Papers

Value of carcinoembryonic antigen (CEA) and cholesterol assays of ascitic fluid in cases of inconclusive cytology

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Abstract

***Aim*—To determine whether assays of carcinoembryonic antigen (CEA) and cholesterol in ascites add diagnostic value to cytology.**

***Methods*—The additional diagnostic efficacy of the biochemical assays was studied in the ascitic fluid from 130 patients, of whom 57 had peritoneal carcinomatosis. All diagnoses were verified by subsequent necropsy and/or histology.**

***Results*—CEA concentrations over 5 ng/ml indicated carcinomas, occasionally without peritoneal involvement of the tumour. However, increased values were significantly more common in cancer with peritoneal involvement ($p < 0.01$), giving a sensitivity of 51% and specificity of 97% for carcinomatosis. A cholesterol value exceeding 1.21 mmol/litre was found in 93% of cancers with peritoneal involvement, but it was not entirely specific (96%) for carcinomatosis. Simultaneous increases in CEA and cholesterol concentrations were specific for carcinomatosis and this combination increased the sensitivity for diagnosing carcinomatosis from 77% with cytology alone to 88%. The correct diagnosis could thus be made in five of 12 cases with inconclusive cytology.**

***Conclusions*—The measurements of both CEA and cholesterol concentrations in ascites give additional specific information about peritoneal carcinomatosis and can therefore be a useful adjunct to cytology—in particular, in inconclusive cases.**

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Keywords: ascitic fluid; carcinoembryonic antigen; cholesterol; cytology

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Conventional cytological examination of ascitic fluid often yields inconclusive results: apart from benign and malignant diagnoses, many cases are "suspicious for malignancy".[1–3] In liver cirrhosis, inflammation, chemotherapy, and irradiation it is often difficult to distinguish exfoliated reactive mesothelial cells from highly differentiated cancer cells. Moreover, the cytology is often false negative because only a few neoplastic cells are present in the fluid,[4] or processing of specimens is suboptimal with lysis of tumour cells.[5] Therefore, a specific cytological diagnosis may have a sensitivity of only 40–80%.[6–8] Samples that are inconclusive because of some cellular atypia can be further evaluated with ancillary morphological methods, such as immunocytochemistry[1–3 9] and DNA cytometry,[2 9 10] although these techniques are of little help when only a few preserved tumour cells are present.

Biochemical parameters, such as the concentrations of carcinoembryonic antigen (CEA)[6–8 10–12] and cholesterol[4 12–15] in the serous effusion, have been studied as markers of malignancy. Such assays can be done rapidly in properly preserved effusions after cytological examination, but their diagnostic value is still debated. It has therefore been stated that the cholesterol content of ascitic fluid is neither specific nor sensitive for a tumour.[5 16] Some authors have suggested that a high concentration of CEA in the ascitic fluid is indicative of peritoneal metastases from a CEA producing tumour,[7 12] although it has been found in carcinomas without serosal involvement.[6 10] These earlier studies were all based on data with incomplete follow ups. The most reliable way to exclude involvement of the peritoneum by a tumour is to perform a thorough postmortem examination, and results that are not corrected for inadequate follow up are difficult to interpret.[13]

In our study, we investigated the possible additional value of cholesterol and CEA assays to ascites cytology—in particular, when the cytological findings alone are inconclusive. In all cases, the results of cytological and chemical analyses were related to the stratified true diagnosis—that is, the presence or absence of peritoneal malignancy, as established at necropsy and/or histological examination.

Material and methods

These studies were carried out on 130 consecutive patients (67 women, aged 28–87 years and 63 men, aged 26–85 years) with ascites, who were admitted to MÁV Hospital in Budapest, Hungary. Their ascitic fluid was submitted for cytological examination and the true diagnosis was established at necropsy and/or by histology within one year of receipt.

Table 1 Origin of 77 carcinomas with or without peritoneal involvement and the proportion of them producing carcinoembryonic antigen (CEA)

Origin	N	With peritoneal involvement: Total no.	With peritoneal involvement: Cases with CEA ⩾ 5 ng/ml	Without peritoneal involvement: Total no.	Without peritoneal involvement: Cases with CEA ⩾ 5 ng/ml
Ovary	18	18	4	0	0
Liver	18	1	1	17	0
Stomach	13	11	8	2	1
Large intestine	11	10	10	1	1
Pancreas	8	8	6	0	0
Lung	3	2	0	1	0
Gall bladder	2	1	0	1	0
Breast	2	1	0	1	0
Kidney	2	2	0	0	0
Total	77	54	29	23	2

Biopsy based histological diagnosis alone was accepted as conclusive only when peritoneal carcinomatosis was shown (17 cases).

