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The D- Dimer and Ferritin Silent Life in Ascites: A Pilot Study

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Abstract

Ascites, regardless of its source (hepatic, neoplastic, cardiac or inflammatory), is a clinical indicator of disease progression. In cirrhosis, ascites forms due to portal hypertension and low circulating blood albumin, which leads to so called third spacing. This study compared blood and ascites samples from 20 consecutive and unselected liver cirrhosis patients and 5 cancer patients. Significant correlations were found in cirrhotic ascites between albumin/D-dimer, albumin/ferritin, albumin/total protein, ferritin/D-dimer, total protein/ferritin, and total protein/D-dimer.

Three out of five malignant ascites patients had low ascitic total protein values (below 25 g/L), whereas five out of twenty cirrhotic ascites patients had high values above 25 g/L. The protein composition of our oncology patients was likely altered due to treatment.

Half of our cirrhotic patients and two cancer patients had high serum ferritin levels. We also observed ascitic ferritin levels up to 100 µg/l in half of the patients and values above the upper reference level for serum (280.00 µg/l) in six of our liver cirrhosis patients. Individual ascites values overlapped in cirrhotic and malignant ascites. D-dimer levels in ascites were 500-1000 times the plasma upper limit of normal (ULN) in three cancer patients and four cirrhosis patients.

This study is the first to simultaneously examine ferritin and D-dimer levels in blood and uncomplicated ascites. Abnormal levels of D-dimer and ferritin in ascites, even in the absence of clinical symptoms, may indicate underlying processes in the ascitic peritoneal fluid such as inflammation and fibrinolysis. Additional research may be needed.

Keywords: Ascites, D- dimer, Ferritin, Cirrhosis, Oncology

Introduction

Ascites is a clinical indicator of disease progression, regardless of its source, be it hepatic, neoplastic, cardiac or inflammatory.
The primary objective of laboratory testing is to distinguish between transudate and exudate through total protein measurement, which has been a standard practice since 1958 (1), and to aid in the identification of the underlying cause through other diagnostic tests. In 1983, Pare and Hoefs discovered that the serum ascites albumin gradient (SAAG) was a superior diagnostic tool for distinguishing between hepatic and oncological ascites, as compared to total protein. (2)
The standard laboratory panel for routine testing of ascites includes: Protein concentration, polymorphonuclear (PMN) count, bacterial culture, glucose, lactate dehydrogenase (LDH), cytology and amylase. (3) Various biomarkers are being sought to better understand the formation of ascites and its processes. Examples of such are: angiogenin, angiopoietin-2, GRO, ICAM-1, interleukins, leptin, MCP-1, MIF NAP-2, osteoprotegerin (OPG), RANTES, TIMP-2, UPAR. (4)
Elevated levels of ferritin have been observed in the serum of patients with neoplasms since 1978, and since 1994, this phenomenon has been noted in malignant and cirrhotic ascites as well. (5)
In 1993, high levels of D-dimer were first detected in the plasma and ascites of patients with liver cirrhosis, and subsequent isolated reports have confirmed this observation. (6,7)
However, there are still no data on parallel evaluation of ferritin, D-dimer, total protein and albumin concentrations in ascites of different etiology as well as on the comparison of their blood and ascitic fluid levels.

Aim

The aim of this study is to concurrently evaluate D-dimer and ferritin levels in ascites, in order to gain a more thorough understanding of the condition, regardless of its underlying cause.

Material And Methods

In this study, parallel blood and ascites samples were tested for total protein, albumin, ferritin and D-dimer. They were obtained from 20 consecutive and unselected liver cirrhosis patients (group A) and 5 cancer patients (group B). Relevant results of two groups were compared. The etiology of liver cirrhosis was predominantly alcoholic (15 patients), 3 patients had hepatitis C (HCV), 1 patient - hepatitis B (HBV) and 1 patient - Wilson's disease. In the oncology group, the primary tumours were with different location- 1 in lung, 2 pancreats, 1 duodenum, 1 ovarium. All cancer subjects received previous or current chemotherapy. The mean age in group A was 60.10 ± 10 years. In group B, they were aged from 43 to 71 years. 14 patients in group A were male and 6 female. In Group B, all patients were female.
Total protein, albumin and ferritin were measured by a clinical chemistry analyser Cobas Integra 400 Plus. D-dimer was measured using Siemens Inovance D-aimer reagent, using a Sysmex CS 2000i automatic coagulometer.
Statistical data were processed with SPSS version 19.0, using descriptive statistics and correlation analysis. P values less than 0.05 were considered statistically significant

Results

Mean values of blood and ascites biomarkers are shown in Table 1. Mean albumin levels in serum and ascites were 31.25±6.7 g/L and 9.6±7.5, respectively. The average D-Dimer value in plasma was 6.1±5.3 mg/L and 318±338 mg/L in ascites. The average ferritin level was 350.8±332 µg/l in serum and 276±382 µg/l in ascites.

