Significance of serum and bile tumor markers in the diagnostic approach of patients with malignant pancreatobiliary disease

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Summary

Purpose: Serum and bile tumor markers are under intense scrutiny for the diagnosis of malignant disease. The purpose of our study was to report the usefulness of serum and bile tumor markers for the discrimination between benign and malignant pancreatobiliary diseases.

Methods: Between March 2010 and May 2013, 95 patients with obstructive jaundice or history of biliary obstruction, were included in the study. During ERCP, bile samples were obtained for measurement of tumor markers CEA, CA19-9, CA125, CA72-4 and CA242. Serum samples were taken before ERCP for the same measurements. The patients were divided into two groups: patients with malignant disease and patients with benign disease.

Results: Serum tumor marker levels were significantly higher in patients with malignant disease. Serum CA242 and CA19-9 exhibited the highest diagnostic accuracy

(76.8% and 73.7%, respectively). CA125 and CA72-4 levels in bile samples were significantly higher in patients with malignant disease. Bile CA125, CEA and CA72-4 achieved the best diagnostic accuracy (69, 65 and 65%, respectively). The combined detection of CA19-9, CA242 in serum and CA125, CA72-4 in bile along with total bilirubin levels, showed the best diagnostic accuracy (81%).

Conclusions: Serum and bile tumor markers, when studied alone, lack the diagnostic yield to discriminate benign from malignant pancreatobiliary diseases. In cases of diagnostic dilemmas the combination of serum and bile markers might be helpful.

Key words: bile tumor markers, cholangiocarcinoma, combined detection, pancreatic cancer, serum

Introduction

Pancreatic cancer and cholangiocarcinoma pose a significant burden for human health. In Europe, pancreatic cancer is the fifth leading cause of cancer-related deaths, with approximately 70,000 estimated deaths each year, while in USA, 46,420 new cases and 39,590 deaths were estimated for 2014 [1,2]. As for cholangiocarcinoma, the predicted numbers for new cancer cases/deaths in the USA for the year 2014 were 33,190/23,000 for intrahepatic disease, and 10,650/3,630, for extrahepatic disease [2-6].

The aforementioned numbers render early diagnosis essential in order to improve prognosis.

Despite the advanced imaging techniques, there are cases with doubtful diagnosis where the differentiation between benign and malignant disease is vital.

Biomarkers (measured in the serum or other biological fluids) are under intense scrutiny throughout numerous clinical studies. Their prognostic as well as predictive value are studied with the hope of identifying earlier patients

with cancer or to distinguish between benign and malignant disease, as well as to help guide treatment [7]. Carcinoembryonic antigen (CEA) is a highly glycosylated cell surface glycoprotein. It is used clinically for more than 20 years and is often found in patients with malignancies of the biliary tract and the pancreas [8,9]. Carbohydrate antigen 19-9 (CA19-9) is a colon-specific antigen, but it is also synthesized by the pancreatic and biliary epithelium. It is elevated in hepatobiliary and pancreatic malignancies. It is also elevated in cases of cholestasis from benign biliary obstruction, but it tends to normalize after the restoration of bile flow [9-11]. Carbohydrate antigen 125 (CA125) is expressed in epithelial ovarian cancer, but it is also elevated in pancreatobiliary malignancies and several cholangiopathies [3,6]. It is not easily influenced in cases of benign biliary obstruction compared with CA19-9 [12]. Carbohydrate antigen 72-4 (CA72-4) is elevated in gastric cancer and other gastrointestinal and ovarian malignancies; including pancreatic and biliary tract cancers [13,14]. Carbohydrate antigen 242 (CA242) is related, but not identical, to CA19-9. It is elevated in patients with pancreatobiliary [15] and colorectal malignancies. It may also be influenced in cases of cholestasis [16,17].

Whilst serum tumor markers have been extensively studied and normal levels have been established, the preoperative measurement of bile tumor markers requires invasive sampling and needs further investigation.

In our prospective institutional study, we report on the usefulness of serum and biliary CEA, CA19-9, CA125, CA72-4 and CA242 concentrations alone or in combination in the diagnosis of patients with malignant pancreatobiliary disease.

Methods

Patient population

Between March 2010 and May 2013, 95 patients with obstructive jaundice or history of recent biliary obstruction, who underwent ERCP in our department, were included in the study. Forty-five of the patients included were males and 50 females with a median age of 69 years (range 29-91) for males and 74 (range 31-93) for females.

