Diagnostic Accuracy of Tumor Markers CYFRA21-1 and CA125 in the Differential Diagnosis of Ascites

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Abstract. *Background: The usefulness of tumor markers in the differential diagnosis of cancer in patients with ascites remains a matter of controversy. Few studies have reported the measurement of cancer antigen 125 (CA125) and cytokeratin 19 soluble fragments (CYFRA21-1) in ascitic fluid. The aim of the present study was to evaluate the diagnostic accuracy of these tumor markers in the detection of malignant ascites. Materials and Methods: We analyzed CA125 and CYFRA21-1 from 143 consecutive undiagnosed patients with ascitis. Results: Use of CA125 gave a sensitivity of 39.7% and a specificity of 98.8%, and CYFRA21-1 a sensitivity of 50.0% and a specificity of 97.6% in differential diagnosis of malignant ascites. For combined use of CA125 plus CYFRA21-1, sensitivity was 65.5% and specificity 96.5%. In patients with negative cytology, these two tumor markers had a sensitivity of 50% and a specificity of 96.5%. Conclusion: The determination of tumor markers in ascitic fluid could be useful for the diagnostic assessment of patients with ascites.*

Ascites is the abnormal collection of fluid in the peritoneal cavity. It is mostly seen in patients with liver disease, pancreatic disease, tuberculous peritonitis, congestive heart failure, kidney disease, AIDS and cancer. Cytology is the gold-standard for confirming the presence of malignant cells in ascitic fluid but its sensitivity only ranges between 50 and 70% (1). The main cause of this low sensitivity is the fact that a primary tumor may infiltrate the peritoneum but does not shed cells and so the cytology result is negative; in these cases, other invasive procedures, such as laparoscopy, may be needed to confirm the presence of malignant cells.

The potential of tumor markers (TMs) for improving the diagnosis of malignant effusions has been mentioned by several authors but there are large discrepancies between different reports regarding their specificity and sensitivity and also in terms of the cut-off values used (2). Specifically, cancer antigen 125 (CA125) (3, 4) and cytokeratin 19 soluble fragment (CYFRA21-1) have been reported as useful diagnostic markers for identifying patients with malignant pleural effusions (5-8). Their sensitivity ranges between 24% and 77% and their specificity from 82% to 100%.

However, few studies have used these tumor markers in the differential diagnosis of the etiology in patients with ascites (9). In a previous study, our group evaluated CA125 and CYFRA21-1, among other TMs, in pleural and peritoneal fluids (10). Analyzing 49 patients with ascites and 52 with pleural effusions, and setting specificity at 100%, our results showed sensitivities of 33% for CA125 and 50% for CYFRA21-1 using specific cut-off values. For patients with negative cytology, the sensitivity of the two TMs was 23% and 43%, respectively. This same study also analyzed the fluid/serum ratio (F/S) of these TMs. In contrast to carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3) and cancer antigen 19-9 (CA19-9), an F/S ratio >1.2 was found for CA125 and CYFRA21-1 in the majority of effusions without malignancy, suggesting that these tumor markers may be produced or secreted by mesothelial cells.

High concentrations of CA125 and CYFRA21-1 have been found in tumors of different kinds, including of the ovary, stomach, colon, pancreas, and breast (11), which are the types most frequently implicated in peritoneal carcinomatosis.

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Key Words: Ascitic fluid, CA125, CYFRA 21-1, differential diagnosis, malignant effusion.

The aim of the present study was to validate the accuracy of fluid and serum determination of CYFRA21-1 and CA125 for the etiological diagnosis of ascites.

Materials and Methods

This study was conducted between January 2004 and December 2011. Samples were collected from 143 patients with newly diagnosed cases of ascites from any of our center's medical specialties.

Diagnostic procedures were performed blind regarding the study data. The reference method used was pathological confirmation of malignant ascites or definitive diagnosis of cases assessed during the three months following the determination of TMs. Effusions were considered malignant when the cytology, biopsy or autopsy was positive for malignant cells. Paramalignant effusions were defined as effusions in which no neoplastic cells were detected by cytology, biopsy or autopsy in patients diagnosed with cancer.

