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ORIGINAL RESEARCH

Innovative Biomarkers for Diagnosing Malignant Ascites in Liver Cancer

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Background: Liver cancer ranks among the most prevalent and lethal malignancies worldwide, with metastatic malignant ascites being a common complication. This study seeks to assess the diagnostic significance of high fluorescent cells (HFCs), biochemical and tumor markers in predicting the development of metastatic malignant ascites in patients with liver cancer.

Methods: We collected ascites samples from 266 patients diagnosed with liver cancer. HFC were analyzed using the BF mode of the BC-7500 hematology analyzer, assessing both relative counts (HF-BF%) and absolute counts (HF-BF#). Additionally, biochemical and tumor markers were evaluated in serum and ascites. The diagnostic accuracy of these indicators, both individually and in combination, was assessed using receiver operating characteristic (ROC) curve analysis.

Results: The malignant ascites group exhibited significantly higher levels of HF-BF%, cancer ratio 2 (Ratio2, ascites LDH: ascites ADA Ratio), and neuron-specific enolase (NSE) compared to the benign group, identifying these markers as independent risk factors for malignant ascites in liver cancer patients. Ratio2 demonstrated limited diagnostic value for malignant ascites, with an area under the curve (AUC) of 0.614. In contrast, HF-BF% and NSE showed moderate diagnostic capabilities, with AUCs of 0.760 and 0.700, respectively. The combined assessment of all three indicators yielded a high diagnostic capability, with an AUC of 0.824. The critical values for NSE, HF-BF%, and Ratio2 were 11.42 U/mL, 4.35/100 WBC, and 32.82%, respectively.

Conclusion: The combined evaluation of HF-BF%, Ratio2, and NSE serves as a valuable indicator for predicting the occurrence of metastatic malignant ascites in liver cancer patients.

Keywords: liver cancer, malignant ascites, high fluorescent cells, ascites LDH: ascites ADA ratio, NSE

Introduction

Liver cancer is the most prevalent fatal malignancy, with an increasing annual incidence. Recurrence and metastasis are widely recognized as the most critical factors affecting the survival and prognosis of liver cancer patients.[1,2] Most advanced liver cancer patients develop ascites, and the presence of metastatic malignant ascites indicates disease progression and is associated with a poor survival rate. Studies indicate that the median survival for patients with positive tumor cytology in peritoneal effusion is merely 26 days, often accompanied by complications such as tumor emboli and pulmonary edema.[1] Therefore, accurate and rapid differentiation of the nature of ascites, as well as the ability to distinguish tumor cells from other cell types, is vital for patient treatment and prognosis, providing essential support for clinical decision-making and research. Pathological biopsy results remain the gold standard for diagnosing metastatic tumors in the abdomen; however, their sensitivity and specificity are relatively low, ranging from 40–70%.[3–6] Cytological examination of ascites serves as a low-cost, non-invasive primary screening method for diagnosing abdominal metastatic tumors. Nevertheless, due to its manual nature, this method has limited sensitivity, is time-consuming, and requires a high level of expertise from the operator, leading to criticism. Ascites is among the serous fluids with a high detection rate of tumor cells, yet the diagnostic accuracy of single tumor markers falls short of expectations. In this study, we employed the BF mode of the BC-7500 automated hematology

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analyzer to count high fluorescent cells (HFCs) in ascites samples from liver cancer patients. We combined HFCs with the assessment of serum and ascitic tumor markers to explore the diagnostic value of these indicators in distinguishing between benign and malignant ascites, aiming to enhance the accuracy, specificity, and sensitivity of diagnosing metastatic tumors in the abdominal cavity.

Methods

Study Design and Patient Selection

We performed a retrospective study of hospitalized liver cancer patients with ascites in Hangzhou Xixi Hospital, which serves as Hangzhou Liver Disease Research Institute, Hangzhou Liver Disease Diagnosis and Treatment Center. We analyzed the clinical parameters of 266 cases from January 2022 to December 2024. The following inclusion criteria were used: (a) patients with new onset ascites; (b) patients who had received diagnostic paracentesis and received serum and ascitic tumor marker assay; (c) patients over 18 years old. Out of 295 patients with new onset ascites, 29 were excluded due to incomplete data, HIV infection, age under 18 years, bloody ascites caused by trauma or surgery, and other highly fluorescent cells (macrophages or mesothelial cells). Finally, patient characteristics and all required information were extracted from 266 medical records and included in the analysis (Figure 1).Among the participants, 200 were male and 66 were female. Based on pathology or the morphology of ascitic cells, 61 cases were classified as malignant ascites, while 205 were deemed benign. The diagnosis of malignant ascites was established according to the following criteria: (a) confirmation of malignant tumors through surgical tissue pathological biopsy; (b) validation of malignant tumors via other imaging examinations and follow-up assessments; and (c) detection of malignant tumor cells in the exfoliated cytology of the ascites.

