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Aim and Scope

This journal, international in scope, is intended for pathologists, clinicians, immunologists and others concerned with all aspects of liver function and diseases.

Members of the editorial board will ensure comprehensive coverage of the many aspects of this speciality.

The general aim of LIVER is to promote and maintain contact between basic and clinically applied liver science. Thus, two main categories of contribution are especially welcomed: articles with morphological emphasis, and related to clinical aspects, as well as immunology and physiology; plus articles mainly based on clinical, immunological or physiological investigations.

Review articles and analyses of technical innovations and concepts will be published from time to time. Case reports will only be accepted if they are of sufficient interest in a wider context.

Book reviews, correspondence relating to articles appearing in previous issues, questions to the editorial board, "controversies in hepatology", "perspectives in hepatology", and announcements of forthcoming meetings will be regular features of the journal.

Within the scope of the journal, articles will be accepted purely on the basis of quality, regardless of their place of origin. The above defined aim and scope will enable LIVER to meet the requirements of all who want to be kept currently informed on recent developments in the field of hepatology.

The journal will be published bimonthly and the Editors and Publishers will endeavour to keep publication time to a minimum, so that the latest results within the field are made rapidly available.

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Key words: *ascitic fluid; cholesterol; differential diagnosis; fibronectin; liver cirrhosis; malignancy related ascites; nonmalignant ascites; peritoneal carcinomatosis; total protein*

Ascitic fluid concentrations of fibronectin and cholesterol: comparison of differential diagnostic value with the conventional protein determination

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ABSTRACT – Ascitic fluid concentrations of fibronectin, cholesterol and protein were determined in 95 patients: 38 with cirrhosis of the liver, 10 with miscellaneous nonmalignant diseases, 43 with peritoneal carcinomatosis and 4 with liver metastases or hepatocellular carcinoma. Fibronectin, cholesterol and protein at discrimination values of 7.5 mg/100 ml, 45 mg/100 ml and 3.0 g/100 ml, respectively, separated patients with peritoneal carcinomatosis from patients with cirrhosis with an efficiency of 94%, 90% and 85%, respectively. Thus, ascitic fluid determinations of fibronectin and cholesterol offer good discrimination of cirrhotic ascites from ascites related to peritoneal carcinomatosis, superior to the conventional protein determination. However, the failure of all parameters to distinguish ascites caused by miscellaneous nonmalignant diseases from malignancy-related ascites underscores the importance of highly specific methods to confirm a suspected diagnosis of malignancy-related ascites.

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The presence of ascites can be related to malignant or nonmalignant diseases; the differentiation between these two conditions is of considerable clinical significance for further diagnostic and therapeutic procedures. Ascitic fluid itself has been examined for parameters which might allow for the differential diagnosis. Cytological examination of ascitic fluid has proven rather insensitive with detection of malignant cells in only 40% to 70% of malignancy-related ascites (1, 2). Therefore, other parameters of ascitic fluid have been investigated for their differential diagnostic value, such as the most widely used total protein concentration. In non-selected patients the diagnostic efficiency of the concentration of ascitic fluid total protein (3, 4), lactic dehydrogenase (5), carcinoembryonic antigen (6, 7) or fibrinogen degradation products (8) and of the serum-ascites albumin concentration difference (9) was found to be below 90%. Recently, an efficiency above 90% was reported for concentrations of fibronectin (10) and cholesterol (11, 12).

The present study was performed to compare these two seemingly superior parameters in the differentiation of malignancy-related ascites from nonmalignant ascites and to evaluate their usefulness, compared to total protein concentration in ascitic fluid.

Patients and methods

Patients

A total of 95 patients with ascites was prospectively studied. Based on the ascites-related diseases, subjects were allocated to the following groups:

Group 1 consisted of 38 patients (23 men, 15 women) with histologically proven liver cirrhosis from various causes (alcoholic in 18, posthepatitic in 11, biliary cirrhosis in 2, mixed or cryptogenic in 7 patients). Malignancy was excluded in these patients by ultrasound, computed tomography or autopsy.

Group 2 included 10 patients (6 men, 4 women) with miscellaneous causes of ascites related to nonmalignant diseases other than liver cirrhosis: congestive heart failure (2 pts.), ovarian overstimulation by gonadotrophic hormones administered for infertility (2 pts.), portal vein thrombosis (2 pts.), pancreatitis (1 pt.), peritoneal tuberculosis (1 pt.), systemic lupus erythematosus (1 pt.) and acute alcoholic hepatitis (1 pt.).