Table 1. Blood and ascitic biomarkers in liver cirrhosis.

Biomarker	Mean (x)	Reference Range
Total Protein Serum[g/L]	72±9.4	64-83
Total Protein Ascitic [g/L]	17.9±11.5	
Albumin Serum [g/L]	31.25±6.7	35-55
Albumin Ascitic [g/L]	9.6 ±7.5	
SAAG	21.6±4.8	<11- exudate >11- transudate
D- Dimer Plasma [mg/L]	6.1±5.3	< 0.55
D- Dimer Ascitic [mg/L]	318±338	
Ferritin Serum [µg/l]	350.8±332	40.00 - 280.00
Ferritin Ascitic [µg/l]	276±382	

Table 2 shows the medians of the studied blood and ascites biomarkers in patients with liver cirrhosis and malignancy.
In cancer patients the median values are: serum total protein- 56 g/L (43- 66), ascitic total protein- 21 g/L (8-45), serum albumin- 31 g/L (27-37), ascitic albumin- 11 g/L (5-30), plasma D- dimer- 4 mg/L (1.8-25), ascitic D- dimer- 709 mg/L (204-1644), serum ferritin- 270 µg/l (120-512), ascitic ferritin- 174 µg/l (59-8683).

Table 2. Median levels of serum and ascitic biomarkers in patients with liver cirrhosis and malignancies.

Biomarker	Liver cirrhosis	Malignancy
	n=20	n=5
Total Protein Serum [g/L]	71.5 (57-99)	56 (43-66)
Total Protein Ascitic [g/L]	13(4-41)	21 (8-45)
Albumin Serum [g/L]	31.25(20-41)	31(27-37)
Albumin Ascitic [g/L]	9.6(1.5-25)	11(5-30)
SAAG	21.6(15-35)	20(6-28)
D- Dimer Plasma [mg/L]	4.2(1.4-23)	4(1.8-25)
D- Dimer Ascitic [mg/L]	167.5(56-1269)	709 (204-1644)
Ferritin Serum [µg/l]	232(28-1368)	270(120-512)
Ferritin Ascitic [µg/l]	95(15-1532)	174(59-8683)

We conducted a study to determine the associations between serum and ascites biomarkers in individuals with liver cirrhosis.
We found no noteworthy correlation in the blood among ferritin, D-dimer, albumin, and total protein.

We observed significant correlations in cirrhotic ascites between albumin and D-dimer, albumin and ferritin, albumin and total protein, ferritin and D-dimer, total protein and ferritin, and total protein and D-dimer. (Table 3)

Table 3. Correlations between ascitic biomarkers in patients with liver cirrhosis (p<0.05)

	Albumin	D- dimer	Ferritin	Total Protein
Albumin		0.559	0.801	0.887
D - dimer	0.559		0.463	0.638

Figures 1-4 illustrate the individual biomarker values in ascites among patients with liver cirrhosis and neoplasms.

