Tomography (CT) or Ultrasound, offer high diagnostic accuracy, but still face challenges in early differentiation between benign pleural effusion (BPE) and MPE[8]. Thoracoscopy, despite having a diagnostic rate of up to 95%, is invasive[9] and may be associated with post-surgical complications[10]. Furthermore, the high requirements for personnel expertise and equipment impede their widespread

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application in primary healthcare institutions. In recent years, new biomarkers such as cell-free DNA[11], CD163+CD14+ macrophages[12], and the microbiota[13] have shown promise in diagnosing MPE. However, most of these biomarkers remain in the research phase and have not yet been widely adopted in clinical practice. In contrast, tumor marker detection in pleural effusion was known as a common and non-invasive method with high sensitivity and specificity for diagnosing MPE. Notably, carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), and cytokeratin 19 fragment (Cyfra21-1) are particularly commonly used in the diagnosis of lung cancer-related malignant effusions[14,15]. However, using these biomarkers alone may not provide sufficient diagnostic accuracy. A multi-biomarker strategy is likely to improve MPE diagnostic precision[16].

Despite the recognized value of the aforementioned indicators in assessing pleural effusion, a definitive biomarker for MPE remains elusive in routine clinical practice. Additionally, the absence of a standardized clinical decision threshold for these indicators poses a challenge. The objective of this study is to determine routine and biochemical parameters, as well as tumor markers, that are significantly different between MPE and BPE, and to evaluate the diagnostic efficacy of the combination of routine and biochemical parameters, along with CEA, NSE, and Cyfra21-1 measurements in pleural effusion for identifying MPE.

Methods

This was a retrospective study.A total of 1,238 patients with pleural effusion were enrolled at the First Affiliated Hospital of Xi'an Jiaotong University, spanning from January 2020 to November 2023. Based on pleural effusion cytology, these patients were divided into two groups: MPE group, comprising 397 patients with confirmed malignant cells, and BPE group, consisting of 841 patients without detectable malignant cells. Comprehensive assessments were conducted to compare routine tests, biochemical analyses, and tumor marker levels in pleural effusion between the two groups. Additionally, the diagnostic efficacy of both individual and combined utilization of significantly differing indicators between MPE and BPE was evaluated specifically for the diagnosis of MPE.This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University.

The inclusion criteria encompassed patients who completed the following diagnostic assessments of pleural effusion: 1) cytological examination, 2) concurrent routine and biochemical analyses, and 3) tumor marker profiling including CEA, NSE, and Cyfra21-1. The exclusion criteria comprised individuals with incomplete datasets for any of these mandatory diagnostic parameters or missing essential baseline demographic/clinical characteristics.

Sample Analysis

Routine Pleural Effusion Examination:Routine examination items encompassed total nucleated cells (TC), HFC, white blood cells (WBC), mononuclear cell count (MN) and its percentage (MN%), polymorphonuclear cell count (PMN) and its percentage (PMN%) and red blood cell count (RBC). Cell classification and counting were accurately performed using the SYSMEX XN9000 hematology analyzer.

Biochemical Testing of Pleural Effusion:Biochemical tests included TP, LDH, GLU, chloride (Cl), and adenosine deaminase (ADA). These were conducted using the Johnson & Johnson Vitros 5600 automated biochemical immunoassay analyzer and its accompanying reagents, with the ADA reagent kit provided by Ningbo Ruiyuan Co., Ltd.

Tumor Markers Detection:Tumor markers CEA, NSE, and Cyfra21-1 were detected using the Roche Cobas e801 automated chemiluminescence immunoassay analyzer and its accompanying reagents. This detection system exhibited high sensitivity and specificity, accurately reflecting the levels of tumor markers in patients.

The experimental results were derived from conditions characterized by optimal instrument performance, standardized operational procedures, and meticulous data recording.

Statistical Analysis

IBM SPSS 25.0 software was utilized for statistical analysis. Given the non-normal distribution of all continuous variables, the data were presented as median and interquartile range (IQR). The Mann-Whitney U test was employed to compare differences between groups, while chi-square tests were utilized for comparing proportions of categorical variables. For assessing the correlation between dichotomous variables and continuous variables, Pearson's correlation coefficient test was selected in this study. Receiver operating characteristic (ROC) curve was performed by GraphPad Prism 8.0 software. Statistical significance was set at $P < 0.05$, and all statistical tests were conducted as two-sided.