Given the differences in biological behavior and clinical manifestations between hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), it is potentially valuable to distinguish between the two for subgroup analysis. However, since this study primarily examined risk factors for malignant ascites specifically related to liver cancer, and the proportion of ICC cases within the 61 malignant ascites patients was low (n=6), conducting a subgroup analysis with sufficient statistical power was not feasible. Therefore, the analysis was performed on the entire cohort of liver cancer patients to identify common risk factors associated with the development of malignant ascites.

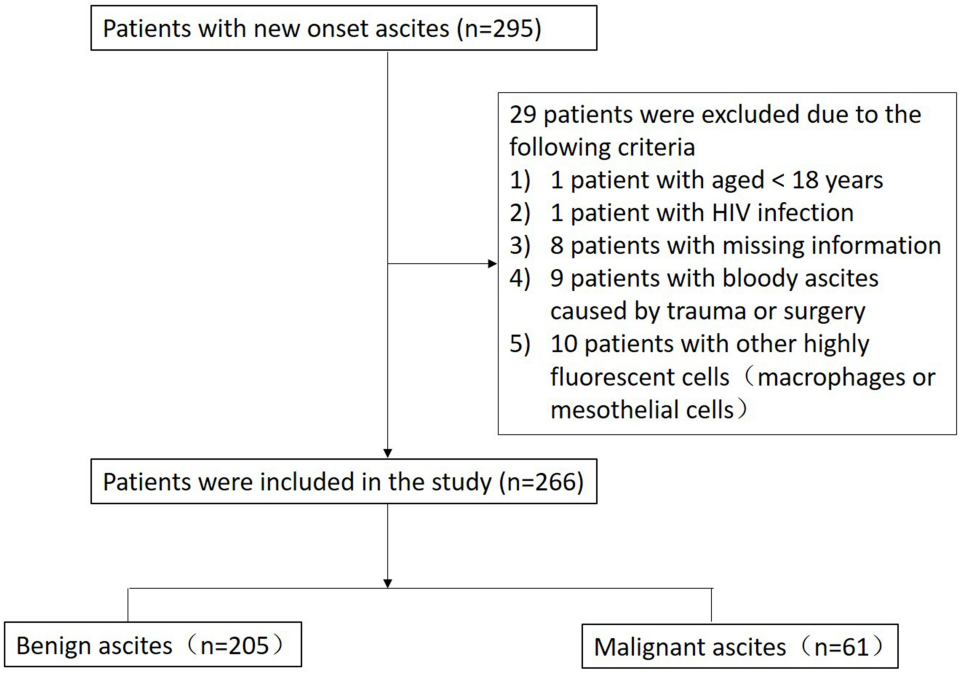

Figure 1 Flowchart of the patients with new onset ascites enrollment.

All ascites samples were collected in K2-ethylenediaminetetraacetic acid (EDTA) tubes. The study protocol received approval from the Medical Ethics Committee of Hangzhou Xixi Hospital. All samples were initially analyzed using the Mindray BC-7500 in BF mode. This body fluid mode (BF mode) provides measurements for red blood cells (RBC-BF) and white blood cells (WBC-BF). Additionally, two key parameters can be obtained in this mode: the percentage of high fluorescent cells (HF-BF%, expressed as /100 WBC) and the absolute counts of high fluorescent cells (HF-BF#, expressed as $\times 10^6$/L). Ascites samples were centrifuged at 3000 g for 10 minutes. The biochemical indices in serum and ascites (ADA, LDH, and GLU) and tumor markers (CEA, AFP, CA199, Fer, CA125, CA153, SCC Ag, cytokeratin 19 fragment (CYFRA21-1), NSE, CA724, CA242, and CA50 were measured using a fully automated chemical analyzer in a clinical laboratory. Ratio 1 is the ratio of serum LDH to ascites ADA, Ratio 2 is the ratio of ascites LDH to ascites ADA. All procedures adhered to the manufacturer's instructions and the laboratory's standard operating procedures (SOPs).