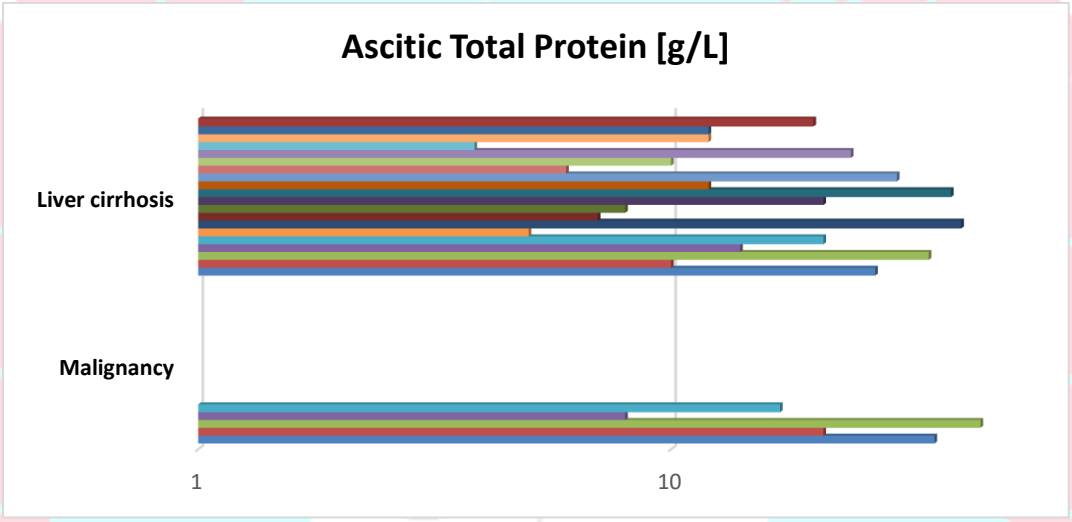

Figure 1. Ascitic Total Protein in patients with liver cirrhosis and malignancies

