Ascitic Fluid Analysis for the Differentiation of Malignancy-Related and Nonmalignant Ascites

Proposal of a Diagnostic Sequence

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The authors tried to differentiate malignancy-related from nonmalignant ascites with a sequence of sensitive followed by specific ascitic-fluid parameters. There were four results of this study. First, of nine parameters investigated in a first series of 48 patients, 28 with nonmalignant and 20 with malignancy-related ascites, ascitic-fluid cholesterol and fibronectin yielded the best negative predictive value of 92% each. Carcinoembryonic antigen (CEA) and cytologic examination both showed a positive predictive value of 100%. Second, combining cytologic examination (sensitivity, 70%) and CEA determination (sensitivity, 45%) increased the sensitivity to 80%. Third, cytologic findings were negative in all ascitic-fluid samples with a cholesterol concentration below the cutoff value of 45 mg/100 ml. Fourth, based on the results of the first series of 48 patients, the diagnostic sequence with cholesterol as a sensitive parameter, followed by the combination of cytologic examination and CEA determination as specific parameters, was tested in a second series of 71 patients, 37 with nonmalignant and 34 with malignancy-related ascites. Again cytologic examination was negative in all samples with cholesterol levels below 45 mg/100 ml. In the total of 119 patients, this diagnostic sequence did not identify 9% of patients with malignancy-related ascites, and 82% of samples classified as malignancy related by cholesterol levels above 45 mg/100 ml were confirmed by positive cytologic examination and/or CEA level above 2.5 ng/ml. Thus, a diagnostic sequence with ascitic-fluid cholesterol determination, followed by cytologic examination and CEA determination, in samples with cholesterol levels above 45 mg/100 ml should permit a cost-efficient routine differentiation of malignancy-related from nonmalignant ascites. *Cancer* 68:1808–1814, 1991.

THE DIFFERENTIATION between malignancy-related (MRA) and nonmalignant ascites (NMA) is important for further diagnostic and therapeutic procedures. Several components of ascitic fluid were tested for their differential diagnostic usefulness. Cytologic investigation of ascitic fluid is specific but may produce a large percentage of false-negative results; its sensitivity ranges between 40% and 70%.[1,2] Therefore, other parameters of ascitic fluid were investigated. In addition to the most widely used total protein determination,[3–5] several other parameters were considered for their usefulness in the differential diagnosis of ascites: ascitic-fluid lactic dehydrogenase (LDH)[6] or carcinoembryonic antigen (CEA)[7,8] and the ratio of concentrations in ascites divided by concentrations in the serum of LDH[6,9,10,11] and protein.[6,9,11] More recently, an excellent differential diagnostic efficiency was reported for the albumin gradient[9,12] (*i.e.*, the difference between the concentrations of albumin in the serum and ascites), ascitic-fluid fibronectin,[10] and ascitic-fluid cholesterol.[13–16]

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For the detection of MRA, sensitive parameters should be preferred over more specific ones in a first screening step. Confirmation of the suspected diagnosis in the subgroup classified as MRA by the sensitive parameter might then be achieved by means of a specific procedure in a second step.

Therefore, we prospectively compared the differential diagnostic power of several ascitic-fluid parameters and found an efficient diagnostic sequence using a sensitive parameter, followed by a specific parameter.

Materials and Methods

The following criteria were applied for patient classification: (1) NMA was identified in those patients in whom malignancy was excluded by ultrasonography, computed tomography, autopsy, or follow-up—the diagnosis of cirrhosis was based mostly on histologic findings and (2) MRA was diagnosed where peritoneal carcinomatosis was found by computed tomography, peritoneoscopy, peritoneal biopsy or autopsy, and follow-up—in most cases histologic proof of the primary tumor was obtained. In MRA patients liver disorders were excluded by ultrasonography or computed tomography. The presence of liver metastases was established likewise. Subjects with neither cirrhotic nor malignant ascites were classified as having miscellaneous NMA and included in this group.

Patients

Two series of patients were investigated. The first series (48 patients) was used to compare several parameters for their differential diagnostic characteristics and to elaborate a diagnostic sequence. The second series (71 patients) was used to test this diagnostic sequence.