Group 3 comprised 43 patients with ascites secondary to peritoneal carcinomatosis (10 men, 33 women). Clinical diagnosis of peritoneal carcinomatosis was confirmed by peritoneoscopy, peritoneal biopsy, computed tomography or autopsy and/or a positive cytology of ascites. The underlying malignancies were: ovarian carcinoma (16 pts.), breast cancer (6 pts.), carcinoma of the stomach (5 pts.), of the pancreas (3 pts.), of the colon (2 pts.), of the kidney (2 pts.), leukemia (2 pts.), adenocarcinoma of unknown origin (2 pts.), carcinoma of the rectum (1 pt.), of the bladder (1 pt.), Hodgkins disease (1 pt.), hepatocellular carcinoma (1 pt.) and lymphosarcoma.

Group 4 (3 men,1 women) consisted of patients with malignant diseases and affection of the liver, but without evidence of peritoneal carcinomatosis: 1 patient with hepatocellular carcinoma and cirrhosis of the liver, 3 patients with liver metastases and carcinoma of the breast, of the stomach and liposarcoma, respectively, as underlying diseases.

Methods

Cholesterol concentration was determined enzymatically with a commercial test kit (Boehringer, Mannheim, Federal Republic of Germany). Fibronectin was measured by means of laser nephelometry with a specific antiserum against human fibronectin, purchased from Boehringer, Mannheim, FRG. Total protein was determined by a commercial biuret method (Merck, Darmstadt, FRG).

Results are given as mean and standard deviation and as median and range, respectively. The Mann-Whitney test was used for comparing data between groups. Receiver-operator curves were calculated by standard procedures (13). Applying cut-off limits for the determined parameters permitted classification into four categories: true positive (a), true negative (b), false positive (c), and false negative (d). Sensitivity was calculated as $(a/(a+d)\times 100)$, specificity as $(b/(b+c)\times 100)$, positive predictive value as $(a/(a+c))$, negative predictive value as $(b/(b+d))$ and diagnostic efficiency as $((a+b)/(a+b+c+d)\times 100)$ (13).

Results

Ascitic fluid concentrations, range and median values, of cholesterol, fibronectin and protein are shown in Fig. 1. For all three parameters there was little overlap between patients with cirrhosis (Group 1) and patients with peritoneal carcinomatosis (Group 3). Whereas values of patients with nonmalignant diseases (Group 2) ranked between Groups 1 and 3, patients with liver metastases or hepatocellular carcinoma (Group 4) exhibited ascitic fluid concentrations in the range of patients with cirrhosis. Mean values and standard deviation as well as median values and range of the ascitic fluid concentrations of cholesterol, fibronectin and protein are displayed in Table 1. In contrast to cholesterol and fibronectin, mean ascitic fluid protein concentration in patients with miscellaneous nonmalignant diseases (Group 2) was not significantly different from that in patients with peritoneal carcinomatosis (Group 3). When patients with malignancy-related ascites (Groups 3 and 4) and those with nonmalignant ascites (Groups 1 and 2) were considered together, the difference of ascitic fluid concentrations was more marked for cholesterol and for fibronectin than for protein (Table 1). The correlation of ascitic fluid concentrations of cholesterol and fibronectin tended to be slightly superior to those of either parameter with ascitic protein concentration (Table 2).

As illustrated by the receiver-operator curves (Fig. 2a), differential diagnostic efficiency of cholesterol and fibronectin was superior to that of protein in separating patients with cirrhosis from patients with peritoneal carcinomatosis. Inclusion of patients with miscellaneous nonmalignant diseases and of patients with liver metastases or hepatocellular carcinoma resulted in a decrease of differential diagnostic efficiency, particularly for protein concentration (Fig. 2b).

