RESEARCH ARTICLE

Tumor Markers in Serum and Ascites in the Diagnosis of Benign and Malignant Ascites

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Abstract

Objective: To evaluate the values of 4 tumor markers in serum and ascites and their ascites/serum ratios in the identification and diagnosis of benign and malignant ascites. Materials and Methods: A total of 76 patients were selected as subjects and divided into malignant ascites group (45 cases) and benign ascites group (31 cases). Samples of ascites and serum of all hospitalized patients were collected before treatment. The levels of carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), cancer antigen 125 (CA125) and carbohydrate antigen 19-9 (CA19-9) were detected by chemiluminescence (CLIA) . Results: CEA, AFP and CA19-9 in both serum and ascites as well as CA125 in ascites were evidently higher in the malignant ascites group than in the benign ascites group ($P<0.01$). Malignant ascites was associated with elevated ascites/serum ratios for AFP and CA125 ($P<0.01$). The areas under receiver operating characteristic (AUROCs) of CEA and CA125 in ascites and the ratios of ascites/serum of AFP, CEA, CA125 and CA19-9 were all >0.7, suggesting certain values, while those of ascites CA19-9 and serum CEA were 0.697 and 0.629 respectively, indicating low accuracy in the identification and diagnosis of benign and malignant ascites. However, the AUROCs of the remaining indexes were <0.5, with no value for identification and diagnosis. Compared with single index, the sensitivity of combined detection increased significantly ($P<0.05$), in which the combined detection of CEA, CA19-9 and CA125 in ascites as well as the ratio of ascites/serum of CEA, CA19-9, CA125 and AFP had the highest sensitivity (98.4%) but with relevantly low specificity. Both sensitivity and specificity of combined detection should be comprehensively considered so as to choose the most appropriate index. Conclusions: Compared with single index, combined detection of tumor markers in serum and ascites can significantly improve the diagnostic sensitivity and specificity.

Keywords: CEA - alpha fetoprotein - CA125 - carbohydrate antigen 19-9 - benign and malignant ascites - diagnosis

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Introduction

Ascites, as a commonly seen symptom in clinic, is caused by malignant tumors of enterocoelia and peritoneum, hepatic diseases, tuberculous peritonitis, cardiac insufficiency and renal diseases, etc, which can be divided into benign and malignant ones. In clinic, the therapeutic protocols and prognosis of ascites induced by malignant tumors are quite different from those by benign lesions, for which the definition of ascites causes is of great significance. However, it is still a difficult issue in clinic to distinguish the benign and malignant ascites, especially the early diagnosis of malignant ascites. At present, in the identification and diagnosis of ascites, cytological detection of ascites has become a gold standard for the confirmation of malignant ascites (Liu et al., 2014). However, though this detection has high specificity, its sensitivity is low, which can easily cause missed diagnosis and repeated tests after multiple ascites collections, leading to the delay of the optimal therapeutic opportunity to some extent and the increase of patients' pain by abdominocentesis. Studies (Cheng et al., 2012; Chen et al., 2012; Zhang et al., 2012; Wu et al., 2014) found that the laboratory indexes such as vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP), Deoxyribose Nucleic Acid (DNA) heteroploid and human leukocyte antigen system-G had certain values in diagnosing malignant ascites, however, their applications were limited in clinic due to the complicated inspection techniques, the difficult operations and the expensive costs. Tumor markers have certain diagnostic sensitivity and specificity in the diagnosis of malignant ascites, but the diagnostic value of each index differs greatly in malignant ascites induced by different causes due to the complex etiology of malignant ascites (Wang et al., 2014). It is said that no tumor marker has been found with high sensitivity and specificity in the malignant ascites induced by all causes. Some scholars (Zhang et al., 2011) believed that the combined detection of tumor markers in serum and ascites could improve their diagnostic values. This study mainly explored the values of tumor markers in serum and ascites as well as the ratios of ascites/serum