Fluid effusions and serum were collected and analyzed the same day. CA125 and CYFRA 21-1 were determined using an electrochemiluminiscence method on an ELECSYS analyzer (Roche diagnostics, Barcelona, Spain). For each TM, we used the cut-off point established in a previous study at a specificity of 100% (10). A fluid effusion was considered malignant when the fluid concentration of CYFRA 21-1 or CA125 was greater then 175 μg/ l or 2,385 kU/l, respectively. The Ethical Committee of clinical investigations of Foundation Union Catalan Hospitals approved this study with this number 12/5.

Statistical analysis. Continuous variables were summarized using means and standard deviation (SD) or medians and interquartile range (IQR). Categorical variables were summarized using absolute values and relative frequency. The Kolmogorov-Smirnov test was used to test for normal distribution. Non-parametric tests (Mann–Whitney *U*-test) were used to compare TM concentrations for independent samples and Wilcoxon test for related samples. Bivariate correlations were tested using Kendall's tau coefficient. The receiver operating characteristics (ROC) curves for each TM were drawn and the areas under the curve (AUC) were calculated in order to determine the diagnostic accuracy of each marker in fluid effusions.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR) were calculated for each TM, for combinations of TMs, and for the combination of both TMs and cytology. F/S ratios for both TMs were also reported. The parameters of diagnostic accuracy are shown, together with their 95% confidence interval (CI). A two-sided 5% significance level was assumed. All statistical analyses were performed using IBM® SPSS® Statistics for Windows v.20 (IBM Corporation, Armonk, New York, NY, USA) and Stata® v.10 (StataCorp LP, College Station,TX, USA).

Results

A total of 143 patients with ascites were included: 80 (55.9%) women and 63 (44.1%) men, with ages ranging from 33 to 94 years. Figure 1 shows a flow chart of this study. Out of the effusions assessed, 85 (59.4%) had a benign etiology and 58 (40.6%) a malignant one (Table I).

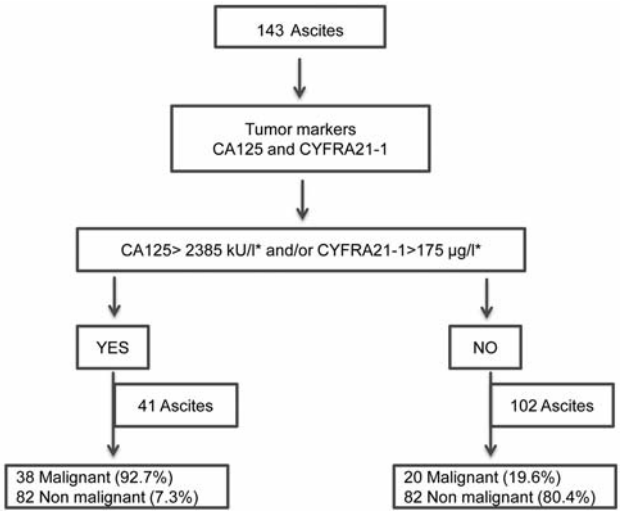

Figure 1. *Flow Chart. *Cut-off values established at a specificity of 100% in previous study (10).*

Table II shows TM concentrations (CA125 and CYFRA21-1) in fluid and serum, and the F/S ratio according to etiology (benign or malignant). In the group of patients with malignant effusion, TM concentrations in fluid and the F/S ratio were significantly higher than in those with benign effusion; in serum, CYFRA 21-1 significantly differed between malignant and benign cases. We found correlations between TMs in fluid and serum (Kendall's Tau; *p*-value) for CA125 (0.485; $p<0.001$) and CYFRA21-1 (0.471; $p<0.001$).

Figure 2 shows the results obtained for the ROCs of the TMs in fluid effusion. An AUC of 0.888 (95% CI=0.832-0.945) was obtained for CYFRA21-1 and of 0.764 (95% CI=0.681-0.848) for CA125.