Exclusion criteria were: age <18 years, previous ERCP and stent placement, active hepatic disease, history of previous chemotherapy or cancer treated in the past. All patients had a definite diagnosis of benign or malignant disease, using ultrasonography, CT, MRI-MRCP, brush cytology or biopsy samples during ERCP and histology of specimen when surgical procedures

were undertaken [18,19]. The indication for ERCP and biliary drainage in patients with malignancy were locally advanced disease, high surgical risk or delayed surgery. Patients with distant metastases were excluded.

During ERCP, bile samples were obtained through an ERCP catheter, for measurement of the tumor markers CEA, CA19-9, CA125, CA72-4 and CA242. Serum samples were collected before ERCP and the same measurements were undertaken.

Serum CEA, CA19-9 and CA125 levels were evaluated by Chemiluminescent Microparticle Immunoassay (Abbott Architect i2000sr System). Serum CA242 levels were evaluated by using the CanAg CA242 Enzyme Immunometric Assay kit (Fujirebio Diagnostics AB, Sweden). Serum CA72-4 levels were evaluated by using TM-CA72-4 ELISA kit (DRG Instruments GmbH, Germany). The normal reference values in serum were as follows: CEA ≤ 5ng/ml, CA19-9 ≤ 37 U/ml, CA125 ≤ 35 U/ml, CA72-4 ≤ 6 U/ml and CA242 ≤ 20 U/ml. Bile levels were evaluated by using the same methodologies as in serum.

Statistics

Categorical variables were compared using the chi square test. Continuous variables were compared using the Mann-Whitney U test. All p values were two-tailed. A p value < 0.05 was considered statistically significant. The diagnostic value of tumor markers was estimated with sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predictive value (PPV), negative predictive value (NPV) and accuracy. In case of bile tumor markers we performed receiver operating characteristic (ROC) curve analysis and we calculated the area under the curve (AUC), the respective 95% confidence interval and the cut-off value that maximized the sum of sensitivity and specificity. Statistical analysis was performed using SPSS version 17.0 statistical software (SPSS Inc., Chicago, IL, United States).

The study was approved by the local Ethics Committee and strictly adhered to the Helsinki Declaration. Written informed consent, after thorough information, was obtained from all patients.

Results

The underlying pathology was choledocholithiasis in 51 (54%), pancreatic cancer in 22 (23%), cholangiocarcinoma in 17 (18%), and ampullary cancer in 5 (5%) patients. The patients were divided into two groups: group 1 included patients with malignant disease and group 2 patients with benign disease. Patient characteristics are shown in Table 1.

Serum CEA, CA19-9, CA125, CA72-4 and CA242 levels were significantly higher in patients with

Table 1. Patient characteristics

	Group I Malignant disease (N=44)	Group II Benign disease (N=51)	p value
Sex (m/f)	23/21	22/29	0.37[a]
Age, years	74 (44-86)	70 (29-93)	0.09[b]
Total serum bilirubin (mg/dl)	10.8 (0.50-28.8)	1.3 (0.37-17)	<0.001[b]

Values are expressed as median (range). [a]chi-square test, [b]Mann-Whitney U test. Normal reference value for total serum bilirubin was 0.2-1.2 mg/dl

Table 2. Comparison of serum tumor markers

Markers	Patients with malignant disease (N=44)	Patients with benign disease (N=51)	p value[a]
CEA	3.74 (0 -110)	2.09 (0.6 -35)	0.02
CA19-9	240.53 (0 -12,000)	22.01 (0.5 -9526)	<0.001
CA125	17.90 (2 - 438)	12.85 (1 -150)	0.03
CA72-4	1.19 (0.8 -162)	0.80 (0.8 -2)	0.001
CA242	30.20 (0.4 -136)	4.50 (0 -119)	<0.001

Values are expressed as median (range); [a]Mann-Whitney U test. The normal reference values in serum were as follows: CEA ≤ 5ng/mL, CA19-9 ≤ 37 IU/mL, CA125 ≤ 35 IU/mL, CA72-4≤ 6 IU/mL, and CA242 ≤ 20 IU/mL.

malignant disease vs benign disease (p≤0.05; Table 2). CA19-9 and CA242 showed the best p value (<0.001). The diagnostic values of serum tumor markers in patients with malignant disease are presented in Table 3. CA242 and CA19-9 exhibited the highest diagnostic accuracy, 76.8% and 73.7%, respectively. The sensitivity of CA242 and CA 19-9 were 63.6 and 75% and the specificity 88.2 and 72.5% respectively.