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Table 1. Comparison of Tumor Marker Levels in the Serum and Ascites Between two Groups

Indexes		Benign ascites group (n=31)	Malignant ascites group (n=45)	*t* value	*P* value
CEA(ng/mL)	Serum	2.69±0.31	54.46±19.23	-14.96	<0.0001
	Ascites	0.64±0.08	203.75±38.24	-29.51	<0.0001
	Ascites/serum	1.03±0.14	33.27±10.06	-17.80	<0.0001
AFP(ng/mL)	Serum	50.17±20.06	102.18±31.55	-8.11	<0.0001
	Ascites	13.46±6.98	62.79±26.84	-9.98	<0.0001
	Ascites/serum	0.96±0.41	0.71±0.03	4.09	0.0001
CA125(U/mL)	Serum	485.47±112.61	552.74±168.57	-1.94	0.0560
	Ascites	753.01±100.75	1577.12±196.48	-21.46	<0.0001
	Ascites/serum	20.18±2.85	4.76±0.71	34.85	<0.0001
CA19-9(U/mL)	Serum	59.26±20.19	201.93±41.38	-17.77	<0.0001
	Ascites	4.09±0.58	220.04±45.56	-26.33	<0.0001
	Ascites/serum	2.09±0.43	3.16±1.04	-5.41	<0.0001

in the identification and diagnosis of malignant ascites by analyzing the clinical data of patients diagnosed with ascites in our hospital in recent years.

Materials and Methods

General data

A total of 76 patients diagnosed with ascites in The First People's Hospital of Anqing and Zhejiang Provincial People's Hospital from June 2012 to June 2014 were selected as study objects and divided into malignant ascites group (45 cases) and benign ascites group (31 cases). The diagnosis and causes of ascites of all patients were confirmed by clinical manifestations, laboratory indexes as well as imageological, cytological and histopathological detections. In malignant ascites group, there were 24 males and 21 females, aged 20~79 years, with average age of (54.36±11.78) years, in whom 6 were with Psendomyxoma peritonei (PMP), 9 with gastric cancer, 1 with pancreatic cancer, 14 with ovarian tumors, 1 with hepatic cancer, 3 with colon cancer, 2 with small intestinal tumors, 2 with lymphoma and 7 with metastatic tumors from unknown primary focuses. In benign ascites group, there were 13 males and 18 females, aged 18~76 years, with average age of (42.71±16.38) years, in whom 21 were with tuberculous peritonitis, 5 with liver cirrhosis accompanied by spontaneous peritonitis, 3 with connective tissue disease (CTD), 1 with acute severe pancreatitis and another 1 with acute purulent cholecystitis.

Methods

Sample collection and disposal: After the patients were hospitalized, abdominocentesis was conducted under aseptic conditions to collect the samples of ascites and serum for detections in Laboratory Department before any treatment.

Detection methods: The levels of carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), cancer antigen 125 (CA125) and carbohydrate antigen 19-9 (CA19-9) were detected by chemiluminescence (CLIA). Kits for the quantitative determinations of above 4 indexes were purchased from German Roche Diagnostics Co. Ltd. All operations were performed according to the instructions and the details were as follows: 10 μL sample (CA125: 20 μL) was incubated together with specific monoclonal antibodies of biotinylation and those marked by ruthenium (RU) so as to form the sandwich compound of antigen and antibody. Magnetic bead particles coated by streptavidin were added to be further incubated, and the compound combined with the magnetic beads via the functions of biotin and streptavidin. The reactants were sucked into measuring wells and made magnetic beads adsorbing on the surface of electrodes by electromagnetic interaction, and those without combination with magnetic beads were removed with ProCell methods. The electrodes were added with proper voltage to make the compounds shining. Then the light intensity was measured by photoelectric multiplier, and the levels were automatically calculated with the calibration curve corrected by TWO POINT.