After the Mindray BC-7500 BF analysis, all samples underwent morphological examination (ME) in the hematology laboratories. For ME, two cytospin slides per body fluid sample were prepared using a cytocentrifuge (Junan Centrifugal Smear Machine, 1,500 rpm for 5 minutes), followed by May Grunwald-Giemsa staining (BASO, China). All slides were examined for the presence of malignant cells (MC) by at least two skilled laboratory professionals, who were blinded to the clinical information, screening a minimum of 100 cells using ×10 and ×50 oil immersion objective lenses. Each sample was specifically assessed for MC presence. The cytological features of non-hematopoietic MC were characterized by large size, multinuclearity, a high nuclear-to-cytoplasmic ratio, variable nuclear shape and size, irregular and smooth nuclear contours, prominent nucleoli, nuclear molding, cannibalism, or the presence of tridimensional clusters.[7,8] Pathological diagnosis was performed using Papanicolaou stained slides and was confirmed by a senior clinical pathologist.

Statistical Analysis

All data were analyzed using SPSS 26.0; both investigators independently entered the data twice and compared the data later. The data were assessed for normal distribution. Since the data in this study did not conform to normal distribution, they were expressed as median (interquartile range) [M (P25, P75)]. The nonparametric Mann–Whitney *U*-test was utilized for comparisons between the two groups. Binary logistic regression analysis was conducted to evaluate the risk factors for the presence of malignant ascites, with variables showing $P < 0.05$ included in the multivariate analysis to calculate the odds ratio (OR) and the corresponding 95% confidence interval (CI). Additionally, receiver operating characteristic (ROC) curve analysis was performed to determine the optimal diagnostic threshold (cutoff value) for NSE, HF-BF%, and Ratio2, with the area under the curve (AUC) calculated. A threshold of $P < 0.05$ indicates statistical significance (two-sided).

Results

Demographic Data and Clinical Characters

Among the 266 patients with ascites, there were 200 males and 66 females, aged between 22 and 86 years, with an average age of 61 years. A total of 61 cases were confirmed as malignant ascites through pathology, cytology, or postoperative peritoneal biopsy. The rate of metastatic malignant ascites in patients with liver cancer was 22.93% (61/266). Overall, 75.19% of the patients were male, with an average age of 61.00 years (interquartile range: 54.00 to 69.75). There was no statistically significant difference in age and gender between the two groups of patients.

Laboratory Features

Comparison of HF-BF# (absolute high-fluorescent cell count), HF-BF% (relative high-fluorescent cell count), and the levels of serum and ascitic tumor markers, as well as biochemical indices, in benign versus malignant ascites.

We conducted a comprehensive analysis of 25 laboratory parameters in patients from the two groups and compared their median levels. The levels of HF-BF#, HF-BF%, CEA, AFP, CA199, Fer, CYFRA21-1, NSE, CA724, CA242, CA50, LDH, aLDH, aCA199, aCEA, aAFP, and Ratio2 in the malignant group were significantly elevated compared to those in the benign group((P<0.001, <0.001, <0.001, 0.045, 0.032, 0.002, 0.007, <0.001, 0.006, <0.001, 0.023, 0.028, 0.009, <0.001, <0.001, 0.009, and 0.024, respectively). Conversely, there was no significant difference in WBC-BF, RBC-BF, CA125, CA153, SCC Ag, aADA, aGLU and Ratio1 between the two groups of patients (Table 1).

Table 1 Comparison of Clinical Parameters Between Malignant Group and Benign Group