Series 1: This series consisted of 28 patients (18 men and ten women; age range, 28 to 81 years) with NMA and 20 patients (six men and 14 women; age range, 18 to 75 years) with MRA. Of the 28 patients with NMA, 22 had cirrhosis (alcoholic in nine, posthepatitic in four, biliary in two, and mixed or cryptogenic in seven patients). Six patients had miscellaneous NMA; two patients showed ovarian overstimulation from administration of gonadotropic hormones for infertility, and one patient each had pancreatitis, peritoneal tuberculosis, Budd–Chiari syndrome, and systemic lupus erythematosus, respectively. Eighteen of 20 patients with MRA had peritoneal carcinomatosis and ovarian carcinoma (seven patients), carcinoma of the stomach (three patients), breast cancer (two patients), carcinoma of the bladder (one patient) and of the kidney (one patient), hepatocellular carcinoma (one patient), adenocarcinoma of unknown origin (one patient), liposarcoma (one patient), and leukemia (one patient), as underlying diseases. One patient with carcinoma of the stomach and another patient with breast cancer had liver metastases but no evidence of peritoneal carcinomatosis.

Series 2: The second series consisted of 71 patients. Thirty-seven (19 men and 18 women; age range, 36 to 79 years) of these 71 patients had NMA. There were 30 patients with cirrhosis of the liver and seven patients with miscellaneous nonmalignant diseases (four patients with congestive heart failure and one patient each with Budd–Chiari syndrome, portal vein thrombosis, and ovarian overstimulation). Thirty-four patients (ten men and 24 women; age range, 27 to 83 years) in the second series had MRA: 32 patients had evidence of peritoneal carcinomatosis with carcinoma of the ovary (14 patients), of the pancreas (five patients), of the breast (four patients), of the stomach (three patients), of the colon (three patients), of the gallbladder (one patient), of the bladder (one patient), and mesothelioma (one patients) as underlying diseases. Two patients had no evidence of peritoneal carcinomatosis: one with hepatocellular carcinoma and one with liposarcoma and liver metastases.

Methods

Samples of blood and ascites were obtained simultaneously. Cytologic examination of ascitic fluid was done independently by two investigators on Papanicolaou and Giemsa stained smears of the sediment obtained by centrifugations of 10 to 20-ml samples. Total protein was determined by a commercially available biuret method (Merck, Darmstadt, Germany). Cholesterol and LDH were measured enzymatically with commercial test kits (Boehringer, Mannheim, Germany). Albumin was analyzed by radial immunodiffusion on Nor-Partigen plates (Behring, Marburg, Germany). Fibronectin was determined by means of laser nephelometry with a commercial antiserum against human fibronectin (Behring). The CEA level was determined with a monoclonal antibody by a commercial kit (Abbott, Wiesbaden, Germany).

Receiver–operator characteristic curves were calculated by standard procedures.[17] Applying cutoff limits for the determined parameters permitted classification into four categories: true positive (a), true negative (b), false positive (c), and false negative (d). The sensitivity was calculated as $a/(a + d) \times 100$, the specificity as $b/(b + c) \times 100$, the positive predictive value as $a/(a + c) \times 100$, the negative predictive value as $b/(b + d) \times 100$, and the diagnostic efficiency as $(a + b)/(a + b + c + d) \times 100$.[17] The significance of differences of sensitivity, specificity, or efficiency between various parameters was evaluated by the chi-square test. A P value less than 0.05 was considered statistically significant for all tests applied.

Results

Ascitic-fluid concentrations of the parameters investigated in the 48 patients in Series 1 are shown in Figure