Statistical data analysis

SAS 9.3 statistical software package was applied for all data analysis. Measurement data was expressed by mean±standard deviation ($\bar{x}\pm s$), and detected with t-test. The receiver operating characteristic (ROC) curves of all tumor markers were drawn to calculate the sensitivity, specificity and accuracy, which was expressed by percentage (%) and detected with χ^2 test between groups. $P<0.05$ was regarded statistically significant.

Results

Comparison of tumor marker levels in serum and ascites between two groups

CEA, AFP and CA19-9 in both serum and ascites as well as CA125 in ascites in malignant ascites group were evidently higher than those in benign ascites group, and the differences were significant ($P<0.01$). However, there was no significant difference in CA125 between two groups

Table 2. The AUROC, Optimal Diagnostic Point, Sensitivity and Specificity of the Indexes

Indexes	AUROC	Optimal diagnostic point	Sensitivity	Specificity
Serum CEA	0.629	7.049	40.95%	96.69%
Ascites CEA	0.859	1.979	67.31%	100%
Ascites CA125	0.706	613.112	80.29%	56.78%
Ascites CA19-9	0.697	14.493	51.07%	100%
AFP Ascites/serum	0.734	0.461	83.51%	36.71%
CEA Ascites/serum	0.879	0.824	67.29%	94.06%
CA125 Ascites/serum	0.726	2.197	71.03%	73.25%
CA19-9 Ascites/serum	0.821	0.533	78.62%	84.16%

Table 3. Values of Combined Detections of Indexes in the Identification and Diagnosis of Benign and Malignant Ascites

Indexes	Sensitivity	Specificity
Ascites: CEA+CA19-9	72.43%	100%
Ascites: CEA+CA19-9+CA125	95.06%	56.71%
ascites/serum: CEA+CA19-9	85.17%	74.97%
ascites/serum: CEA+CA125+CA19-9+AFP	95.06%	56.71%
CEA(ascites)+CEA(ascites/serum)	72.21%	93.32%
CA125(ascites)+CA125(ascites/serum)	88.49%	50.01%
CA19-9(ascites)+CA19-9(ascites/serum)	85.19%	83.16%
CEA(ascites)+CA19-9(ascites)+CEA(ascites/serum)	83.46%	93.27%
CEA(ascites)+CA19-9(ascites)+CA125(ascites)+ CEA(ascites/serum)+CA125(ascites/serum)+CA19-9(ascites/serum)+AFP(ascites/serum)	98.38%	33.53%

(P>0.05). Malignant ascites group was apparently higher in the ratios of ascites/serum of CEA and CA19-9 and obviously lower in those of AFP and CA125 than benign ascites group (P<0.01), as shown in Table 1.

ROC analysis of all indexes and their values in the identification and diagnosis of benign and malignant ascites

The areas under receiver operating characteristic (AUROCs) of CEA and CA125 in ascites and the ratios of ascites/serum of AFP, CEA, CA125 and CA19-9 were all >0.7, suggesting certain values, while those of ascites CA19-9 and serum CEA were 0.697 and 0.629 respectively, indicating low accuracy in the identification and diagnosis of benign and malignant ascites. However, the AUROCs of the rest indexes were <0.5, which revealed no value in the identification and diagnosis, as shown in Table 2.

Values of combined detections of the indexes in the identification and diagnosis of benign and malignant ascites

The optimal diagnostic points in Table 2 were applied. It was suggested that the sensitivity of the combined detection of CEA, CA19-9 and CA125 in ascites was 95.06%, markedly higher than their single detections (P<0.05); that the sensitivity of the combined detection of the ratio of ascites/serum of CEA, CA19-9, CA125 and AFP was 95.06%, significantly higher than each single detection (P<0.05); and the combined detection of CEA, CA19-9 and CA125 in ascites as well as the ratio of ascites/serum of CEA, CA19-9, CA125 and AFP had the highest sensitivity (98.38%) but with relevantly low specificity, demonstrating that both the sensitivity and specificity of combined detections should be comprehensively considered to choose the most appropriate index, as shown in Table 3.