Conventional cytological examination of ascitic fluid was carried out on haematoxylin and eosin and Giemsa stained cytospin (⩾ 4 glasses/specimen) sediments. The evaluation was performed independently by two cytopathologists, using three diagnostic categories: benign (negative), inconclusive (mainly including "borderline" and "suspicious for malignancy"), and malignant (positive). When calculating specificity, sensitivity, and predictive values, only "malignant" was considered abnormal, because inconclusive cytology was incorporated with the "negatives". Aliquots from the supernatant were taken for chemical analysis. CEA was determined by a two stage sandwich enzyme linked immunosorbent assay method, using mouse monoclonal antibody (Dako, Glostrup, Denmark) and total cholesterol was measured enzymatically, using a colorimetric analysis adapted for the autoanalyser system (Technicon RA-100, Tarrytown, New York, USA). Repeated analyses were performed with standard deviations of 2% and 3% for CEA and cholesterol, respectively. All assays were run in duplicate, at least. Cut off limits for the chemical analyses were calculated on the basis of receiver operating characteristics (ROC).[17] The independence of the true diagnosis and the classification by the test used was checked by χ^2 statistics at a rejection value of $p < 0.05$.

Results

Of the 130 patients, 50 showed no sign of neoplastic disease at necropsy, the cause for the benign ascitic effusion being liver cirrhosis (41 cases), peritonitis (two cases), and cardiac decompensation (seven cases). The remaining 80 patients had a malignancy. Of 57 patients with malignant involvement of the peritoneum, 54 had a carcinoma accompanied by peritoneal carcinomatosis and three had non-epithelial malignancies: one mesothelioma, one melanoma, and one rhabdomyosarcoma. In 23 others, the ascites resulted from metastases in the liver and/or portal lymph nodes or a primary liver cancer, with no malignant involvement of the peritoneum at necropsy (table 1).

Of the 51 cases classified cytologically as benign (negative), 50 were correctly identified as non-neoplastic with regard to peritoneal involvement of the tumour, but one was false negative. In nine of these 50 cases, tumours were found in organs, without involvement of the peritoneum. All 44 cases with malignant cytology had carcinomatosis, and in 35 cases (27%), the cytological report was inconclusive. Twelve of these proved to have peritoneal carcinomatosis on later biopsy or necropsy, 14 had a carcinoma without carcinomatosis, whereas no tumour was found in the remaining nine inconclusive cases.

Figure 1 shows the distribution and covariation of CEA and cholesterol values. Only one patient without a tumour—a patient with liver cirrhosis and freshly perforated diverticulosis—had a CEA concentration close to the 5 ng/ml cut off value. All the remaining 31 cases with raised CEA concentrations had a carcinoma; the specificity of CEA for a tumour was 100% whereas the sensitivity was 39%, regardless of whether the peritoneum was involved. Although CEA production is most common in gastrointestinal tumours, it also occurs in various other types of carcinomas and, in 31 cases,

