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Determination of pH, Fibronectin, Cholesterol, Lactate Dehydrogenase and Sialic Acid in the Differentiation of Nonmalignant and Malignant Ascites

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Abstract: Ascites, being an accumulation of excess fluid within the peritoneal cavity, is most frequently encountered in patients with cirrhosis or certain other diseases such as congestive cardiac failure, peritoneal tuberculosis and some primary or secondary peritoneal tumours. This study included 17 patients (5 females, 12 males) with malignant ascites (MA) and 23 patients (13 females, 10 males) with nonmalignant ascites (NMA). We aimed to determinet the values for total sialic acid (SA), pH, cholesterol, fibronectin, total protein concentrations and lactate dehydrogenase (LDH) activity in ascitic fluid for the differential diagnosis of ascites.

While total SA, fibronectin, cholesterol, LDH and total protein values in MA cases were found to be higher ($p<0.05$, $p<0.001$, $p<0.05$, $p<0.05$ and $p<0.05$ respectively), pH values were lower ($p<0.05$) in the MA group than in the NMA group. From these parameters, fibronectin proved to be the most specific and total SA proved to be the most sensitive test for differential diagnosis of ascites.

It was is concluded that the measurements of total SA, cholesterol and fibronectin in ascitic fluid may be useful for differential diagnosis and appropriate treatment of ascites. The use of these three parameters, in combination, may provide higher sensitivity and specificity for the differentiation of MA.

Key Words: Sialic acid, Ascitic fluid, cholesterol, fibronectin, pH.

Introduction

The accumulation of fluid within the peritoneal cavity is a common clinical finding with a wide range of causes (1). There are no distinctive features and no single diagnostic test is accurate in differentiating MA from NMA (2). Recent studies have drawn attention to the surface properties of cancer cells, suggesting new possible markers of malignant effusions. Lipids, mainly cholesterol and fibronectin, a glycoprotein derived from the extracellular matrix, have been found to be elevated in MA (2-4). SA is a common terminal saccharine of cell surface constituents (glycoproteins and glycolipids) (2). It has been found in elevated concentration in neoplastic cells taken from breast, lung, stomach, colon, ovary, prostate and liver tumours. In these malignancies, sialoglycoprotein and sialoglycolipid concentrations are often elevated in the blood as well because of shedding or secretion by tumour cells (5). Fibronectin is a glycoprotein of high molecular weight present in plasma and other body fluids and tissues (6, 7). Fibronectin plays an important role in diverse biological phenomena including cell adhesion, cell migration, embryonic development, wound healing and homeostasis as well as in the pathogenesis of such diseases as cancer and bacterial infection (8, 9). Malignant, transformed cells may shed their matrix fibronectin (6), which may then be found in the body fluids. We have assessed the value of measuring the fibronectin, total SA, pH, cholesterol, total protein and LDH values of ascitic fluid in the diagnosis of malignant ascites.

Materials and Methods

We studied 40 consecutive patients admitted because of ascites of unknown origin. There were 23 NMA patients (10 male, 13 female, mean age 50.7 yrs). Of these patients, 12 had cirrhosis of the liver, 7 had right heart failure, 2 had chronic renal failure, 1 patient with ulcerative colitis and 1 patient with nephrotic syndrome. There were 17 MA patients (12 male, 5 female, mean age 49.8 yrs). Of these patients, 4 had carcinoma of the stomach, 3 had hepatocelluler carcinoma, 4 had

intraperitoneal lymphoma, 2 had cholangiocarcinoma, 2 had cancer of the bladder, 1 had colonic carcinoma and 1 had pancreal cancer. Diagnoses were confirmed by biopsy. None of the NMA patients had any malignancy. Diagnostic paracentesis was performed on every patient 24 hours after admission to the hospital and before the beginning of any treatment. Part of the sample was sent for bacteriologic examination. For determination of pH, 5 ml of ascitic fluid was collected anaerobically in a non-heparinized plastic syringe, and analyzed on a blood gas analyzer (Nova, USA). The fibronectin concentration was determined with commercial kits of antibody against human fibronectin (Boehringer Mannheim, Germany). Total SA was determined by enzymatic-colorimetric assay (Boehringer Mannheim, Germany). Determination of LDH and cholesterol was performed by autoanalyzer (Hitachi 717, Japan). Total protein concentration was determined by sulphosalycilic acid. We selected the cutoff values for each test according to the data of previous studies (2, 6, 9, 23).