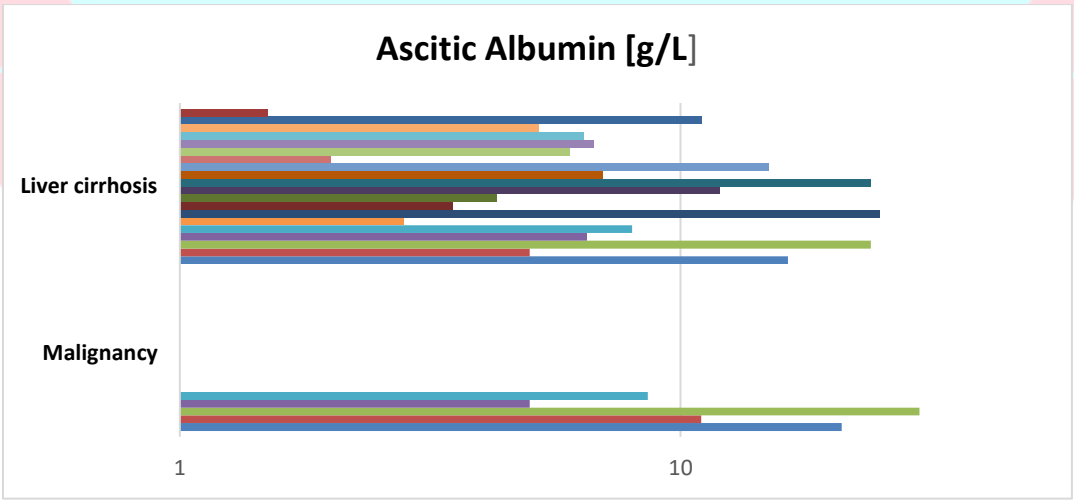

Figure 2. Ascitic Albumin in patients with liver cirrhosis and malignancies

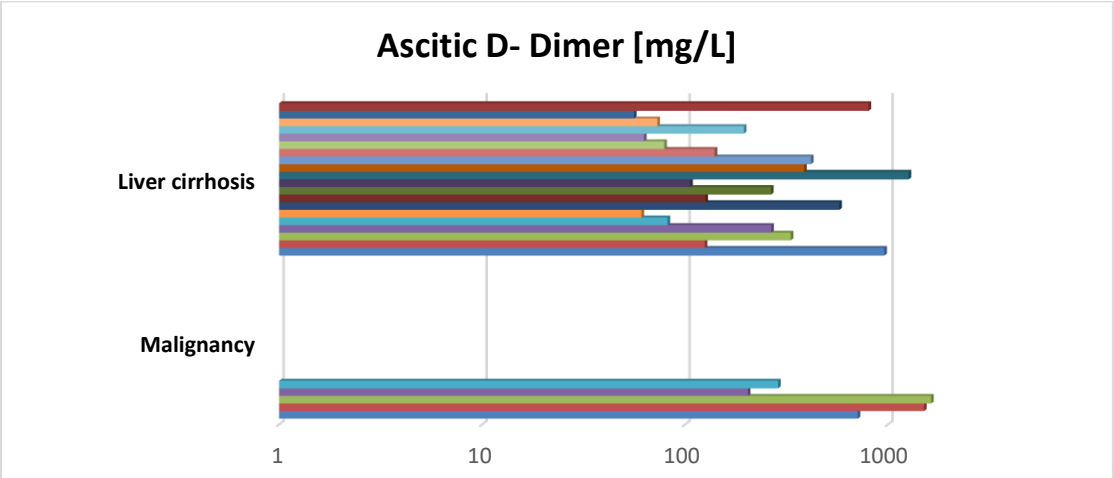

Figure 3. Ascitic D- Dimer in patients with liver cirrhosis and malignancies

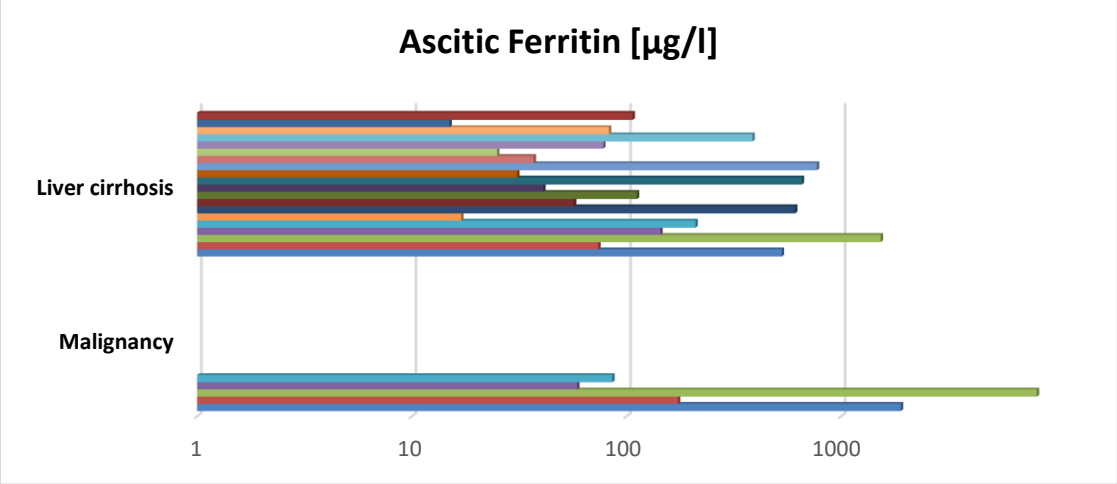

Figure 4. Ascitic Ferritin in patients with liver cirrhosis and malignancies

Discussion

The peritoneum is the largest dynamic membrane that can adapt according to different pathologies. In 1923, Putnam described the peritoneal membrane as a "living membrane". (8) Fluid exchange between the plasma and the interstitium depends on the hydraulic and oncotic pressures in each compartment. Macromolecules, proteins, and cells can return to the systemic circulation via the peritoneal lymphatic system via the lymphatic stomata.

In cirrhosis due to portal hypertension and a low amount of albumin in the circulating blood, ascites forms the so-called third spacing. (9) Decreased lymph absorption contributes to its formation.

The mechanism for the accumulation of fluid and protein in the intraperitoneal space associated with malignancy appears to involve impaired lymphatic drainage (obstructed by tumour infiltration) and increased vascular permeability. (10,11) The high protein content indicates a change in vascular permeability that allows the accumulation of large molecules in the intraperitoneal space. (12)

Exudate and transudate

Samples of ascitic fluid are usually used for differential diagnosis. The concept of transudate/exudate is based on a total protein concentration in ascites below and above 25 g/L. (13)
Despite this postulate, up to 25% of cirrhotic patients may have high ascites protein levels, and 18% of malignant ascites may have low protein levels. (14,15)

The serum-ascites albumin gradient (SAAG) corrects the concentration protein mismatch. If the serum gradient is above 11 g/L (transudative ascites), this is highly suggestive of portal hypertension, which is usually caused by liver cirrhosis. SAAG less than 11 g/L (exudative ascites) indicates another origin of ascites (e.g. peritoneal malignancies, peritoneal infection, nephrotic syndrome). (3,13,16)
In our study, we found that all patients with cirrhosis had a serum-ascites albumin gradient (SAAG) greater than 11 g/L. In the oncology group, three patients had SAAG greater than 11 g/L and two had SAAG less than 11 g/L. This corresponds to Chen's observations about low sensitivity of SAAG < 11 g/L in the differential diagnosis of malignancy-related ascites. (17)

We observed an overlap between cirrhotic and malignant ascites for four biomarkers:

1.Low values of **ascitic total protein** (below 25 gl/L) had three of five patients with malignant ascites, and with high values above 25 g/L were five of twenty subjects with cirrhotic ascites (Figure 1). This observation is consistent with AASL guidelines and the finding of Henderson et al, who divided cirrhotic ascites into ascites with total protein less than 25 g/L and with greater than or equal to 25 g/L. (13,18)
The treatment received by our oncology patients may have impacted the protein composition in ascites.