CA125 and CA72-4 levels in bile samples were significantly higher in patients with malignant disease vs benign disease (Table 4). The estimated AUC and the cut-off values for distinguishing malignant vs benign disease are shown in Table 5 and Figure 1. The best cut-off values were 63.2 ng/ml for CEA, 272.8 IU/ml for CA19-9, 11.6 IU/ml for CA125, 19.8 IU/ml for CA72-4 and

Table 4. Comparison of bile tumor markers

Markers	Patients with malignant disease (N=44)	Patients with benign disease (N=51)	p value[a]
CEA	29.2 (1-6450)	27.3 (1.4-352)	0.1
CA19-9	12000 (7-12000)	12000 (10-12000)	0.76
CA125	26.2 (1-215)	7.7 (1-59)	<0.001
CA72-4	22.5 (0.8-200)	13.6 (0.8-144)	0.012
CA242	1136.3 (16-2000)	1114.7 (21-2000)	0.92

Values are expressed as median (range). [a]Mann-Whitney U test. The normal reference values in bile were as follows: CEA ≤ 63.2 ng/mL, CA19-9 ≤ 272.8 IU/mL, CA125 ≤ 11.6 IU/mL, CA72-4≤ 19.8 IU/mL, and CA242 ≤ 1407.1 IU/mL

1407.1 IU/ml for CA242. The diagnostic values of bile tumor markers using the above cut-off values are presented in Table 6. CA125, CEA and CA72-4 achieved the best diagnostic accuracy (69, 65 and 65% respectively). The sensitivity of CA125, CEA and CA72-4 were 75, 40.9 and 54.5% and the specificity 64.7, 86.3 and 74.5% respectively.

In an attempt to increase the diagnostic yield of tumor markers we analyzed different combinations of the best tumor markers (CA242 and CA19-9 in serum and CA125, CA72-4 in bile) along with total bilirubin levels. The detection of any 3 out of 5 had the best diagnostic accuracy (81%) with a sensitivity of 81.8% and specificity of 80.4% (Table 7).

Discussion

Several tumor markers have been detected in clinical samples, such as tissues, bile and serum, collected from patients with pancreatobiliary diseases. The uncertainty of their overall accuracy limits the use of these markers in early detection. However, they may be useful in conjunction with other diagnostic modalities for the differential diagnosis of benign vs malignant disease and may have some value in monitoring disease progression (i.e. CA19-9 can be used to guide treatment and follow-up and may have a prognostic value, in

Table 3. Diagnostic values of serum tumor markers

Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	Diagnostic accuracy (%)
CEA	25.0	92.2	73.3	58.8	3.20	0.81	61.0
CA19-9	75.0	72.5	70.2	77.1	2.72	0.35	73.7
CA125	31.8	88.2	70.0	60.0	2.69	0.77	62.1
CA72-4	9.1	100.0	100.0	56.0	NA	0.91	57.9
CA242	63.6	88.2	82.4	73.8	5.39	0.42	76.8

PPV: positive predictive value, NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio, NA: not applicable

Table 5. Area under the curve (AUC) and cut off value of bile tumor markers

Markers	AUC % (95% CI)	Best cut-off value
CEA	59.6 (47.8-71.5)	63.2 ng/ml
CA19-9	48.9 (37.2-60.6)	272.8 IU/ml
CA125	73.1 (62.6-83.5)	11.6 IU/ml
CA72-4	65.0 (53.7-76.3)	19.8 IU/ml
CA242	49.4 (37.6-61.3)	1407.1 IU/ml

Figure 1. Receiver Operating Characteristics (ROC) curves of bile tumor markers. ROC showing the diagnostic performance of CA19-9, CA125, CA72-4, CA242, and CEA.

the absence of cholestasis, for pancreatic cancer) [3,6,10,11,20,21].

In our study, we prospectively measured the concentration of tumor markers in the serum and bile of patients with pancreatobiliary diseases (malignant or benign) and tried to measure their diagnostic values as well as their ability to distinguish malignant from benign disease.