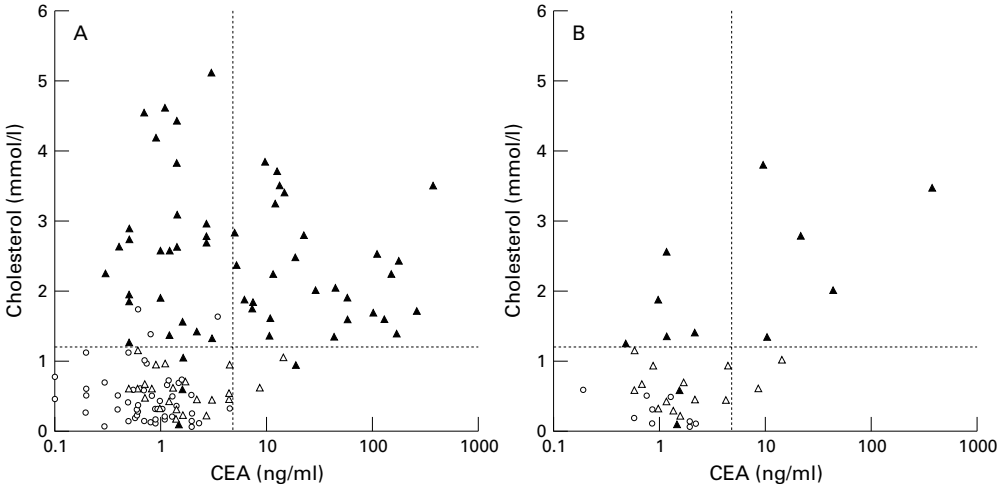

Figure 1 Concentrations of carcinoembryonic antigen (CEA) and cholesterol in ascitic fluid. (A) All 130 cases and (B) 35 cases with inconclusive cytology. Benign effusion, open circles; carcinoma without peritoneal involvement, open triangles; peritoneal carcinomatosis, closed triangles. The cut off values for the two analyses are shown as broken lines.

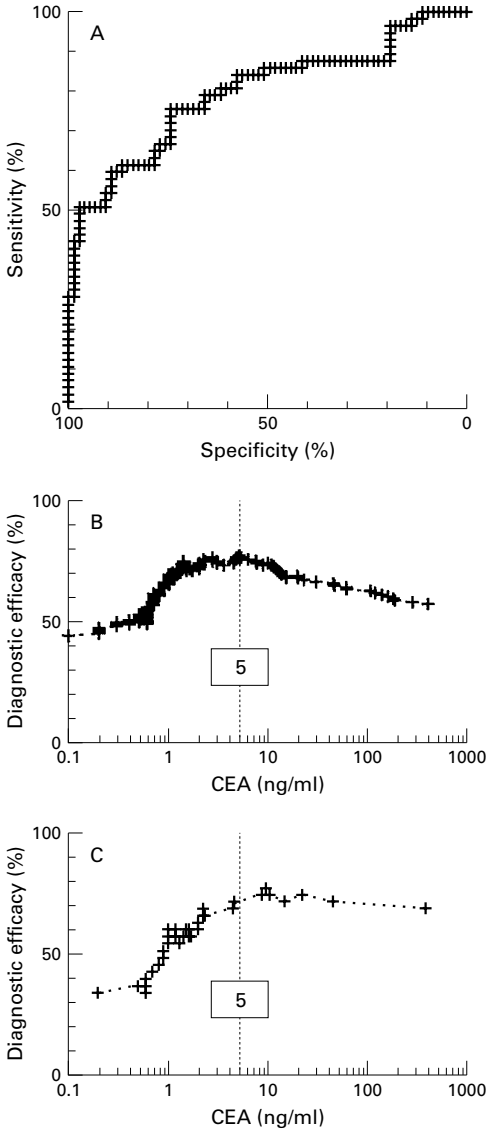

Figure 2 Performance of the carcinoembryonic antigen (CEA) analysis as a measure to detect peritoneal carcinomatosis: (A) receiver operating characteristic plot and (B) diagnostic efficacy as a function of cut off values for the total material and (C) for cases with inconclusive cytology. The last two curves are similar in shape, suggesting an optimal cut off of 5 ng/ml.