Table 1. The results of analyses of MA and NMA

Parameter	NMA (x±SD)	MA (x±SD)	*p
pH	7.5±0.14	7.3±0.18	p<0.05
Sialic acid (mg/l)	251.4±264	459.0±294	p<0.05
Fibronectin (µg/ml)	45.9±67	152.2±97	p<0.001
Cholesterol (mg/dl)	32.5±34	47.4±27	p<0.05
LDH (U/l)	40.3±46	172.0±321	p<0.05
Total protein (gr./dl)	2.2±1.71	3.1±1.70	p<0.05

*NMA vs. MA

Statistical Analysis

Values are expressed as mean ± SD. The significance of the mean differences between groups was assessed by the Student's two-tailed unpaired t test. The correlation coefficient was determined by linear regression analysis. Differences were considered significant at $p<0.05$. For each parameter we also calculated the sensitivity, specificity, predictivity (of a positive test) and diagnostic accuracy.

Results

There were significant differences between the MA group and the NMA group in values for fibronectin, total SA, pH, LDH, total protein and cholesterol levels. Results are summarized in Table 1. Ascitic concentrations of total SA above 300 mg/L (the cut-off value 16) were found in 6 patients with NMA and in 13 patients with MA (sensitivity 76%; specificity 73%; predictivity of positive test 68% and diagnostic accuracy 75%) (Table 2). Ascitic concentrations of fibronectin higher than 100 mg/ml (the cutoff value was 4.9) were found in 2 patients with NMA and in 11 patients with MA (sensitivity 64%; specificity 91%; predictivity of positive test 84% and diagnostic accuracy 80%). Table 2 shows a comparison of the diagnostic accuracy of the parameters which seemed to be useful for separating MA from NMA. We found a significant positive correlation between ascitic concentration of fibronectin and SA in the MA group ($r=0.517$, $p<0.05$; Figure 1). A significant correlation

Table 2. In differential of NM and NMA diagnostic values of some parameters

Parameter	True Positive	False Positive	Sensitivity	Specificity	Positive predictivity	Diagnostic accuracy
Sialic acid (≥300 mg/l)	13/17	6/23	76%	73%	68%	75%
Fibronectin (≥100 µg/ml)	11/17	2/23	64%	91%	84%	80%
Cholesterol (≥45mg/dl)	7/17	4/23	41%	82%	63%	65%
pH (≤7.45)	8/17	4/23	47%	82%	66%	67%
LDH (≥150 U/l)	4/17	3/23	23%	86%	57%	60%
T.prot (≥3gr/dl)	9/17	7/23	52%	69%	56%	62%

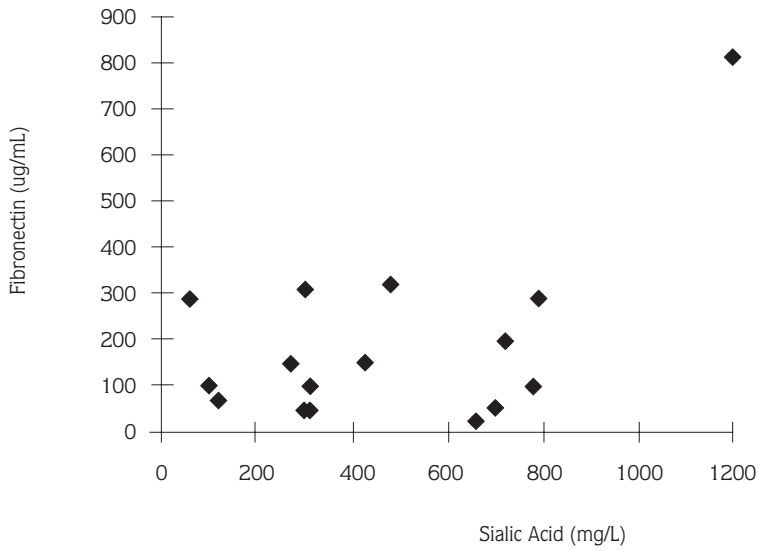

Figure 1. Correlation between SA and Fibronectin in patients with MA (r=0.517;p<0.05)