2. There was a presence of **ascitic albumin** above 20 g/L in one patient with cancer and three patients with cirrhosis. (Figure 2)

3.**Serum ferritin** was high in half of our cirrhotic patients (maximum value of 1368 µg/l in alcohol-induced cirrhosis) and in two of cancer patients (maximum value of 512 µg/l in a lung carcinoma patient). (Figure 4)

In 1994, AKEAY et al reported the average ferritin levels in ascites for the benign ascites group to be 242.4±232 µg/l and for the malignant group to be 282±210 µg/l, which is consistent with our findings. (19)
Ferritin levels were assumed to be related to tumour mass and behaved as non-specific markers. (20)
We observed a extremely elevated level of ferritin in the ascitic fluid (8683 µg/l.) of one of our oncology patients. The therapy for ovarian carcinoma with peritoneal metastases was ineffective in subject.
Our observations of ascites ferritin levels revealed that half of the patients had values up to 100 µg/l. Furthermore, six patients with liver cirrhosis exhibited values exceeding 280.00 µg/l, which is the upper reference level for serum ferritin.
Elevated levels of peritoneal ferritin are regarded as a reliable indicator of malignancy, with greater sensitivity compared to serum-ascites albumin gradient (SAAG). (21) A discriminant value of ferritin levels (>375 ng/ml) in malignant and cirrhotic ascites was calculated. (5) The observed cut-off does not align with our findings of very high ferritin levels in cirrhotic ascites, and lower levels in ascites associated with neoplasms.
Our findings revealed ferritin levels in ascites that were consistent with the mean values reported in 1994 for benign ascites (242.4±232 µg/l) and malignant ascites (282±210 µg/l). (19)

4. It appears that both liver cirrhosis and malignancy patients in the study had high levels of **plasma D-dimer**, ranging from 3 to 50 times upper limit of normal (ULN). Additionally, ascites D-dimer levels were also elevated in both groups, with a mean of 318±338 mg/L in ascites of cirrhotic patients. (Figure 3) There was overlap in individual ascites values between the two groups, with three cancer patients having ascites D-dimer levels 500-1000 times higher than the plasma ULT for D-dimer. Four cirrhotic patients also showed this same pattern. These findings suggest that elevated levels of D-dimer in ascites may be indicative of concurrent processes in the fluid, regardless of the underlying condition. Further study is recommended to better understand the relationship between D-dimer and ascites in liver cirrhosis and malignancy.

Activation of fibrinolysis was demonstrated in 1993 by Toschi et al both in ascitic fluid and to a lesser extent in plasma, confirmed in 2000 by Agarwal et al and studied in details by J Thaler, in 2022. (7, 22,23) D - dimer levels are significantly lower in plasma than in ascitic fluid. (6,7)

In 2008, A. Spadaro et al. found higher plasma levels of D-dimer in the presence of ascites (649 ± 420 g/L) than in the group without ascites B (359 ± 219 g/L). (24) After resolution of ascitic fluid, D-dimer levels decreased in all patients with high basal levels.

In pancreatic cancer A. Durczyński et al measured plasma D-dimer levels in portal and peripheral blood. (25) Peripheral plasma D-dimer levels above normal limits were found in 10/15 patients, while D - dimer values above normal were confirmed in all portal blood samples. Mean D - dimer values were 297% higher in portal than in peripheral blood (3279.37 vs. 824.64).

Conclusion

This is the first study to simultaneously investigate ferritin and D-dimer levels in uncomplicated ascites, regardless of the etiology. Until now there are a limited number of communication for separate evaluation of the D-dimer and ferritin levels in ascites. Our findings indicated elevated levels of ferritin in both cirrhosis and malignancy patients. Of particular note was the high D-dimer levels in both types of ascites, which in cirrhotic patients was likely due to fibrinolysis. We believe that the genesis of elevated D-dimer levels in cancer patients is similar. Interestingly, after chemotherapy, albumin, D-dimer, and ferritin concentrations in malignant ascites were comparable to those in cirrhotic ascites. The presence of abnormal D-dimer and ferritin levels in liver and malignant ascites, in the absence of clinical manifestations, suggests that silent high levels of these markers may be indicative of concomitant processes in the ascitic fluid (such as fibrinolysis and inflammation) and warrant further research.

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