All 5 serum tumor markers were significantly higher in patients with malignant disease (p ≤0.05). Moreover, CA19-9 and CA242 revealed a p value ≤0.001 (Table 2). As regards to diagnostic values of serum tumor markers, the results were not optimal. Overall, CA242 and CA19-9 exhibited the highest diagnostic accuracy (76.8% and 73.7%, respectively), confirming previous reports [22-24]. CEA has been tested for the last 20 years and there are many reports regarding pancreatic cancer, with a varying sensitivity (25-54%) and specificity (78-81%) [25]. In our study the sensitivity of diagnosing malignancy was also low
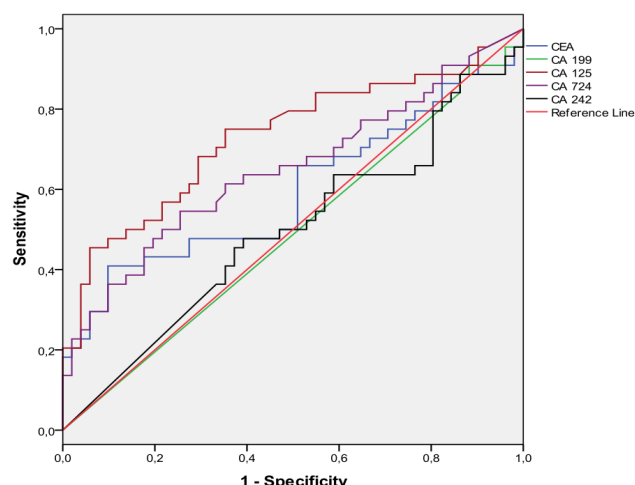
(25%). The unacceptable low sensitivity has limited its role in clinical practice and it has been replaced by other recent tumor markers. CA19-9 is the most used serum tumor marker in gastrointestinal malignancies. However, its sensitivity and specificity varies. Serum 19-9 has shown a sensitivity ranging from 70 to 90% and specificity from 68 to 91% for differentiating pancreatic cancer from chronic or recurrent pancreatitis [26,27]. Also, it has been reported that CA19-9 had a sensitivity ranging from 53 to 79% and specificity from

Table 6. Diagnostic values of bile tumor markers

Markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	Diagnostic accuracy (%)
CEA	40.9	86.3	72.0	62.9	2.99	0.68	65.3
CA19-9	90.9	11.8	47.1	60.0	1.03	0.77	48.4
CA125	75.0	64.7	64.7	75.0	2.12	0.39	69.5
CA72-4	54.5	74.5	64.9	65.5	2.14	0.61	65.3
CA242	47.7	60.8	51.2	57.4	1.22	0.86	54.7

PPV: positive predictive value; NPV: negative predictive value, PLR: positive likelihood ratio, NLR: negative likelihood ratio

Table 7. Diagnostic values of different combinations of bile and serum tumor markers

Number of positive markers	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	Diagnostic accuracy (%)
1 item	100.0	25.5	53.7	100.0	1.34	NA	60.0
2 items	95.5	51.0	62.7	92.9	1.95	0.09	71.6
3 items	81.8	80.4	78.3	83.7	4.17	0.23	81.1
4 items	61.4	92.2	87.1	73.4	7.87	0.42	77.9
5 items	22.7	98.0	90.9	59.5	11.4	0.79	63.2

NA: not applicable. PPV: positive predictive value, NPV: negative predictive value, PLR: positive-likelihood ratio, NLR: negative likelihood ratio. For the analyses of different combinations we used the 4 best tumor markers: CA242, CA19-9 in serum and CA125, CA72-4 in bile along with total serum bilirubin levels. The "3 items" row shows that the detection of any 3 out of 5 markers had the best diagnostic results