This observation was confirmed by the calculation of sensitivity, specificity, positive and negative predictive values and diagnostic efficiency (Table 3). Fibronectin, cholesterol and protein at

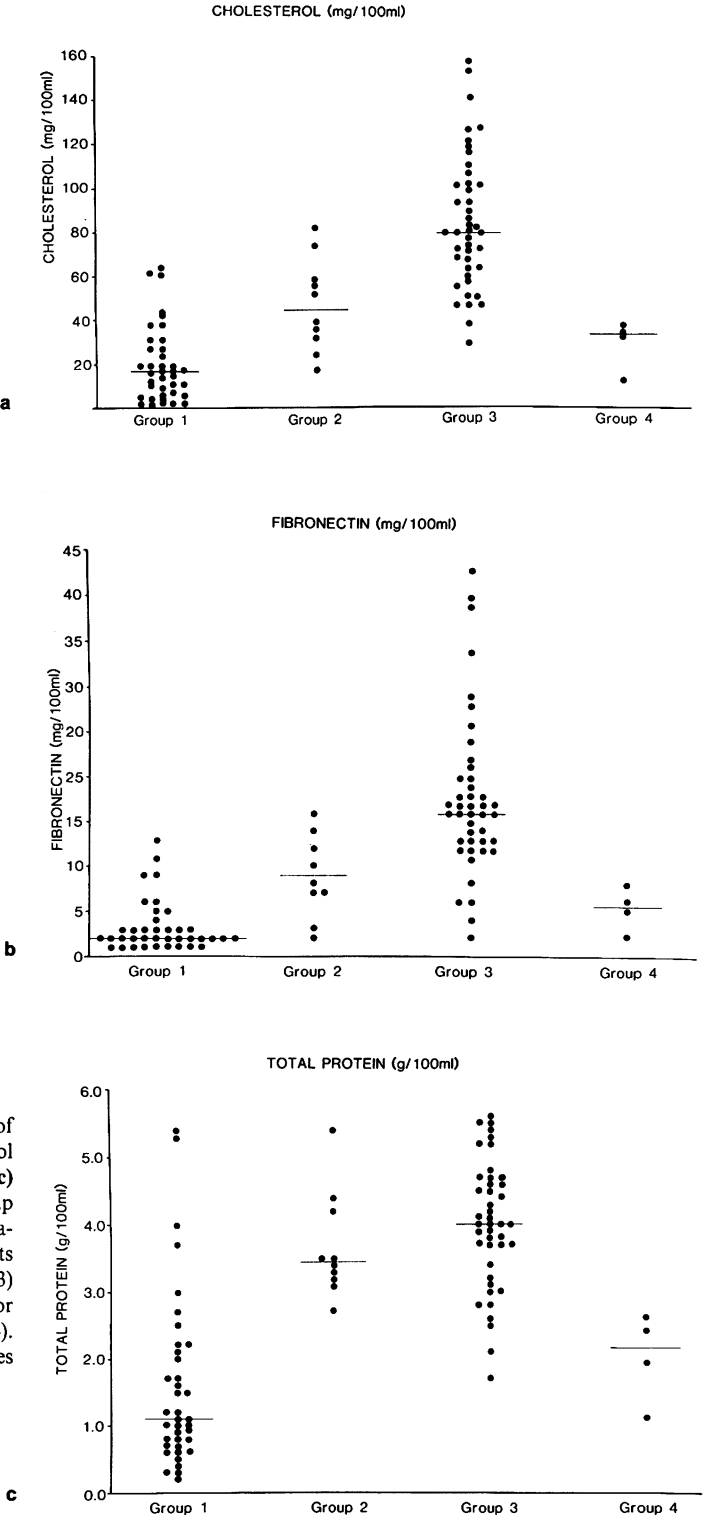

Fig. 1 (a–c). Scattergram distribution of ascitic fluid concentrations of cholesterol **(a)**, fibronectin **(b)** and total protein **(c)** in 38 patients with liver cirrhosis (Group 1), 10 patients with miscellaneous nonmalignant diseases (Group 2), 43 patients with peritoneal carcinomatosis (Group 3) and 4 patients with liver metastases or hepatocellular carcinoma (Group 4). Horizontal bars indicate median values within groups.