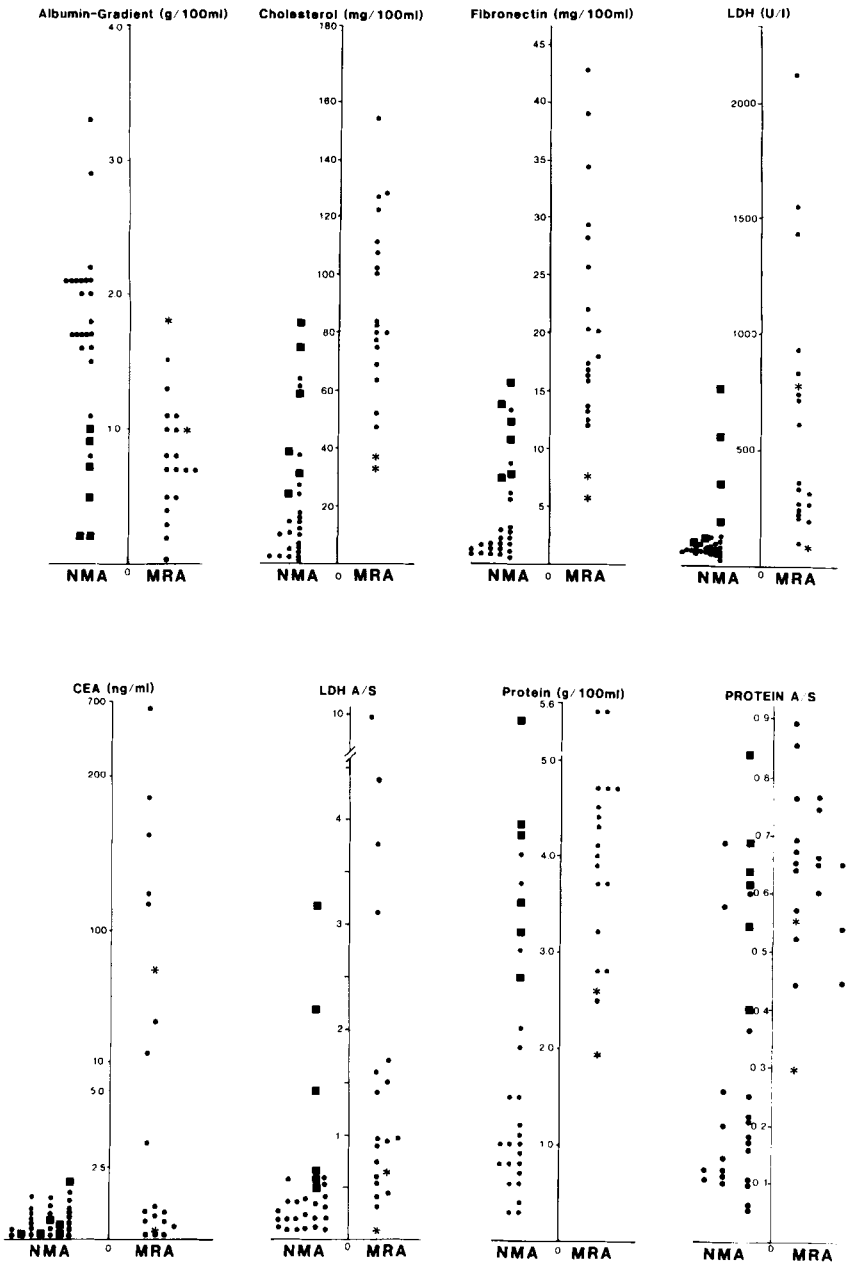

FIG. 1. (Top) Albumin gradient (serum − ascites concentration difference of albumin) and ascitic fluid concentrations of cholesterol, fibronectin, LDH, and of (bottom) CEA, ascites/serum concentration ratios of LDH (LDH A/S), of protein, and ascites/serum concentration ratios of protein (protein A/S). Data obtained from 48 patients of series 1: 28 patients with nonmalignant ascites (NMA), 22 patients with cirrhosis (●), six patients with miscellaneous nonmalignant disease (■), and from 20 patients with malignancy-related ascites (MRA), 18 patients with peritoneal carcinomatosis (•), and two patients with liver metastases (*).

1. This scattergram illustration shows the overlap between MRA and NMA was different for various parameters. The two patients with hepatic metastases without peritoneal carcinomatosis were ranked by most parameters in the concentration range of NMA.

As illustrated by the receiver-operator characteristic curves (Fig. 2), the differential diagnostic efficiency of cholesterol and fibronectin, closely followed by LDH, was superior to that of the other parameters. This observation was confirmed when sensitivity, specificity, positive and negative predictive values, and diagnostic efficiency were calculated (Table 1). At discrimination points of 45 mg/100 ml and 10.0 mg/100 ml, respectively, cholesterol and fibronectin reached 90% sensitivity and 82% specificity. Protein determination showed 90% sensitivity at a cutoff value of 2.5 g/100 ml; however, the specificity was only 68%. Specificity and differential diagnostic efficiency of both cholesterol and fibronectin were significantly better ($P < 0.05$) than the corresponding values of protein determination. The LDH (200 U/l) and albumin gradient (1.1 g/100 ml) yielded 85% sensitivity with 89% and 71% specificity, respectively.