Variable	Total (n=266)	Benign Group (n=205)	Malignant Group (n=61)	*P*-Value
Male, n (%)	200(75.19)	155(75.61)	45(73.77)	0.770*
Age (years)	61.00(54.00,69.75)	61.00 (54.00, 69.00)	62.00 (53.00, 70.00)	0.907
WBC-BF (10^9/L)	0.40 (0.20, 1.22)	0.39 (0.18, 1.54)	0.46 (0.24, 0.81)	0.785
RBC-BF (10^9/L)	2.00 (1.00, 12.00)	2.00 (1.00, 13.00)	2.00 (1.00, 9.00)	0.595
HF-BF# (10^6/L)	12.00 (5.00, 31.00)	10.00 (4.00, 22.00)	30.00 (8.00, 74.25)	<0.001
HF-BF%(/100WBC)	3.40 (1.35, 6.80)	2.90(0.95,5.80)	6.50 (2.68, 12.35)	<0.001
CEA (ug/L)	3.27 (1.85, 5.98)	2.96 (1.70, 4.78)	4.88 (2.84, 14.43)	<0.001
AFP (ug/L)	3.67 (2.27, 30.30)	3.59 (2.07, 21.12)	4.25(2.56,228.12)	0.045
CA199 (kU/L)	26.45(10.88,73.12)	24.90 (10.80, 61.60)	31.10(14.10,488.70)	0.032
Fer (ug/L)	152.20(47.80,478.00)	122.75 (39.38, 434.47)	338.00 (84.90, 598.60)	0.002
CA125 (kU/L)	299.50 (111.45, 615.45)	293.35 (99.37, 585.43)	312.80(153.30,617.10)	0.268
CA153 (kU/L)	9.35(6.32,15.47)	8.80 (6.40, 14.40)	12.5(5.60,23.40)	0.202
SCC Ag (ng/mL)	0.74 (0.54, 1.17)	0.74 (0.55, 1.19)	0.74 (0.53, 1.14)	0.937
CYFRA21-1 (ng/mL)	4.27 (2.94, 7.24)	4.07 (2.85, 5.99)	4.98 (3.56, 13.03)	0.007
NSE (U/mL)	11.32 (9.06, 14.62)	10.55(8.79,13.96)	13.73 (10.71,16.45))	<0.001
CA724 (U/mL)	1.62(0.61,7.16)	1.28(0.56,5.71)	3.27(0.90,12.49)	0.006
CA242 (U/mL)	3.84(1.90,10.17)	3.11(1.72,7.38)	6.46 (2.53, 152.44)	<0.001
CA50 (U/mL)	21.37 (9.87, 63.27)	20.79(9.32,43.20)	33.85 (11.64,170.10)	0.023
LDH (U/L)	230.00(178.50,308.25)	217.00 (172.00, 296.75)	259.50 (204.50,345.50)	0.028
aLDH (U/L)	87.00(54.00,221.00)	77.00(52.00,195.00)	112.50(75.75,243.50)	0.009
aADA (U/L)	3.60(2.40,6.00)	3.60 (2.50, 5.60)	3.85 (2.38, 6.42)	0.618
aGLU (mmol/L)	8.37(6.83,10.37)	8.48(6.86,10.37)	8.12(6.39,10.01)	0.514
aCA199 (U/mL)	5.00 (2.05, 18.55)	4.30 (1.90, 12.95)	17.30 (3.35, 406.90)	<0.001
aCEA (ng/mL)	0.73 (0.33, 1.72)	0.61 (0.30, 1.20)	1.44 (0.66, 38.02)	<0.001
aAFP (ng/mL)	1.19 (0.66, 4.04)	1.09 (0.56, 3.83)	1.89 (0.97,15.79)	0.009
Ratio1 (%)	60.57(30.86,118.30)	58.52(28.28,115.36)	65.76(34.85,129.29)	0.496
Ratio2 (%)	26.18(18.50,44.29)	24.07(17.14,41.78)	32.93(20.58,56.04)	0.024

Notes: *Continuous correction chi-square test.

Abbreviations: HF-BF#, absolute high-fluorescent cell count; HF-BF%, relative high-fluorescent cell count; SCC Ag, squamous cell carcinoma antigen; CYFRA21-1, cytokeratin 19 fragment; NSE, neuron-specific enolase; LDH, lactate dehydrogenase; aLDH, ascetic LDH; aADA, ascetic ADA; aGLU, ascetic GLU; aCA199, ascetic CA199; aCEA, ascetic CEA; aAFP, ascetic AFP; Ratio1, LDH/aADA; Ratio2, aLDH/aADA.

Risk Factors for Prediction of Malignant Ascites

Through binary logistic regression analysis, significant laboratory tests identified in the univariate analysis were included as independent variables in a forward step-wise multivariate analysis. The odds ratio (OR) values and corresponding 95% confidence intervals (CI) were calculated. The results indicated that NSE (OR = 1.066, 95% CI: 1.002–1.135; P=0.004), HF-BF% (OR = 1.087, 95% CI: 1.015–1.164; P=0.017), and Ratio2 (OR = 1.031, 95% CI: 1.003–1.060; P=0.028) emerged as independent risk factors for malignant ascites in patients with liver cancer (Table 2). Among these parameters, HF-BF% exhibited the best performance in predicting mortality. The predictive probability model for death in these patients was determined as logit (P) = −3.143 + 0.064 * NSE + 0.083* HF-BF% + 0.031 * Ratio2.