Discussion

In normal people, there is a small quantity of fluid with lubricating effect in the abdominal cavity, whose excessive accumulation because of various pathological factors will become ascites. Ascites can be divided into malignant and benign ones according to various causes. The former is mainly caused by primary or metastatic tumors in enterocoelia while the latter by other factors such as cardiac insufficiency, liver cirrhosis, nephritis and tuberculous peritonitis, etc. In clinic, the therapeutic protocols and prognosis for malignant ascites is quite different from those for the benign ones, and the immediate definition of the causes is of great importance.

Tumor markers refer to the bioactive substances in body fluids or tissues that are synthesized, secreted or shed off from tumor cells during the development and proliferation of malignant tumors, or substances, which are produced through the reaction of hosts to tumor tissues, with significantly higher levels in the fluids or tissues than normal reference values (Baser et al., 2014). Therefore, the detections of tumor markers (including proteins, hormones, enzymes and cancer gene products) in blood or body fluids have certain values in the diagnosis, efficacy observation and prognostic evaluation of tumors.

It was reported in many studies (Liu et al., 2014; Tampellini et al., 2014; Wang et al., 2014) that CEA, as a soluble glycoprotein compound, expressed highly in multiple malignant tumors like gastric cancer, colon cancer, pancreatic cancer and non-small cell lung cancer, etc, whose level in serum had certain value in the prognostic evaluation. Other reports (Tuzun et al., 2009; Kaleta et al., 2013) showed that the diagnostic sensitivity, specificity and accuracy of ascites CEA were 31%, 90% and >98%, respectively. AFP, as kind of serum glycoprotein synthesized by liver and yolk sac in the early stage of fetal development, will disappear gradually with the ages, and it is the most classical marker for hepatic cancers. A research (Kaleta et al., 2013) indicated that AFP was obviously higher in malignant ascites than in benign ascites, with diagnostic sensitivity and specificity being 17% and 95%, respectively. CA125, as a kind of macromolecular poly-glycoprotein, will increase evidently in the blood of patients when the cellular structures are damaged and changed in malignant tissues. Some researches (Bozkurt et al., 2013; Bilen et al., 2014; Povolotskaya et al., 2014) demonstrated that CA125 level had different-degree increase in ascites and serum of patients with cancers of lung, liver, stomach, uterus and prostate, etc. CA19-9, also known as gastrointestinal cancer associated antigen, can be used as a complementary diagnostic index for patients with malignant tumors such as pancreatic and colorectal cancers. Trape et al (Trape et al., 2004) found that with the ratio of ascites/serum of CA19-9 >1.19 as the positive critical value, the sensitivity and specificity of CA19-9 could be 47% and up to 100%

in the diagnosis of malignant ascites, respectively.

Partial results of this study were consistent with above reports that the diagnostic values of different tumor markers differed significantly in different ascites caused by distinct causes, so it was believed that the combined detections of multiple tumor markers were more valuable in clinic. In this study, the combined detection of CEA, CA19-9 and CA125 in ascites as well as the ratio of ascites/serum of CEA, CA19-9, CA125 and AFP had the highest sensitivity (98.38%) but with relevantly low specificity, demonstrating that both the sensitivity and specificity of combined detections should be comprehensively considered to choose the most appropriate index. However, the sensitivity and specificity of combined detections of ascites CEA, ratio of ascites/serum and ascites CA19-9 were 83.46% and 93.27%, whereas those of ascites CEA and CA19-9 were 72.43% and 100% respectively, suggesting that they could be considered as the complementary therapies for the diagnosis of malignant ascites.

To sum up, the combined detection of tumor markers, which can improve the positive rate of diagnosis, is of great value in the identification and diagnosis of benign and malignant ascites. As to partial ascites patients with unknown causes, the diagnosis should be combined with the medical histories, symptoms, signs, other laboratory indexes and imaging detection in clinical practices.

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