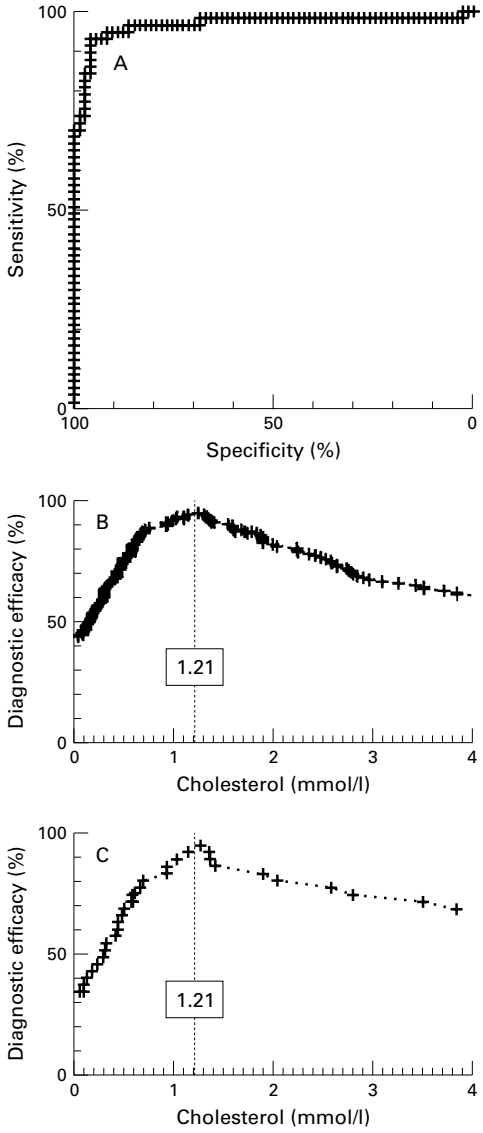

Figure 3 Performance of the cholesterol analysis in detection of carcinomatosis: (A) receiver operating characteristic plot and (B) diagnostic efficacy as a function of cut off values for the total material and (C) for cases with inconclusive cytology. The optimal cut off value of 1.21 mmol/litre is the same for the two groups.

the secretion of CEA was sufficient to raise the values above the cut off point (table 1). In two of these carcinomas there was no carcinomatosis; however, high CEA values were significantly more common in carcinomas with peritoneal involvement ($p < 0.01$). All three non-epithelial malignancies had low CEA concentrations. The diagnostic performance of the CEA analysis (fig 2) shows that the selected cut off value is also suitable for analysing cases of the considerably smaller subpopulation with inconclusive cytology (fig 2C).

Whereas an increased concentration of CEA is seen not only in peritoneal carcinomatosis, but rather indicates the presence of a CEA producing carcinoma, a raised concentration of cholesterol in the ascitic fluid seems to be a sign solely of neoplastic peritoneal involvement. The optimal diagnostic efficacy is obtained with a cut off point of 1.21 mmol/litre, and the same threshold is obtained for the cytologically inconclusive samples (fig 3). At this cut off point, the groups of cancers with or without carcinomatosis can be almost entirely separated from each other (fig 1), giving a sensitivity of 93% and a specificity of 96% for carcinomatosis (table 2). In a few cases, raised cholesterol concentrations are seen in ascites associated with non-malignant conditions; two such patients had purulent peritonitis and the third chronic heart failure. Such high values, in combination with non-inflammatory cytology, are therefore most common in association with peritoneal malignancy, but occasional other causes of increased values prevent this parameter from being used as a conclusive ancillary analysis alone.

Simultaneous increases in CEA and cholesterol concentrations in ascitic fluid were specific for carcinomatosis (fig 1). When the two chemical assays are combined, they add

Table 2 Diagnostic value of cytology, carcinoembryonic antigen (CEA), cholesterol, and their combined use in distinguishing between 57 ascites from patients with peritoneal carcinomatosis and 73 cases without malignant involvement of the peritoneum

Assays	Definition of abnormality	Specificity (n/N)	Sensitivity (n/N)	Predictive value (n/N)	
				Positive	Negative
Cytology alone	Malignant cytology*	100% (73/73)	77% (44/57)	100% (44/44)	85% (73/86)
CEA alone	Raised CEA†	97% (71/73)	51% (29/57)	94% (29/31)	72% (71/99)
Cholesterol alone	Raised cholesterol‡	96% (70/73)	93% (53/57)	95% (53/56)	95% (70/74)
Combined CEA + cholesterol	High concentrations of CEA† and cholesterol‡	100% (73/73)	49% (28/57)	100% (28/28)	72% (73/102)
Cytology and combined CEA + cholesterol	Malignant cytology* and/or high concentration of CEA† and cholesterol‡	100% (73/73)	88% (50/57)	100% (50/50)	91% (73/80)

*Malignant diagnoses: "positives" only; inconclusive cytology is incorporated with "negatives".
†Cut off point for CEA, 5 ng/ml.
‡Cut off point for cholesterol, 1.21 mmol/litre.

diagnostic value (table 2) by increasing the sensitivity of the specific diagnosis of peritoneal carcinomatosis from 77% (95% confidence interval (CI), 71.6% to 82.8%) with cytology alone to 88% (95% CI, 83.4% to 92.1%). In five of the 12 carcinomatosis samples with inconclusive cytology, both markers exceeded the respective cut off values, indicating neoplastic peritoneal involvement. This gives a 42% diagnostic sensitivity among inconclusive cases, the 95% CI being 27% to 56%. Two cases with borderline cytology had raised CEA values alone, suggestive of a carcinoma not yet accompanied by carcinomatosis. Among the cases with negative cytology (n = 51), the only case with carcinomatosis was detected by the simultaneous increase of cholesterol and CEA.