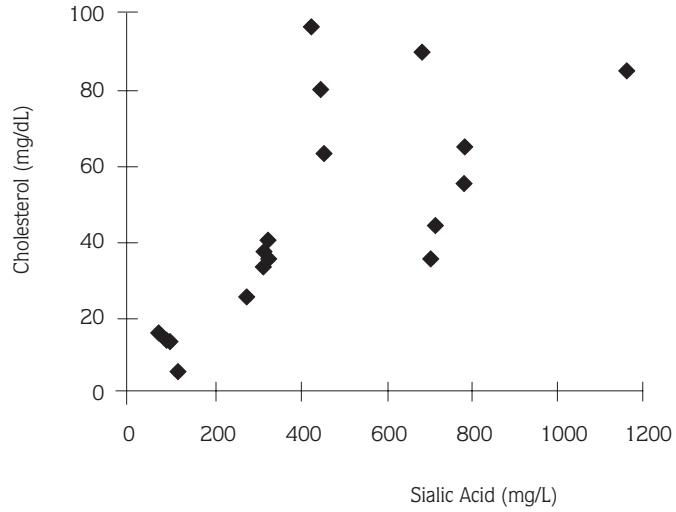

Figure 2. Correlation between SA and Cholesterol in patients with MA (r=0.679;p<0.01).

between ascitic concentration of cholesterol and SA was found in both MA and NMA (r =0.679, p<0.01 for MA; r = 0.587, p<0.01 for NMA; Figure 2 and 3, respectively).

Discussion

Ascites may be caused by various diseases, the most common of which are chronic liver disease and malignancy. Attempts to achieve a complete differentiation of patients with MA and NMA by means of a simple laboratory test have so far failed (6). We studied 40 consecutive patients with ascites, 23 of which had benign disease and 17 malignancy. We found no patient with infected ascites. Ascitic fluid total protein levels were higher in malignant patients than in non malignant patients, while sensitivity and specificity were low. These findings support the view of various investigators that protein concentration is not a definite criterion for differentiating MA from NMA. The ascitic LDH levels of MA patients were significantly higher than those of NMA patients. The sensitivity of ascitic LDH was low (23%). Our findings were in agreement with the findings of other researchers (4, 6, 10). Also as in previous studies (6, 7, 11), a significantly lower pH value was found in the MA group than in the NMA group. Rovelstad et al. (19) reported elevated total lipid concentrations in the ascites of patients with malignant neoplasms. This was later mentioned by other researchers (2, 15, 16, 20-22). Furthermore, our results show that the cholesterol level

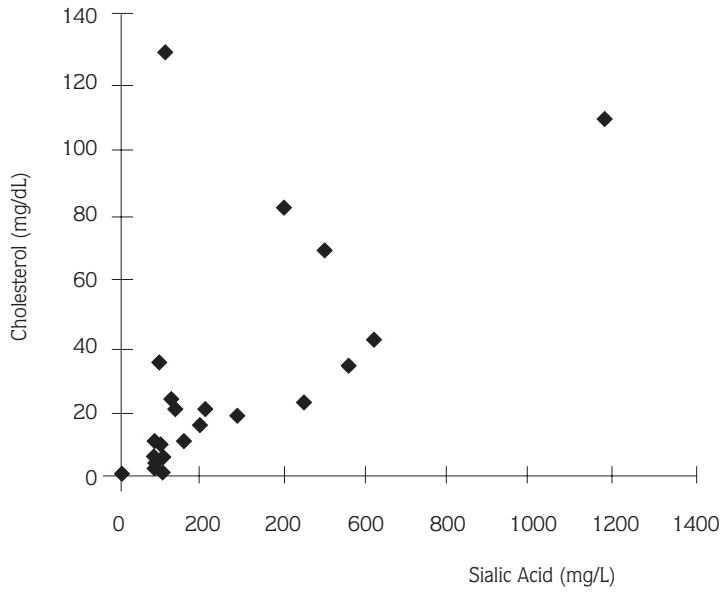

Figure 3. Correlation between SA and Cholesterol in patients with NMA (r=0.587;p<0.01).