81 to 99% in differentiating cholangiocarcinoma [28-30]. In our study, CA19-9 had 75% sensitivity, 72.5% specificity and an accuracy of 74% in distinguishing malignant disease. The PLR and NLR, which are the best indicators for ruling in and out diagnosis, were low. In addition CA19-9 is elevated in jaundiced patients with biliary obstruction from benign conditions, such as choledocholithiasis or benign biliary strictures, but it tends to normalize after biliary drainage. If it still remains high, then the possibility of malignancy is increased [27,29,30]. The sensitivity and specificity of CA125 were 32 and 88% respectively, and generally serum CA125 shows poor sensitivity and specificity for the diagnosis of pancreatic cancer [31]. Our results failed to confirm CA125 as a more specific marker than CA19-9 in distinguishing between benign and malignant disease [25]. It is also increased in patients with cirrhosis, hepatitis, pancreatitis and in patients with jaundice [3,6]. When tumor markers were measured in bile (Table 4), only CA125 and CA72-4 were identified as possible tumor markers for discriminating patients with malignant or benign disease. The concentrations of CA19-9 and CA242 in bile, for both groups of patients, were very high and surpassed by far those in serum. Biliary obstruction or inflammation is supposed to induce the destruction of the barrier of the biliary epithelium and increases the permeability between the blood vessels and the bile. The increase of serum CA19-9 in patients with jaundice may be due to the reflux from the bile, where it is present in high concentrations [32,33]. CA125 achieved the best statistical significance between malignant and benign disease, as it is not so influenced by biliary obstruction or inflammation [33]. As regards to diagnostic values (Table 6), the results of our study were not optimal, as the sensitivity or specificity of bile tumor markers was relatively low. CA125 exhibited 69.5% diagnostic accuracy with CEA and CA72-4 having 65.3% each. CEA measured in bile, has been found to be increased in patients with cholangiocarcinoma and pancreatic cancer [34]. Increased levels of CEA are excreted in the bile of patients with perihilar cholangiocarcinoma and intrahepatic stones compared with patients with benign strictures [35]. Our results, revealed that biliary CEA, although yielding higher specificity (86.3%) than previously described (<70%) [12], has not reached a significant diagnostic yield to be considered as a sole marker for diagnosing malignant pancreatobiliary diseases. Moreover, the results of our

study confirmed that the bile markers CA19-9 and CA125 lack a significant diagnostic efficacy, so as to be considered as single tumor markers in the diagnosis of pancreatobiliary malignancies. According to literature, CA72-4 measured in bile has only 41.3% sensitivity for carcinomas of the pancreatobiliary system and 86.7% specificity [14]. Our study revealed even lower specificity (74.5%) without a significant increase of sensitivity (54.5%).

Combinations of tumor markers in serum and in bile specimens have been described in an attempt to improve the diagnostic efficacy in pancreatobiliary malignancies [12,22]. In the most recent publication the diagnostic accuracy was highest (69.2%) [22] with different combinations of serum CA19-9, CA242, CA125 and CEA. In our study, analyses of different combinations of 4 tumor markers (CA19-9, CA242 in serum and CA125, CA72-4 in bile) along with total bilirubin levels showed the best diagnostic values for distinguishing malignant disease, when any 3 out of 5 exceeded the critical values (Table 7). However, sensitivity, specificity and diagnostic accuracy demonstrated significant improvement (81,80 and 81% respectively) but cannot be considered optimal. In clinical practice, we are not willing to perform ERCP just for measuring bile tumor markers, but in doubtful cases when ERCP is required either for drainage or for sampling, it is worth sending a bile sample for measurement of tumor markers.

There are several drawbacks in our study. First of all we considered pancreatic cancer, cholangiocarcinoma and ampullary cancer cases all together, as group 1 or patients with malignant disease. The common feature of patients with malignant disease was the painless jaundice. The number of patients was too small to perform separate analysis. Anyway, the primary clinical objective point was to distinguish patients with malignant disease. The benign disease group included only patients with choledocholithiasis and not patients with chronic pancreatitis, Mirrizi syndrome or other causes of benign biliary obstruction. The diagnosis of choledocholithiasis can be easily made clinically and by imaging techniques, but we tried to establish cut-off levels for tumor markers differentiating between malignant disease and benign biliary obstruction.

In conclusion, serum and bile tumor markers, when studied alone, lack the diagnostic yield to discriminate benign from malignant pancreatobiliary disease. In cases of diagnostic dilem-

mas the combination of serum and bile markers might be helpful. New technologies are required in order to find more sensitive and specific tumor markers. Gene expression analysis and protein profiling techniques are under evaluation for the development of tumor markers [36-39] despite the criticism of their increased cost. Hopefully, new studies will come up with more accurate tumor markers in order to reduce the time to diagnosis and estimate the prognosis.

Conflict of interest

The authors declare that «Abbott Laboratories Hellas ABEE» and «A&L Medical Supplies Ltd» contributed to the cost of the diagnostic Kits.

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