Cytologic examination and CEA determination (2.5 ng/ml) were 100% specific. The sensitivity of cytologic examination was superior to that of CEA determination: 70% *versus* 45%. A combination of these specific parameters increased their sensitivity. Whereas only nine of 20 patients with MRA were detected by CEA and 14 of 20

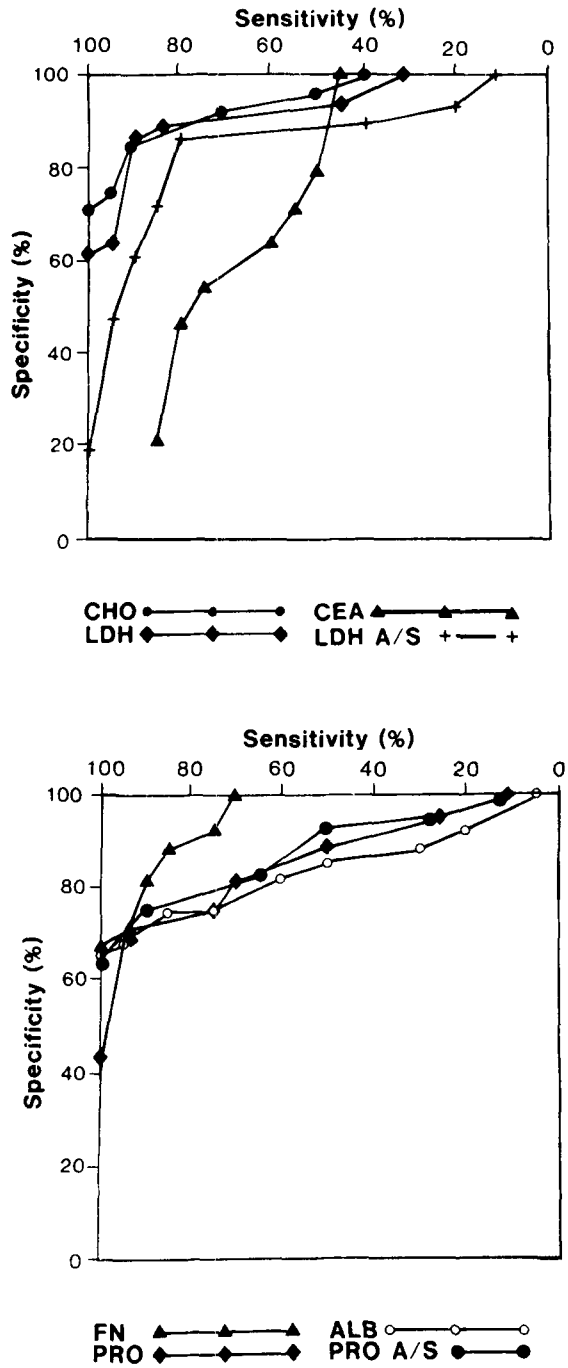

FIG. 2. Receiver-operator characteristic curves (ROC), displaying sensitivity and specificity at various discrimination levels for (top) cholesterol (CHO), LDH, CEA, ascites/serum concentration ratio of LDH (LDH A/S), and of (bottom) fibronectin (FN), protein (PRO), ascites/serum concentration ratio of protein (PRO A/S), and the serum − ascites concentration difference of albumin (ALB). Data were obtained from 48 patients of series 1: 28 with nonmalignant and 20 with malignancy-related ascites. With improving differential diagnostic efficiency, the curves approach the left upper corner (100% sensitivity and 100% specificity) of the illustration.

by positive cytologic findings, 16 patients had either positive cytologic findings and/or CEA values above the cutoff level.

The idea of using the sequence of a sensitive parameter and confirmation of positive findings by means of a specific parameter was tested in the 48 patients in Series 1. A sequence was examined with fibronectin or cholesterol (the parameters with the best negative predictive value of 92% in Series 1) or the conventional protein determination, as the first step, followed by a combination of cytologic examination and CEA determination for samples above the discrimination point. One patient with peritoneal carcinomatosis and positive cytologic findings was misclassified by protein determination, but ascitic fluid with cholesterol or fibronectin concentrations below the respective discrimination values did not include peritoneal carcinomatosis samples. Two patients with MRA and liver metastases without peritoneal carcinomatosis showed cholesterol and fibronectin concentrations below the cutoff level. However, cytologic findings were negative in these patients and thus did not provide any additional information. All 18 patients with peritoneal carcinomatosis showed ascitic-fluid values of cholesterol, fibronectin, and protein above their respective cutoff levels. Sixteen of these samples could be confirmed as MRA by positive cytologic findings and/or CEA concentrations more than 2.5 ng/ml.