ROC Curve Analysis of NSE, HF - BF% and Ratio 2 Levels

In conjunction with the clinical diagnostic results of the enrolled patients, levels of NSE, HF-BF%, and Ratio2 were assessed individually and collectively, followed by ROC curve analysis. HF-BF% and NSE demonstrated moderate diagnostic values for malignant ascites (AUCs =0.760 and 0.700, respectively), while HF-BF% exhibiting higher specificity (0.889) in diagnosing malignant ascites. In contrast, Ratio2 showed a lower diagnostic value for malignant ascites, with an AUC of 0.614. Based on the ROC curve analysis, optimal thresholds (cutoff values) were established for diagnosing malignant ascites, yielding NSE at 11.42 U/mL, HF-BF% at 4.35%, and Ratio2 at 32.82. The combined assessment of NSE, HF-BF%, and Ratio2 significantly enhanced clinical diagnostic efficiency, achieving an AUC of 0.824, as presented in Table 3 and Figure 2.

Table 2 Logistic Regression Analysis of Risk Factors for Prediction of Malignant Ascites

Variable	B	Standard Error	Waldχ2	*P*-Value	Odds Ratio	(95% CL)
NSE	0.064	0.032	4.070	0.004	1.066	(1.002~1.135)
HF-BF%	0.083	0.035	5.694	0.017	1.087	(1.015~1.164)
Ratio2	0.031	0.014	4.802	0.028	1.031	(1.003~1.060)
Constant	−3.143	0.681	21.314	0.000		

Table 3 ROC Curve Analysis of NSE, HF-BF%, and Ratio 2 in the Prediction of Malignant Ascites

Variable	AUC	Standard error	*P*-value	95% (CL)	Sensitivity	Specificity	Cutoff value	Youden index
NSE	0.700	0.051	0.000	0.600~0.800	0.705	0.667	11.42	0.371
HFBF%	0.760	0.045	0.000	0.672~0.847	0.636	0.889	4.35	0.403
Ratio2	0.614	0.051	0.033	0.515~0.713	0.545	0.667	32.82	0.212
Prediction probability P	0.824	0.041	0.000	0.743~0.904	0.727	0.867	0.37	0.594

Discussion

Malignant ascites is a prevalent complication among patients with liver cancer, indicating an advanced stage of the disease and correlating closely with poor prognosis.[9] Consequently, the early and precise diagnosis of malignant ascites is crucial for guiding clinical treatment and enhancing patient outcomes. However, distinguishing between benign and malignant ascites poses a significant challenge in clinical practice due to the high similarity in their clinical manifestations and fluid characteristics, coupled with the low positive rates of traditional cytology and histopathology. In this context, this study developed a novel combined diagnostic model that integrates parameters from blood cell analyzers