in ascitic fluid of patients with malignancies is significantly high, a finding that is in accordance with previous results. The pathogenesis of increased cholesterol level in malignant ascites was not completely explained. However, based on previous studies, it was thought that the source of cholesterol originated from malignant cell membrans (13).

Malignant transformed cells may shed matrix fibronectin (6) which may then be found in the body fluids. The ascitic fibronectin level was the most specific test for malignancy of the various tests evaluated (91%). The specificity is similar to that reported by Colli et al. (93%) (7), and superior to the 88% reported by Gerbes et al. (23), but inferior to the 100% accuracy observed by Schölmerich (6) and Archimandritis et al. (24) and the 98% observed by Prieto et al. (15). It should be noted that the cutoff levels for malignancy were different in the five studies mentioned. Although many cell types have the capacity to synthesize and secrete fibronectin, several lines of evidence suggest that most, if not all, circulating fibronectin is produced by hepatocytes. Therefore, patients with decompensated cirrhosis of fulminate hepatic failure have low concentrations of plasma fibronectin (10). In our study, we found fibronectin levels above 100 µg/ml in only 1 out of 12 patients with cirrhosis and in only 1 out of 7 patients with congestive cardiac failure. All other NMA patients had low levels of fibronectin. SA is a common terminal saccharide in glycoproteins and glycolipids found on membrane surfaces. SA concentration has been reported to be higher in cancer cells obtained from tumors. The concentrations of sialoglycoprotein and sialoglycolipid are frequently high because of their secretion by tumor cells both in blood and ascitic fluid (2). We found higher concentrations of total SA in the MA group than in the NMA group and, taking 300 mg/l as the cut-off value (2), found the false positive levels to be 6 out of 23, the false negative levels to be 4 out of 17 and the overall diagnostic accuracy to be 75%. The sensitivity found in the study was higher than that found by Colli et al. (2) while the specificity was lower. This difference may be due to different disease groups. There were statistically significant correlations between total SA and cholesterol, and total SA and fibronectin. These findings may indicate that cholesterol, total SA, and fibronectin are synthesized and secreted from malignant cells.

References

1. Bender MD. Diseases of Peritoneum, Mesentery and Omentum. In: Wyngaarden JB, Smith L H(eds.). Cecil Textbook of Medicine 18 Th edition Philadelphia WB Sounders Company 737-739,1988.
2. Colli A, Bussan G, Cecile M, Parravicini R, Mariani F, Scaltrini G. Diagnostic Accuracy of Sialic Acid in the Diagnosis of Malignant Ascites. Cancer 63 : 912-916,1989.
3. Deverbizier G, Beauchant M, Chapron A, Touchard G, Reiss D. Fibronectin a Marker for Malignant Ascites. The Lancet 10:1104,1984.

4. Lee C, Changchien C, Liaw YF. Serum-Ascites Albumin Concentration Gradient and Ascites Fibronectin in the Diagnosis of Malignant Ascites. Cancer 70:2057-2060,1992.

5. Bolmer SD, Davidson EA. Preparation and properties of a glycoprotein associated with malignancies. Biochemistry 20;1047-1054,1981.

6. Schölmerich J, Volk BA, Köttgen E, Ehlers S, Gerok W. Fibronectin Concentration in Ascites Differentiates Between Malignant and Non-malignant Ascites. Gastroenterology 87: 1160-1164,1984.