Results

General Characteristics

The median age of patients in the MPE group was 66years (57-72), comprising 218 males and 179 females. Lung cancer was the predominant primary disease, accounting for 71.5% (284 cases), with lung adenocarcinoma making up 88.4% (251 cases) of these. Other primary diseases included ovarian cancer, gastric cancer, breast cancer, colon cancer, mesothelioma, endometrial cancer and lymphoma. In the BPE group, the median age was 67 years (55-75), with 502 males and 339 females. This group primarily consisted of patients with lung-related conditions, such as lung cancer, pneumonia, and chronic obstructive pulmonary disease, along with other conditions, including pleurisy, cardiac insufficiency, decompensated cirrhosis and some other tumors. There were no significant differences in age ($Z = -0.430$, $P = 0.667$) or gender distribution ($\chi^2 = 2.311$, $P = 0.128$) between the two groups.

Comparison of Routine Parameters Between BPE and MPE

In the MPE group, the routine pleural effusion parameters, including TC [1378 (702-2253) vs. 1146 (429-2625); p=0.039], HFC [60 (20-162) vs. 13 (4-41); P <0.001], PMN [122.0 (58.0-361.0) vs. 92.0 (35.5-356.5); P =0.005], and RBC [9000 (2000-39000) vs. 5000 (2000-18500); P <0.001] were significantly higher than those in the BPE group. However, no significant differences were observed between the two groups for WBC, MN, MN%, PMN% or MN/PMN. (Table 1)

Comparison of Biochemical Indices Between BPE and MPE

In the MPE group, the biochemical indices of pleural fluid demonstrated significantly higher levels of TP [42.10 (36.50-46.70) vs. 37.50 (27.45-45.15); P <0.001] and LDH [304

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Table 1. Comparison of Routine Parameters Between BPE and MPE

	BPE group（n = 841）	MPE group（n = 397）	Z	P
TC (×10⁶/L)	1146 (429-2625)	1378 (702-2253)	-2.068	0.039
WBC (×10⁶/L)	1096 (407.5-2572)	1212 (620-2029)	-0.916	0.360
HFC (×10⁶/L)	13 (4-41)	60 (20-162)	-12.795	<0.001
MN (×10⁶/L)	749.0 (286.0-1949.5)	944.0 (500.0-1564.0)	-1.898	0.058
MN% (%)	88.20 (66.65-94.85)	87.50 (72.10-93.30)	-0.748	0.454
PMN (×10⁶/L)	92.0 (35.5-356.5)	122.0 (58.0-361.0)	-2.825	0.005
PMN% (%)	11.80 (5.15-33.35)	12.50 (6.70-28.00)	-0.959	0.338
MN/PMN	7.43 (2.00-18.48)	6.98 (2.57-13.88)	-0.921	0.357
RBC (×10⁶/L)	5000 (2000-18500)	9000 (2000-39000)	-4.536	<0.001

Table 2. Comparison of Biochemical Indices Between BPE and MPE

	BPE group (n = 841)	MPE group (n = 397)	Z	P
TP (g/L)	37.50 (27.45-45.15)	42.10 (36.50-46.70)	-6.988	<0.001
LDH (U/L)	211 (120-417)	304 (183-652)	-6.45	<0.001
GLU (mmol/L)	6.33 (5.21-7.87)	5.81 (4.80-7.15)	-3.666	<0.001
Cl (mmol/L)	104.3 (100.3-107.5)	104.7 (101.4-107.1)	-0.969	0.332
ADA (U/L)	9 (5-20)	9 (6-13)	-1.494	0.135

Table 3. Levels of CEA, NSE and Cyfra21-1 in BPE and MPE

	BPE group (n = 841)	MPE group (n = 397)	Z	P
CEA (ng/mL)	1.23 (0.70-2.27)	90.00 (10.50-613.00)	-22.259	<0.001
NSE (ng/mL)	5.67 (2.97-12.24)	13.76 (5.45-40.21)	-11.487	<0.001
Cyfra21-1 (ng/mL)	19.73 (9.39-48.12)	93.00 (32.59-346.20)	-15.454	<0.001

Table 4. Diagnostic Efficacy of HFC, CEA, NSE and Cyfra21-1 for MPE

	AUC (95% CI)	Cutoff value	Sensitivity	Specificity
HFC (×10⁶/L)	0.726 (95% Cl:0.696-0.756)	17.5	78.5%	57.4%
CEA (ng/mL)	0.894 (95% Cl:0.873-0.914)	5.78	80.7%	88.6%
NSE (ng/mL)	0.703 (95% Cl:0.672-0.735)	10.97	59.3%	71.9%
Cyfra21-1 (ng/mL)	0.774 (95% Cl:0.746-0.802)	34.61	74.7%	67.9%
HFC+CEA	0.868 (95% CI: 0.847-0.889)	0.249	88.4%	70.7%