Discussion

Ascites can have many causes. In about 80% of cases, it occurs during decompensation of chronic hepatic cirrhosis, but tumours account for 10% of cases, thereby constituting the second most common cause of ascitic fluid.[18] Less frequent reasons include congestive heart failure (3%) and inflammatory conditions (3%), whereas other causes, such as nephrotic syndrome, exudative enteropathy, and chylous ascites, are still less common. In about two thirds of tumour associated effusions, peritoneal carcinomatosis is found, but in one third of cases, the cancer gives rise to the effusion by mechanisms other than those related to peritoneal involvement.[5] The presence of tumour cells in an effusion sediment is seen only in peritoneal carcinomatosis, but the sensitivity of detecting such cells by cytology is limited, as a result of the low effusion volume with a small yield and/or poor preservation of cells. At the same time, benign mesothelial cells may be growth stimulated, and the resulting "mesotheliosis" is sometimes impossible to distinguish from malignant cells by routine morphology alone. These problems add to the number of inconclusive cytological reports of a "borderline lesion".

Very high concentrations of CEA are primarily caused by adenocarcinomas,[11] and this antigen can be traced in serum and effusions. Usually, high CEA values in effusions occur in carcinomas of the gastrointestinal tract, mucinous ovarian cancer, breast cancer, and non-small cell carcinomas of the lung.[10] Raised CEA concentrations in effusions have been described in carcinomas with[7 12] or without carcinomatosis.[6 10] In this last case, the effusion is often caused by hepatic involvement and portal hypertension, or by obstruction of lymphatic flow from massive abdominal lymph node metastases. In our study, two cases of carcinomas with increased CEA values had inconclusive changes on cytology, although involvement of the peritoneum by the tumour could not be seen. These morphological changes may be explained by non-malignant growth stimulation and proliferation, although the presence of macroscopically undetected metastases[7] cannot be excluded. Increased CEA concentrations have also been reported in benign ascites with perforations of gastrointestinal organs.[8 11] We had one benign sample with a CEA close to the cut off point, and this effusion was associated with cytological evidence of faecal contamination from a perforated colon.

The increased concentration of cholesterol in effusions is more specifically related to tumour involvement of the serosal cavity. This can be the result of various mechanisms that act together. The cholesterol may originate in cell membranes, perhaps as a result of disintegration of tumour cells and/or surrounding benign cells.[4 19] It can also enter the cavity from the interstitial space because of obstructed lymph vessels or be related to increased permeability of the carcinomatous serous membrane.[13 20] Raised cholesterol concentrations have also been reported in inflammatory conditions involving the peritoneum and associated with chronic cardiac congestion.[5 13]

The diagnosis of a malignant condition in the peritoneum necessitates maximal specificity to be useful in clinical practice. The examination of exfoliated cells provides the first line analysis, allowing a correct diagnosis in most cases with carcinomatosis. The need for specificity prevents higher sensitivity, and some samples are reported as inconclusive. Ascitic cholesterol values over 1.21 mmol/litre are a sensitive, although not entirely specific, marker for carcinomatosis, whereas CEA values exceeding 5 ng/ml indicate a carcinoma. In our series, there was no benign case with simultaneous increases of both biochemical parameters. The determination of both cholesterol and CEA concentrations in ascitic fluid thus adds information to the cytological diagnosis of peritoneal carcinomatosis. Although a high sensitivity is obtained with cytology alone, the diagnostic improvement is significant, and the combined analyses can be used as an adjunct to conventional cytology; in particular, when the

cytology is inconclusive. When both parameters are raised, the diagnosis of a carcinomatosis can be made, thereby avoiding the need of a second paracentesis.

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