Table 1
Ascitic fluid concentrations of cholesterol, fibronectin and protein

n =	Group 1 38	Group 2 10	Group 3 43	Group 4 4	Groups 1+2 48	Groups 3+4 47
Cholesterol (mg/100 ml)						
Mean±SD	19.7±17.2	46.8±21.3	85.1±30.1	28.8±11.4	25.4±21.0	80.3±33.4
Median	16,6[bcf]	44.9[acf]	80.0[abde]	33.3[cf]	19.2[cf]	79.8[abde]
Range	0.9–64.0	17.1–82.3	28.2–157.6	11.9–36.7	0.9–82.3	11.9–157.6
Fibronectin (mg/100 ml)						
Mean	3.1±2.9	9.1±4.4	17.8±8.9	5.1±2.4	4.4±4.1	16.7±9.3
Median	2.0[bcf]	8.9[acf]	16.4[abde]	5.3[cf]	2.8[cf]	16.1[abde]
Range	0.6–13.4	2.1–15.6	2.4–42.7	2.0–7.7	0.6–15.6	2.0–42.7
Total protein (g/100 ml)						
Mean	1.6±1.3	3.7±0.8	4.0±0.9	2.0±0.7	2.0±1.5	3.8±1.1
Median	1.1[bcf]	3.5[ad]	4.0[ade]	2.2[bcf]	1.6[cf]	4.0[ade]
Range	0.2–5.4	2.7–5.4	1.7–5.6	1.1–2.6	0.2–5.4	1.1–5.6

Significant ($p < 0.05$) difference to [a]Group 1 (liver cirrhosis), [b]Group 2 (miscellaneous nonmalignant disease), [c]Group 3 (peritoneal carcinomatosis), [d]Group 4 (liver metastases or hepatocellular carcinoma), [e]Groups 1+2, [f]Groups 3+4.

discrimination values of 7.5 mg/100 ml, 45 mg/100 ml and 3.0 mg/100 ml, respectively, separated patients with peritoneal carcinomatosis from patients with cirrhosis with an efficiency of 94%, 90% and 85%, respectively. Inclusion of patients with miscellaneous nonmalignant diseases, liver metastases or hepatocellular carcinoma reduced differential diagnostic efficiencies of the three investigated parameters to 85%, 82% and 74%, respectively.

Discussion

Stimulated by recent observations on the excellent differential diagnostic qualities of ascitic fluid concentrations of fibronectin and cholesterol (10, 11), these parameters were prospectively evaluated in the present study in 95 patients in comparison with the routine protein determination. In 81 patients with liver cirrhosis (n = 38) or peritoneal carcinomatosis (n = 43), an excellent differential diagnostic efficiency of 94% for cholesterol and of 90% for fibronectin was found. Total protein concentration had an efficiency of 85% only. Inclusion of patients with miscellaneous nonmalignant diseases (n = 10) and with hepatic metastases or hepatocellular carcinoma without peritoneal carcinomatosis (n = 4), however, reduced diagnostic accuracy by about 10% to 85%, 82% and 74% for cholesterol, fibronectin and protein, respectively. Thus, determination of cholesterol and fibronectin offer about the same differential diagnostic power, superior to protein determination. The mechanisms behind this observation remain to be elucidated; possibly alterations of transperitoneal diffusion may play a role (12).

Table 2
Correlation of ascitic fluid concentrations of cholesterol, fibronectin and total protein

		Groups 1+2 n = 48	Groups 3+4 n = 47	Groups 1–4 n = 95
Cholesterol-fibronectin	r =	0.77	0.61	0.80
Cholesterol-protein	r =	0.72	0.60	0.76
Fibronectin-protein	r =	0.74	0.55	0.71

r: correlation coefficient. All correlations have a p-value below 0.001. Groups 1 (liver cirrhosis, n = 38) and 2 (miscellaneous nonmalignant ascites, n = 10) comprise nonmalignant ascites, Groups 3 (peritoneal carcinomatosis, n = 43) and 4 (hepatic metastases and hepatocellular carcinoma, n = 4) comprise malignancy-related ascites.