This diagnostic sequence for the differentiation of MRA from NMA was tested in Series 2 with 71 patients (Fig. 3). Because cholesterol was shown to be equally effective to fibronectin in the first series of patients and cholesterol offers considerable advantages over fibronectin in terms of simplicity and costs of determination, it was chosen as the sensitive parameter to be tested in Series 2. The cholesterol concentration was compared with the total protein concentration; the latter is the most widely used parameter for ascitic-fluid differential diagnosis. There were two of 37 patients with NMA with a cholesterol level above the cutoff value (one patient with congestive heart failure and another with portal vein thrombosis). Three of the 34 patients with MRA had ascitic-fluid cholesterol levels less than 45 mg/100 ml. Cytologic examination, however, was negative in these three samples (one patient with peritoneal carcinomatosis, one with hepatocellular carcinoma, and one with liver metastases). Cholesterol determination was superior to protein determination in this second series of patients. Of 37 patients with NMA, 11 patients (six with cirrhosis and five with miscellaneous diseases) were classified incorrectly by protein concentration. Of 34 patients with MRA, seven patients (five with peritoneal carcinomatosis and one each with liver metastases and hepatocellular carcinoma) had a total protein less than 2.5 g/100 ml. Three of these seven patients had positive cytologic findings in their ascitic fluid.

After applying the sequence of cholesterol or protein as the initial sensitive parameter and a combination of cytologic findings and CEA determination as the specific parameter to the total number of 119 patients in Series 1

TABLE 1. Sensitivity, Specificity, Efficiency, and Positive and Negative Predictive Value of the Investigated Variables in Separating 28 Patients With Nonmalignant Ascites From 20 Patients With Malignancy-Related Ascites (48 Patients of Series 1)

	Sensitivity (%)	Specificity (%)	Efficiency (%)	Predictivity		Cutoff value
				Positive (%)	Negative (%)	
Cholesterol	90	82	85	78	92	45 mg/100 ml
Fibronectin	90	82	85	78	92	10 mg/100 ml
Protein	90	68	77	67	90	2.5 g/100 ml
LDH	85	89	88	85	89	200 U/l
Albumin gradient	85	71	77	68	87	1.1 g/100 ml
Protein A/S	80	79	79	73	85	0.5
LDH A/S	70	86	79	78	80	0.6
Cytology	70	100	88	100	82	
CEA	45	100	77	100	72	2.5 ng/ml

LDH: lactic dehydrogenase; CEA: carcinoembryonic antigen.

and 2, six patients with peritoneal carcinomatosis were classified incorrectly by protein, four of whom would have been detected by positive cytologic findings. Cholesterol determination, however (Fig. 4), missed only one patient with peritoneal carcinomatosis, but cytologic findings were negative in this sample. With both parameters, three and four patients, respectively, with hepatocellular carcinoma or liver metastases without peritoneal involvement were classified incorrectly; cytologic examination was negative in each of these samples. Forty-nine of 50 patients with peritoneal carcinomatosis showed an ascitic-fluid cholesterol level of more than 45 mg/100 ml, but only 44 had an ascitic-fluid protein level more than 2.5 g/100 ml. The suspected diagnosis of MRA was confirmed by positive cytologic findings and/or CEA concentration more than 2.5 ng/ml in 46 patients with elevated cholesterol and in 42 patients with elevated protein. Thus, only ten of 119 patients showed an ascitic-fluid cholesterol concentration more than 45 mg/100 ml but negative cytologic findings and CEA below cutoff level: seven patients with NMA and three patients with peritoneal carcinomatosis. The corresponding scheme with protein determination classified 22 patients, 20 with NMA and two with peritoneal carcinomatosis with suspected but not confirmed MRA.