7. Colli A, Buccino G, Cocciolo M, Parravicini R, Mariani F, Scaltrini G. Diagnostic Accuracy of Fibronectin in the Differential Diagnosis of Ascites. Cancer 58: 2489-2493,1986.

8. Gomez-Lechon MJ, Castell JY. Measurement of Fibronectin in Human Body Fluids. J Clin Chem Clin Biochem 24: 333-339,1986.

9. Adamsen S, Jönsson P, Bradin B, Lindberg B, Jorpes P. Measurement of Fibronectin Concentration in Benign and Malignant Ascites. Eur J Surg 157:325-328,1991.

10. Salmanyeli N, Sivas A. Significance of Fibronectin in Cirrhotic and Malignant Ascites. J Clin Biochem Nutr 9: 191-196,1990.

11. Stassen W, Bacon BR, McCullough AJ, Gutnik S, Kalhan S, Tavill AS. Superior Diagnostic Value of Ascitic Fluid pH and Lactate Levels as Immediate Indicators of Infected Ascites. Gastroenterology 86:1341,1984.

12. Buchwald B. Cholesterol Ynhibition, Cancer and Chemotherapy. The Lancet 339:1154-1156,1992.

13. Ryngenberg S, Doll DC, Loy TS, Yarbro JW . Malignant Ascites of Unknown Origin. Cancer 64: 753-755,1989.

14. Pyna Cabral JE, Correya-Leitao M, Guerra C, Tome L, Pinto ML, Costo D. Ascitic Cholesterol: Accurate Parameter to the Differential Diagnosis of Ascites. Dyg Dis Sci 34: 964,1989.

15. Prieto M, Gomez-Lechon MJ, Hoyos M, Castel JV, Carrasco D, Berenguer J. Diagnosis of Malignant Ascites. Dig dis Sci 33:833-838,1988.

16. Jüngst D, Gerbes AL, Martin R, Paumgartner G . Value of Ascitic Lipids in the Differentiation Between Cirrhotic and Malignant Ascites. Hepatology 6: 239-243,1986.

17. Olt G, Berchuck A, Soisson AP, Boyer CM, Bast RC. Fibronectin is an Ymmunosuppressive Substance Associated With Epithelial Ovarian Cancer. Cancer 70:2137-2142,1992.

18. Topçu S, Atik A, Aslan L, _encan M. Diagnostic value of the ascitic fluid cholesterol can concentration in malignancy. Turkish J Clin Gastroenterohepatologia 1:59-62,1990.

19. Salmanyeli N, Sivasly A. Ascitic Fluid Lipid Concentrations in Malignant Disorders and Cirrhosis. Turkish J Med Biol Res 1:103-106,1990.

20. Olmos M, Martinez A, Raffo C, Valdue I, Mendelluk G, Orcinoli MI, Magnanini F. Evaluation of the Usefulness of the Physico-Chemical Study of Ascitic Fluid in Patients With Chronic Hepatopathies, Neoplasms and Spontaneous Peritonitis. Acta Gastroenterol Latinoam 20:145-158,1990.

21. Garg R, Sood A, Arora S, Bhatia KL, Chawla AS, Gupta R, Chawla LS. Ascitic Fluid Cholesterol in Differential Diagnosis of Ascites. Jb Assoc Physicians 41:644-646,1993.

22. Gupta R, Misra SP, Wivedi M, Misra V, Kumar S, Gupta SG. Diagnosing ascites:value of ascitic fluid total protein,albumin ,cholesterol their ratios ,serum-ascites albumin and cholesterol gradient. J Gastroenterol Hepatol 10:295-9,1995.

23. Gerbes AL, Jüngst D, Xie Y, Permanetter W, Paumgartner G. Ascitic Fluid Analysis for the Differentiation of Malignancy-Related and Non-malignant Ascites. Cancer 15: 1808-1814,1991.

24. Archimandritis A, Kapsalos D, Douvara M, Tjivras M, Tsirantonaki M, Fertakis A. Value of ascitic fibronectin and cholesterol concentration in differantiation between malignancy-related and non-malignant ascites. Ann Med Ynterne 147:145-50,1996.