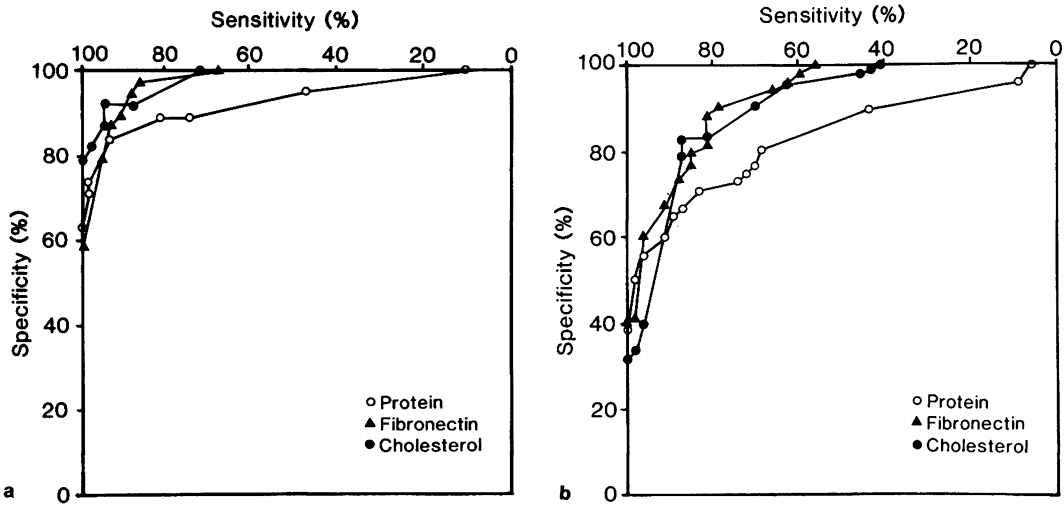

Fig. 2 (a, b). Receiver-operator characteristics (ROC), displaying sensitivity and specificity at various discrimination levels for cholesterol, fibronectin and protein. As differential diagnostic efficiency improves, the curve approaches the left upper corner (100% sensitivity and 100% specificity) of the illustration. **a:** ROC for a total of 81 patients, including 38 patients with liver cirrhosis and 43 patients with peritoneal carcinomatosis. **b:** ROC for a total of 95 patients, comprising 81 patients with cirrhosis or peritoneal carcinomatosis, 10 patients with miscellaneous nonmalignant diseases and 4 patients with hepatic metastases or hepatocellular carcinoma.

There were two limitations which precluded any of the investigated parameters from providing a complete separation of malignancy-related from nonmalignant ascites: malignancy-related ascites without peritoneal carcinomatosis could not be separated from nonmalignant ascites by any of the parameters in this study. This finding confirms observations by ourselves (12) as well as by others (14) and underscores that investigation of ascitic fluid cannot provide information other than the usually negative cytological examination in these patients. This may explain the rather low (< 80%) differential diagnostic efficiency of fibronectin, observed in a study comparing 18 patients with malignancy-related ascites, most of them without peritoneal carcinomatosis, to 30 patients with chronic liver disease (15). Furthermore, increased ascitic fluid concentrations of protein or any other parameter are not totally specific for malignancy-related ascites (16, 17); therefore a combination

Table 3
Diagnostic value of cholesterol, fibronectin and total protein in ascitic fluid

Discrimination value	Cholesterol 45 mg/100 ml		Fibronectin 7.5 mg/100 ml		Total protein 3.0 g/100 ml	
Groups	1,3	1–4	1,3	1–4	1,3	1–4
Sensitivity (%)	95	87	91	85	81	75
Specificity (%)	92	83	89	80	89	73
Efficiency (%)	94	85	90	82	85	74
Pos. predictivity (%)	93	84	91	80	90	73
Neg. predictivity (%)	95	87	89	84	81	74

Diagnostic value of ascitic fluid cholesterol, fibronectin and total protein for separating ascites related to liver cirrhosis (Group 1, n = 38) from ascites related to peritoneal carcinomatosis (Group 3, n = 43) and for separation of nonmalignant ascites (Group 1 and Group 2: n = 10, miscellaneous nonmalignant ascites) from malignancy-related ascites (Group 3 and Group 4: n = 4, hepatic metastases and hepatocellular carcinoma).

of these parameters might increase sensitivity but will invariably decrease specificity and hence will not improve diagnostic discrimination. In the present study, protein concentrations in ascites due to nonmalignant diseases other than cirrhosis were more often in the range of concentrations found in peritoneal carcinomatosis than fibronectin or cholesterol concentrations, resulting in reduced specificity, particularly of protein. The failure of all investigated parameters to distinguish ascites caused by miscellaneous nonmalignant diseases from malignancy-related ascites underscores the importance of highly specific methods to confirm a suspected diagnosis of malignancy-related ascites.

However, in patient populations with a high percentage of cirrhotic and peritoneal carcinomatosis ascites and a consequently small fraction of both noncirrhotic, nonmalignant ascites and malignancy-related ascites without peritoneal carcinomatosis, cholesterol and fibronectin determination offer useful differential diagnostic qualities, superior to those of protein determination. Since cholesterol can be determined more easily and cheaply than fibronectin, it may be recommended as a first-line routine parameter of ascitic fluid investigation.

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