Discussion

Our results in patients with MRA and NMA show that none of the investigated ascitic-fluid parameters could separate these two groups of patients totally. In the first series of 48 patients, cholesterol, fibronectin, and protein were the most sensitive parameters, closely followed by the albumin gradient and LDH, confirming earlier studies[6,9-16] Each of the 18 samples of peritoneal carci-

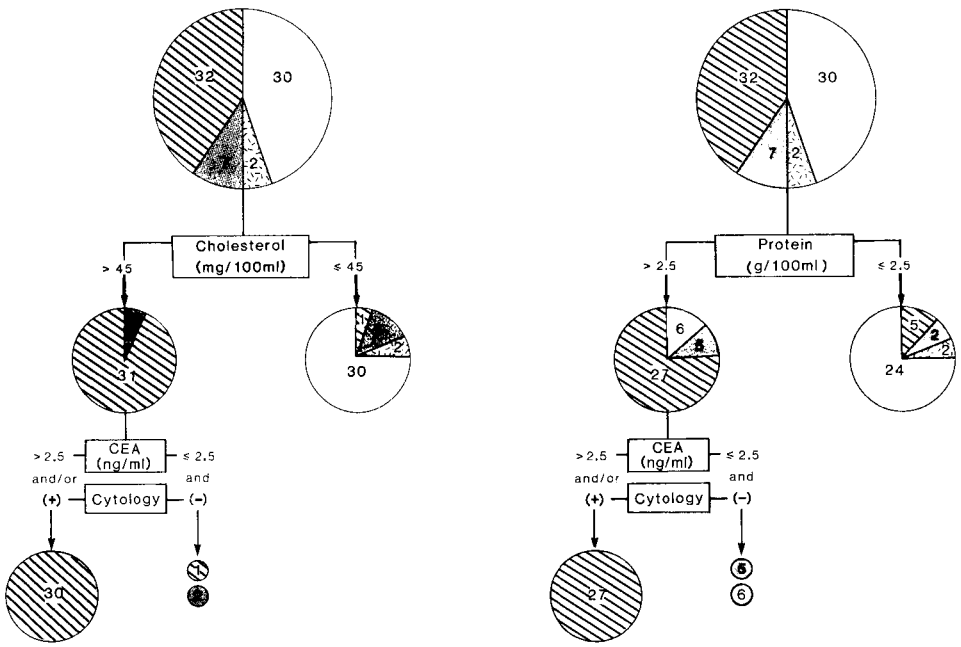

FIG. 3. Classification of 71 patients of series 2: 37 patients with nonmalignant ascites (30 patients with cirrhosis, seven patients with miscellaneous diseases) and 34 patients with malignancy-related ascites (32 patients with peritoneal carcinomatosis, one patient with liver metastases, and one patient with hepatocellular carcinoma) by determination of rather sensitive variables, such as cholesterol (left) or protein (right) in ascitic fluid. Samples with values above the respective cutoff levels were classified further by cytology and CEA determination. ▨: Peritoneal carcinomatosis; □: cirrhosis of the liver; ▩: liver metastasis-HCC; ▢: miscellaneous nonmalignant diseases.

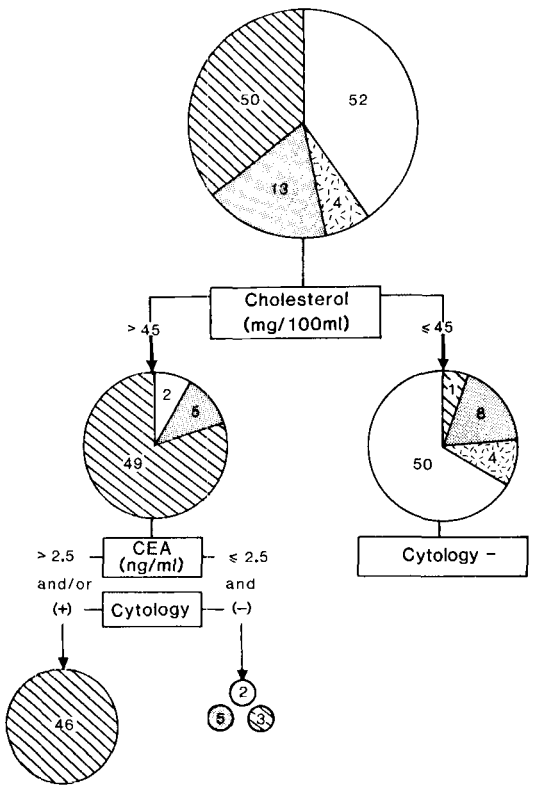

FIG. 4. Classification of the total of 119 patients: 65 patients with nonmalignant ascites (52 patients with cirrhosis, 13 patients with miscellaneous diseases) and 54 patients with malignancy-related ascites (50 patients with peritoneal carcinomatosis, four patients with liver metastases or hepatocellular carcinoma) by the proposed diagnostic sequence with cholesterol as the sensitive variable. ▨: Peritoneal carcinomatosis; □: cirrhosis of the liver; ▨: liver metastasis-HCC; □: miscellaneous nonmalignant diseases.