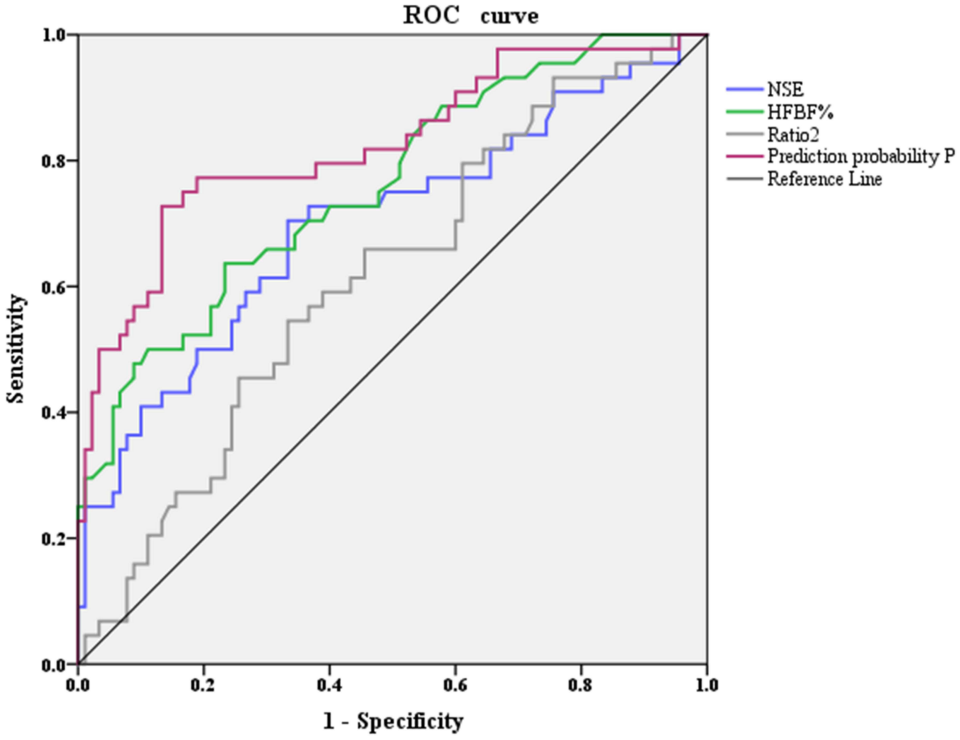

Figure 2 The ROC curve of NSE, HF - BF% and Ratio 2 levels.

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(HF-BF%/HF-BF#) with a multi-group of serum and ascitic biomarkers (NSE/LDH/ADA), offering an innovative approach to address this pressing clinical issue.

The Mindray's blood cell analyzer utilizes 3D fluorescence technology in its body fluid mode for high-fluorescence cell detection. This approach offers advantages of rapid processing, high automation, and low cost. However, it exhibits reduced sensitivity for low-concentration samples and lacks precise cell typing capabilities. Flow cytometry provides highly specific cell typing, but at a significantly higher cost. Manual microscopy remains the gold standard for morphological diagnosis, yet its accuracy is compromised by variability in staining quality, susceptibility to subjective bias, and poor reproducibility. Taking the above into consideration, we used the body fluid mode of Mindray's blood cell analyzer to screen and detect tumor cells. However, the presence of highly fluorescent cells, such as macrophages or mesothelial cells, in the peritoneal fluid can interfere with test results.[10] To mitigate this issue, we excluded these confounding cases through microscopic examination, thereby significantly enhancing the diagnostic utility of HF-BF% and HF-BF# values for malignant peritoneal effusion. Our findings revealed that the absolute count and relative percentage of highly fluorescent cells in malignant peritoneal effusion were markedly elevated compared to the benign control group, with this difference being statistically significant. Nonetheless, it is important to emphasize that while HF-BF% demonstrates high specificity when used alone for diagnosing malignant peritoneal effusion, its sensitivity is inadequate, aligning with previous research findings.[10,11] Consequently, HF-BF% is more appropriately utilized as part of a combined diagnostic model rather than as a standalone diagnostic indicator.

Neuron-specific enolase (NSE) serves as a specific marker for neurons and peripheral neuroendocrine cells, typically found only in certain tissues. However, in malignant tumors such as small cell lung cancer and neuroblastoma, NSE levels are significantly elevated and have been extensively utilized for diagnosis, prognosis assessment, and treatment monitoring.[12] In this study, we observed that NSE levels were also markedly increased in patients with malignant ascites; however, its diagnostic capability for malignant ascites when considered alone was moderate, with an area under the curve (AUC) of 0.700. This finding indicates that while NSE has limited diagnostic value for malignant ascites in liver cancer patients, it still holds potential for application. In the field of liver cancer, its limitations as an independent biomarker may be related to the following mechanisms. First, it is affected by tumor heterogeneity. Liver cancer has a high degree of molecular heterogeneity, and NSE mainly reflects the characteristics of neuroendocrine differentiation. Secondly, the formation of malignant ascites is accompanied by a complex inflammatory response, in which macrophages, necrotic cells, etc. can release non-tumor-derived NSE. Notably, related studies suggest that NSE, when integrated into a combined diagnostic model, can predict the early onset of liver cancer.[13,14] In this study, the actual clinical value of the diagnostic model based on HF-BF%, NSE, and Ratio2 is reflected in the early identification of malignant ascites. When the risk of malignancy is indicated, imaging suspicious areas can be preferentially selected for puncture to improve the diagnosis rate and optimize the treatment strategy. At the same time, for benign patients, excessive diagnosis and treatment can be avoided and the risk of complications can be reduced. This implies that NSE may play a significant role in diagnosing liver cancer and its complications through synergistic interactions with other biomarkers. Therefore, future research should further investigate the biological mechanisms of NSE in liver cancer metastatic ascites and optimize its application within a combined diagnostic model to enhance clinical utility.