Table III shows the sensitivity, specificity, NPV, PPV NLR and PLR of CA125 and CYFRA21-1 for cut-off points of 2385 kU/l and 175 μg/l respectively, and combining the two TMs in ascitic fluid both for all effusions and for effusions with negative cytology. Table III also shows sensitivity and specificity of 65.5% and 96.5%, respectively, combining the two TMs, and of 82.8% and 96.5% when combining them with cytology. It also shows sensitivity and specificity of 50% and 96.5% when the cytology was negative.

Data obtained from the F/S ratio and serum concentrations of TMs gave lower sensitivity and specificity than their determination in fluid. The sensitivity of the F/S ratio was 28.3% for a cut-off for CA125 of 8.76, and 18% for a cut-off for CYFRA21-1 of 85 at 97.6% specificity. Likewise, the optimal cut-offs for differentiating malignant ascites using

Table I. *Etiology of ascites*.

Aetiology	n=143
Benign	n=85 (59.4%)
Cirrhotic	49
Paraneoplastic	13
Cardiogenic	11
Peritonitis	5
Pancreatitis	2
Post-traumathic	1
Fibrothecoma	1
Viral	1
Unknown	2
Malignant	n=58 (40.6%)
Ovarian cancer	15
Gastric cancer	10
Pancreatic cancer	9
Papillary serous carcinoma	5
Cholangiocarcinoma	3
Colonic carcinoma	2
Mesothelioma	2
Lymphoma	2
Bladder cancer	1
Breast cancer	1
Endometrial cancer	1
Leukemia	1
Unknown primary cancer	6

Table II. *Concentrations of tumor markers cancer antigen 125 (CA125) and cytokeratin 19 soluble fragment (CYFRA21-1) in ascitic fluid, serum and fluid/serum ratio (F/S) according to etiology of effusions*.

Tumor marker	Benign N=85	Malignant N=58	*p*-Value Mann–Whitney *U*-test
CA125 (kU/l)*			
Fluid	803±585	6329±1211	<0.001
	666 (0.6-4020)	1449 (18-54896)	
Serum	612±763	950±1334	0.917
	451 (48-4522)	297 (16-6555)	
F/S	2.1±1.9	20.8±65.8	<0.001
	1.4 (0.04-9.79)	5.3 (0.4-393)	
CYFRA21-1 (µg/l)*			
Fluid	37.9±164	590±1155	<0.001
	8.6 (0.6-1498)	180 (3.6-6962)	
Serum	19.7±71.3	57.6±194	<0.001
	3.2 (0.7-430)	8.8 (1.4-1290)	
F/S	7.3±15.5	47.9±15.5	<0.001
	3.3 (0.3-82.6)	7.0 (0.64-404)	

Values are the mean±standard deviation and median (minimum value – maximum value). F/S: Fluid/serum. *Significant differences Wilcoxon test between serum and ascitic fluid in CA125 and CYFRA21-1 (p<0.001).

serum TMs were 1,580 kU/l for a sensitivity of 24% for CA125 and 130.7 µg/l for a sensitivity of 8% for CYFRA21-1, maintaining specificity at 97.6%.

Table IV shows the sensitivity of each TM and combination of TMs according to tumor type detected in our patients with ascites.

Discussion

In patients with ascites and a suspicion of cancer, cytology is the gold-standard for confirming the presence of tumor cells. Unfortunately, cytology is negative in between 30% and 50% of patients with malignant ascites. In these cases, the use of other invasive and expensive procedures, such as laparoscopy, may be necessary (12).

The results of our study in newly-diagnosed cases of ascites validate previously published data in patients with ascitic and pleural effusions (10). Determination of CA125 in ascitic fluid using cut-off values >2385 kU/l gave a sensitivity of 39.7% and a specificity of 98.8% for the diagnosis of peritoneal cancer. Our training study showed a sensitivity of 33%, fixing specificity at 100% (10). With regard to the diagnostic accuracy of determination of CYFRA21-1 in ascitic fluid using cut-off values above 175 µg/l, that study found a sensitivity of 50% and a specificity of 97.6%. Previous data reported by our team with this TM gave a sensitivity of 50%, fixing specificity at 100%. In the present study, combining the two TMs led to a sensitivity of 65.5% and specificity of 96.5%.