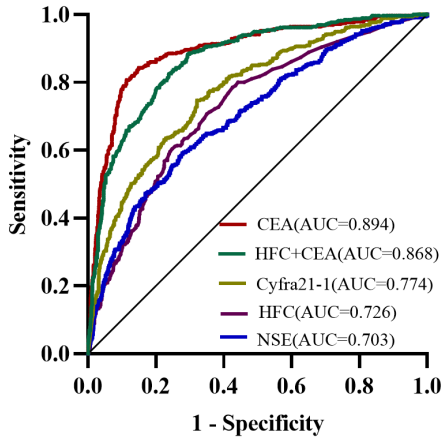

Figure 1. ROC of HFC, CEA, NSE, Cyfra21-1, and HFC+CEA in distinguishing MPE from BPE. HFC: High-fluorescent cell counts, CEA: carcinoembryonic antigen, NSE: neuron-specific enolase, Cyfra21-1: cytokeratin 19 fragment, HFC+CEA: combination of HFC and CEA, AUC: Area Under the Curve

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(183-652) vs. 211 (120-417); P <0.001] compared to BPE group.

Conversely, the GLU levels were significantly lower in the MPE group than in the BPE group [5.81 (4.80-7.15) vs. 6.33 (5.21-7.87); P <0.001]. While Cl and ADA showed no significant difference between the two groups. (Table 2)

Comparison of CEA, NSE, and Cyfra21-1 between BPE and MPE

Compared to the BPE group, significantly elevated levels of CEA [90.00 (10.50-613.00) vs. 1.23 (0.70-2.27); P < 0.001], NSE [13.76 (5.45-40.21) vs. 5.67 (2.97-12.24); P < 0.001], and Cyfra21-1 [93.00 (32.59-346.20) vs. 19.73 (9.39-48.12); P < 0.001] were observed in the MPE group. (Table 3)

Correlation and Diagnostic performance of Significant Indicators between Groups for MPE

We analyzed the correlations between MPE and the indicators which showed significant differences between the BPE and MPE groups. Significant positive correlations were observed between MPE and the following indicators: HFC (r = 0.157, P < 0.001), TP (r = 0.200, P < 0.001), CEA (r = 0.219, P < 0.001), NSE (r = 0.234, P < 0.001), and Cyfra21-1 (r = 0.375, P < 0.001). In contrast, GLU exhibited a significant negative correlation with MPE (r = -0.077, P = 0.007).

To assess the diagnostic efficacy of these significantly correlated indicators for MPE, we conducted ROC curve analysis. The results demonstrated that HFC, CEA, NSE, and Cyfra21-1 exhibited high diagnostic efficacy for MPE, with high areas under the ROC curve (AUC): 0.726 (95% CI: 0.696-0.756), 0.894 (95% CI: 0.873-0.914), 0.703 (95% CI: 0.672-0.735), and 0.774 (95% CI: 0.746-0.802), respectively. The AUC of TP and GLU were lower than 0.7(data not shown). The corresponding cutoff values, sensitivity, and specificity for these indicators were as follows: (17.5, 78.5%, 57.4%) for HFC; (5.78, 80.7%, 88.6%) for CEA; (10.97, 59.3%, 71.9%) for NSE; and (34.61, 74.7%, 67.9%) for Cyfra21-1.Combining HFC and CEA demonstrated the highest diagnostic efficiency when performing ROC curve analysis for combined diagnosis among the four indicators. The combined analysis achieved an AUC of 0.868 (95% CI: 0.847-0.889), with a cutoff value of 0.249, sensitivity of 88.4%, and specificity of 70.7%. (Table 4, Figure 1)

Discussion

This study employed a large sample size and used pleural effusion cytology as the gold standard for diagnosis. It systematically assessed the diagnostic value of routine parameters, biochemical markers, and tumor markers in pleural effusion for differentiating MPE. Significant differences in various indicators, particularly HFC, CEA, NSE, and Cyfra21-1, were observed between the two groups. The introduction of HFC as a novel indicator offers a promising approach for distinguishing BPE from MPE.