nomatosis ascites showed cholesterol concentrations more than 45 mg/100 ml and fibronectin concentrations greater than 10 mg/100 ml whereas in one of these samples ascitic protein content was less than 2.5 g/100 ml. Cytologic examination was negative in four patients with peritoneal carcinomatosis and also in the ascites of the two patients with liver metastases. Thus, additional cytologic examination did not increase the sensitivity in ascitic samples with cholesterol or fibronectin concentration above the discrimination level.

None of the examined parameters could classify ascites from patients with liver metastases without peritoneal carcinomatosis as MRA. This observation confirms earlier reports[18,19] and could explain the rather low sensitivities observed in studies with a considerable prevalence of such samples.[20] In a clinical setting where the underlying disease is to be diagnosed, however, such patients cannot be excluded from analysis and "subgrouping" of patients,[18] although useful in pathophysiologic studies, is not helpful. When MRA is indicated, further investigations, applying imaging techniques, will be preferred to define the nature and exact location of the tumor.

With the idea of a simple and cost-efficient diagnostic sequence for the differentiation of MRA and NMA in mind, we looked for a parameter with high negative predictive value to exclude patients with NMA and a parameter with high positive predictive value to confirm the suspected diagnosis of MRA in a second step. This second step of the diagnostic sequence was necessary to exclude false-positive results obtained by the sensitive parameter. Both fibronectin and cholesterol (each with a 92% negative predictability) appeared to be suitable candidates as sensitive parameters. Cholesterol determination was chosen for further investigation in a second series of patients because it is available and low priced in most laboratories, whereas fibronectin determination can be more complicated. Protein determination, despite its rather low specificity, was selected for comparative testing because it may be regarded as the most widely used routine parameter in ascitic fluid.

Both cytologic examination and CEA determination had 100% positive predictivity. Thus, the combination of both parameters could increase sensitivity without reducing specificity. In the first series of patients, the combination of cytologic examination (70% sensitivity) and CEA determination (45% sensitivity) yielded 80% sensitivity at unchanged specificity of 100%. Thus, a combination of cytologic examination and CEA measurement was chosen as the second parameter of the differential diagnostic test sequence.

In a second series of 71 patients, it was confirmed that no ascitic sample of a patient with peritoneal carcinomatosis and positive cytologic findings was missed by cholesterol determination, whereas three were missed by protein determination. Cytologic examination thus could be regarded as unnecessary in ascitic-fluid samples with a cholesterol level less than 45 mg/100 ml. In 30 of 31 patients, the classification of MRA (by cholesterol determination) was confirmed by cytologic examination and/or CEA determination. Two samples misclassified as MRA by cholesterol were negative by cytologic findings and/or CEA determination.

In the total of 119 patients, cholesterol had a sensitivity of 91% and protein, of 83%. Pursuing the idea of a sequence of a sensitive and, in the case of classification as MRA, a specific parameter, we found in 119 patients that 82% of the samples, classified as MRA by cholesterol (with only 66% classified by protein), were confirmed by positive cytologic examination and/or CEA level more than 2.5 ng/ml. Thus all false-positive classifications were eliminated by negative cytologic examination and CEA level less than 2.5 ng/ml. Therefore, the following diagnostic sequence for an efficient differentiation of MRA and NMA is proposed. Ascitic-fluid cholesterol determination should be followed by cytologic examination and CEA determination in samples with a cholesterol concentration more than 45 mg/100 ml. When proceeding according to

this sequence, only 8% of patients could not be classified correctly, namely those with ascitic cholesterol levels more than 45 mg/100 ml, but negative cytologic findings and ascitic CEA levels less than 2.5 ng/ml. Such patients, most of them with nonmalignant diseases, will require additional investigations to establish the correct diagnosis. Furthermore it was observed that none of the investigated parameters was able to detect liver metastases without peritoneal carcinomatosis. Therefore, any clinical suspicion in this regard will have to be clarified by imaging techniques. However, with these limitations in mind, the proposed diagnostic test sequence should permit routine cost-effective differentiation of MRA from NMA.

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