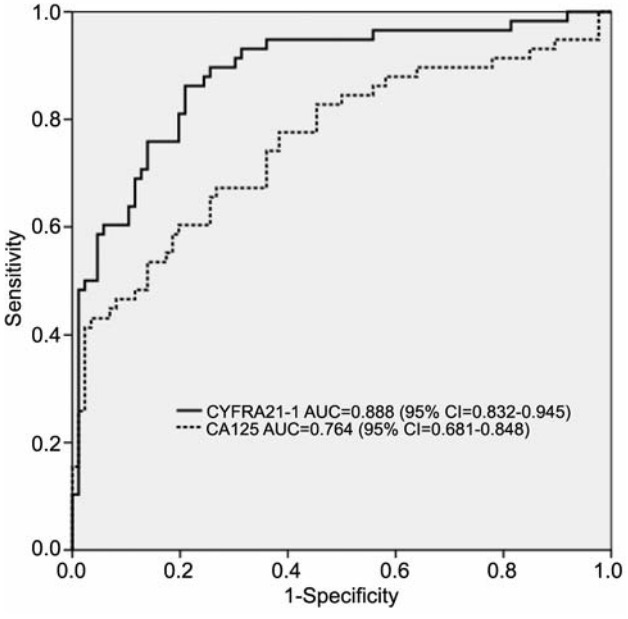

Figure 2. *Receiver operating curves for each tumor markers*.

Table III. *Diagnostic accuracy of tumor markers and cytology in ascitic fluids.*

Tumor marker	Sensitivity 95% CI	Specificity 95% CI	NPV 95% CI	NLR 95% CI	PPV 95% CI	PLR 95% CI
All effusions						
CYFRA 21-1	50.0%	97.6%	74.1%	0.51	93.5%	20.83
	36.7-63.3%	91.0-99.6%	64.8-81.7%	0.41-0.66	77.2-98.9%	5.6-85.6
CA125	39.7%	98.8%	70.6%	0.61	95.8%	33.08
	27.3-53.3%	92.7-99.9%	61.4-78.4%	0.50-0.75	76-9-99.8%	4.7-242
CA125+CYFRA21-1	65.5%	96.5%	80.4%	0.36	92.7%	18.71
	51.8-77.2%	89-3-99.1%	71.1-87.3%	0.25-0.51	79.0-98.1%	6.0-57.3
Cytology	65.5%	100.0%	81.0%	0.35	100.0%	>9999
	51.8-77.2%	94.6-100%	71.9-87.7%	0.24-0.49	88.6-100%	
TM + cytology	82.8%	96.5%	89.1%	0.18	94.1%	23.65
	70.1-90.1%	89.3-99.1%	80.5-94.4%	0.10-0.31%	82.8-98.5%	7.7-71.7
Effusions with negative cytology						
CYFRA21-1	40.0%	97.6%	87.4%	0.61	80.0%	17.00
	20.0-63.6%	91.0-99.6%	78.6-93.0%	0.43-0.88	44.2-99.6%	3.9-74.0
CA125	30.0%	98.8%	85.7%	0.71	85.7%	25.50
	12.8-54.3%	92.7-99.9%	76.8-91.7%	0.53-0.94	42.0-99.2%	3.2-20
CA125+CYFRA21-1	50.0%	96.5%	89.1%	0.52	76.9%	14.17
	27.8-72.1%	89.3-99.1%	71.1-87.3%	0.33-0.80	46.0-94.4%	4.3-46.8

NPV: Negative predictive value; NLR: negative likelihood ratio; PPV: positive predictive value; PLR: positive likelihood ratio; 95% CI: confidence interval. Cut-off: CYFRA21-1 >175 μg/l; CA125 >2385 kU/l.