MN primarily consist of lymphocytes and monocytes, while PMN are mainly composed of neutrophils and eosinophils. In this study, the levels of TC, PMN and RBC were significantly higher in the MPE group compared to the BPE group, reflecting inflammation induced by malignant tumor growth. HFC, which encompasses tumor cells, histiocytes, and mesothelial cells, exhibited a robust correlation with MPE (P < 0.001), yielding an AUC of 0.772 (95% CI: 0.695-0.855). These findings align with previous research[6,17]. Notably, the sensitivity and specificity of diagnostic tests are highly dependent on threshold selection[18,19]. For instance, extremely high thresholds for tumor markers such as CEA or CA 15-3 can achieve 100% specificity for MPE[20,21]. Furthermore, we identified an optimal cutoff value for HFC in MPE at 17.5 × 106/L. As a screening test, HFC demonstrated a sensitivity of 78.5%, providing timely and valuable guidance for the clinical diagnosis and management of pleural effusions.

This study demonstrates that TP and LDH levels were significantly higher, while GLU levels were significantly lower in the MPE group compared to the BPE group. Notably, TP and GLU showed significant correlations with MPE, whereas the correlation with LDH was less prominent. Although these indicators exhibited limited diagnostic efficacy for MPE (AUC < 0.7), they may hold prognostic value for MPE patients. A retrospective study reported that MPE patients with elevated LDH or reduced TP levels in pleural effusions had shorter survival durations[22].

CEA, NSE and Cyfra21-1 were recommended by the National Academy of Clinical Biochemistry (NACB) and the European Group on Tumor Markers (EGTM) for broad clinical use as indicators of primary lung cancer[23]. Similarly, CEA, NSE and Cyfra21-1 have demonstrated significant diagnostic value for lung cancer-related MPE[24-27]. In this study, the levels of CEA, NSE, and Cyfra21-1 were significantly elevated in the MPE group compared to BPE group, with strong positive correlations to MPE (P < 0.001). ROC curve analysis highlighted the superior diagnostic performance of CEA (AUC: 0.894; 95% CI: 0.873-0.914), achieving 80.7% sensitivity and 88.6% specificity at a 5.78 ng/mL cutoff. NSE and Cyfra21-1 also demonstrated substantial diagnostic value, with AUC of 0.703 and 0.774, and cutoff values of 10.97 ng/mL and 34.61 ng/mL, respectively. NSE exhibited higher specificity, while Cyfra21-1 provided greater sensitivity, suggesting that combining these markers could improve diagnostic precision. The strong performance of these biomarkers likely reflects the predominance of lung cancer, particularly lung adenocarcinoma, in the MPE group.

When focusing on multi-biomarker strategy, we identified that the combination of HFC and CEA offered the highest diagnostic efficiency (AUC: 0.868; 95% CI: 0.847-0.889), with a sensitivity of 88.4% and a specificity of 70.7%. Although the AUC was marginally lower compared to CEA alone, the combined approach significantly improved sensitivity, underscoring its potential value in enhancing diagnostic accuracy for MPE.

This study highlights the utility of multiple indicators in distinguishing MPE from BPE, though certain limitations should be noted. As a single-center retrospective analysis, the findings are subject to selection bias, potentially limiting their generalizability. Moreover, the heterogeneity of primary diseases contributing to pleural effusions, including a significant proportion of lung cancer patients in the BPE group, may have influenced diagnostic accuracy. Despite these challenges, HFC, CEA, NSE, and Cyfra21-1 demonstrated substantial clinical value. Future multicenter prospective studies are needed to validate these findings and explore tumor markers tailored to specific malignancies, aiming to enhance diagnostic precision, facilitate early detection, and support personalized treatment strategies.

Conclusion

Despite the proven effectiveness of routine pleural effusion parameters and tumor markers in diagnosing MPE, a

unified threshold standard remains undefined. This study, leveraging a large sample size and comprehensive ROC curve analysis, identified HFC, CEA, NSE, Cyfra21-1 and combined HFC+CEA as robust diagnostic indicators, offering optimized cutoff values to support clinical practice. By leveraging existing medical resources, these findings not only improve diagnostic accuracy but also yield favorable socioeconomic outcomes.

Statements and Declarations

Author Contributions

Ning Zhang and Xiaoqin Wang contributed to the conception and design of the study. Ning Zhang and Thomas Stuart Mughogho contributed to the statistical analyses and manuscript preparation. Aubrey Nothale, Rashid Kaseka and Zhonglin Wang participated in data collection. Dokani Michael Ndovi,Jie Zheng and Jian Hu reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

All procedures performed in our studies involving human participants were following the ethical standards of the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (approval number: XJTU1AF2018LSK-228). Due to the retrospective nature of the study, the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University waived the need of obtaining informed consent.

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