Lactate dehydrogenase (LDH) and adenosine deaminase (ADA) are critical indicators of cellular metabolism and immune activity. LDH is widely distributed across various cell types, with elevated levels typically indicating cellular damage and increased tumor metabolic activity.[15–17] Conversely, ADA is primarily found in the lymphatic system, and its activity is closely linked to immune function, serving as a classic marker for diagnosing tuberculous pleural effusion.[18–21] Research has demonstrated that ADA levels may also rise in pleural effusions resulting from malignant tumors, empyema, or autoimmune diseases.[22,23] In this study, we noted that serum LDH and ascitic LDH levels in patients with malignant ascites were significantly higher than those in the benign control group, while there was no notable difference in ascitic ADA levels between the two groups. Previous studies have indicated that the ratio of serum LDH to ADA in pleural effusion is a valuable marker for diagnosing malignant pleural effusion.[16,17] Building on this, we innovatively introduced the ratio of serum LDH to ascitic ADA (Ratio1) and the ratio of ascitic LDH to ascitic ADA (Ratio2). Our findings revealed that Ratio2 differed significantly between the two patient groups and emerged as an independent risk factor for malignant ascites in liver cancer patients. Although its diagnostic ability for malignant ascites

when considered alone is limited, it combined diagnostic performance with HF-BF% and NSE is robust (AUC = 0.824). This discovery offers a fresh perspective on diagnosing malignant ascites associated with liver cancer.

The strength of this study lies in its utilization of real clinical data, integrating parameters from blood cell analyzers with serum and peritoneal effusion tumor indicators to systematically analyze the risk factors for malignant peritoneal effusion in patients with liver cancer. We constructed a joint diagnostic model based on HF-BF%, NSE, and Ratio2. The results demonstrated that the combined detection of these three indicators yielded an area under the curve (AUC) of 0.824, significantly surpassing the diagnostic capability of any single indicator. This outcome not only confirms the superiority of the combined diagnostic model but also highlights its potential value in clinical applications. The indicators utilized are commonly employed in routine clinical practice, ensuring high practicality and feasibility. This is particularly beneficial for cases with negative cytology, as the combined diagnostic model can offer highly accurate diagnostic insights without incurring additional costs or time. Consequently, it enables clinicians to more proactively identify patients requiring further evaluation.

This study has several potential limitations. First, the small sample size and single-center design may impact the statistical power and generalizability of the findings. Second, the retrospective nature of the study constrains the ability to infer causal relationships. Finally, the risk factors identified in this study represent potential predictors of malignant ascites in patients with liver cancer. However, their relative contributions and underlying mechanisms may vary between HCC and ICC subtypes. These distinctions warrant further investigation in larger, prospective, specifically designed subtype-stratified studies. Additionally, exploring the application value of these indicators in prognostic evaluation and personalized treatment will be essential for enhancing the clinical utility of the combined diagnostic model.

Conclusion

In conclusion, this study is the first to demonstrate the potential for extracting additional diagnostic information from hyperfluorescent cells, biochemical markers, and tumor markers in the peritoneal effusion of patients with liver cancer. We confirmed the significant role of combined detection using HF-BF%, NSE, and Ratio2 in differentiating benign from malignant ascites. As markers suitable for routine laboratory testing, HF-BF%, NSE, and Ratio2 can accurately indicate the malignant nature of ascites without incurring additional costs or time. This screening strategy enhances the detection rate of malignant ascites and provides clinicians with earlier and more precise diagnostic evidence, ultimately improving treatment options and patient prognosis. Future studies should focus on exploring new biomarkers and diagnostic models to further advance diagnostic technology for malignant ascites.

Data Sharing Statement

The data will be made available upon request by the corresponding author.

Ethics Approval and Consent to Participate

The study protocol was in accordance with the ethical standards of the institutional research committee and the ethics guidelines of the 1975 Declaration of Helsinki. The study protocol was in accordance with the ethical standards of the institutional research committee. Hangzhou Xixi Hospital's institutional ethics review committee approved the study (2023 Science Ethic No.64). Written informed consent was not required due to the retrospective nature of this study. All the data used in this study were anonymized.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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