Few studies have assessed the diagnostic capacity of TMs in ascitic fluid (10, 13-15), and even fewer have done so focusing on CA125. There is a great deal of variability between authors regarding the diagnostic accuracy of CA125. Hunter *et al*. reported a sensitivity and a specificity of 96% and 99%, respectively, in ascitic fluid, at a cut-off of 200 kU/l (4). Studying patients with ascites and ovarian cancer, and using patients with ascites and benign gynecological disease as controls, Kucokgoz *et al*. found a sensitivity of 76.3% and a specificity of 62.5% with a cut-off of 928 kU/l (16). For CYFRA21-1, Tuzun *et al*. reported a correlation between CYFRA21-1 in ascitic fluid and serum, but did not evaluate the diagnostic accuracy (9).

Our results show higher concentrations of CA125 and CYFRA21-1 in ascitic fluid than in serum, both in benign and in malignant etiologies, and therefore F/S ratios greater than 1, as previously described in patients with ascites and other effusions (3, 4, 10, 17-19). These findings indicate that these two markers are secreted in non-cancerous conditions by mesothelial cells, consistent with the observations of Zeillemaker *et al*. who demonstrated the secretion of CA125 by mesothelial cells of the peritoneum (20). However, other TMs such as CEA, CA15-3 and CA19-9 showed a different behavior, with F/S ratios of below 1 in cases with benign effusions (10, 21, 22).

Table IV. *Sensitivity of each tumor marker and combination of tumor markers according to tumor type.*

Tumor type	n	CYFRA21-1	CA125	CYFRA21-1 and/or CA125
Ovarian cancer	15	73.3%	80.0%	100.0%
Gastric cancer	10	40.0%	10.0%	50.0%
Pancreatic cancer	9	44.4%	22.2%	44.4%
Papillary serous carcinoma	5	60.0%	80.0%	100.0%
Cholangiocarcinoma	3	66.7%	0.0%	66.7%
Colonic carcinoma	2	50.0%	0.0%	50.0%
Mesothelioma	2	50.0%	50.0%	50.0%
Lymphoma	2	0.0%	50.0%	50.0%
Bladder cancer	1	0.0%	0.0%	0.0%
Breast cancer	1	0.0%	0.0%	0.0%
Endometrial cancer	1	0.0%	0.0%	0.0%
Leukemia	1	0.0%	0.0%	0.0%
Unknown primary cancer	6	50.0%	33.3%	66.7%

Cut-off: Cytokeratin 19 soluble fragment (CYFRA21-1)> 175 μg/l; cancer antigen (CA125)> 2385 kU/l.

Serum concentrations of CA125 in patients with effusions (either malignant or benign) exceeded the upper reference limit, as described previously (23, 24). No significant differences were found between the concentrations of CA125

in serum in patients with malignant and benign ascites. In the case of CYFRA21-1, even though nearly half of all patients had values above the upper reference limit, we found significant differences between those with malignant and those with benign effusions. Unfortunately, the sensitivities of these markers in serum are very low, especially in the case of CYFRA21-1. The F/S ratio also gave less sensitivity than single determination in the fluid, and was higher for CA125 than for CYFRA21-1. We found a correlation between fluid and serum concentrations of TMs similar to that found by Tuzun *et al*. (9), but we obtained the highest diagnostic sensitivity in the determination in fluid for both CA125 and CYFRA21-1; therefore, TM determination in serum cannot replace determination in fluid.

In our study, the combination of CA125 and CYFRA21-1 in fluid detected all ovarian cancer and papillary serous carcinoma of the peritoneum. However, it only detected just over half (54%) of cancer of the digestive system (stomach, colon and pancreas).

The main limitations of the study are that the results are from a single Institution and that the combination included only two markers. Our results need to be validated in larger samples and in multi-centric studies.

We conclude that the determination of TMs CYFRA21-1 and CA125 in ascitic fluid may be useful in the differential diagnosis of neoplastic ascites in conjunction with cytology. In patients with negative cytology, it offers high specificity for selecting those that require invasive tests.

Conflicts of Interest

The Authors declare they have no conflicts of interests with regard to this study.

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