Laminin concentration in ascites of patients with hepatic cirrhosis and peritoneal carcinomatosis

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Abstract

Laminin levels in ascitic fluid have been proposed as a marker for neoplastic ascites. We compared the concentration of laminin in serum and in ascitic fluid from patients with hepatic cirrhosis and peritoneal carcinomatosis and assessed the diagnostic value of serum laminin levels in differentiating neoplastic from benign ascites. Laminin concentrations were determined by ELISA with antibodies against laminin extracted from the human placenta, in patients with ascites due to peritoneal carcinomatosis (N = 20) and hepatic cirrhosis (N = 33). Patients with infected or hemorrhagic ascites were excluded. The receiver operating characteristic curve was used to determine the sensitivity and specificity of serum laminin for the diagnosis of neoplastic ascites. When compared to the group with cirrhosis, the carcinomatosis group presented significantly higher mean laminin levels in serum (3.3 ± 0.5 vs. 2.1 ± 0.4 µg/ml, mean ± SD, P < 0.05) and ascites (2.8 ± 0.5 vs. 1.6 ± 0.4 µg/ml, P < 0.05). Although laminin concentration was higher in serum than in ascites, the laminin serum/ascites ratio and serum-ascites gradient did not differ between the studied groups. A significant correlation (r = 0.93, P < 0.0001) was observed between the serum and ascites laminin values. Serum laminin levels >2.25 µg/ml showed 100% sensitivity and 73% specificity for the diagnosis of neoplastic ascites. Serum concentration seems to be the main determinant of laminin levels in ascitic fluid and its values can be used as a diagnostic parameter in the study of neoplastic ascites.

Key words
- Ascites laminin
- Serum laminin
- Peritoneal carcinomatosis
- Cirrhosis

Introduction

Laminin, a basement membrane glycoprotein, participates in a series of biological phenomena such as adhesion, migration, differentiation, and cell growth (1), in addition to the maintenance of essential membrane architecture (2,3). Its serum levels have been studied in patients with portal hypertension and neoplastic diseases. Increased serum laminin levels have been observed in chronic liver diseases with fibrosis and/or liver cirrhosis and can be correlated with portal pressure levels (4-6) and with the functional status of the liver (6,7). The increase in circulating glycoprotein has
been attributed to a progressive increase in laminin deposition along the sinusoids during capillarization of the Disse’s space (4,8,9). Furthermore, increased serum laminin levels are also found in patients with neoplasms, especially in cases of basement membrane alterations associated with tumor proliferation and invasion (10,11). The serum values tend to increase significantly with the emergence of metastases, independent of tumor lineage or neoplasm origin (12-15).

The levels of laminin in ascitic fluid have also been proposed as a neoplastic ascites marker (16), but no systematic study has been reported in the literature.

Patients and Methods

Twenty-five healthy adult individuals, 10 men and 15 women, with a mean age of 44 ± 15 years (mean ± SD) were studied as a reference population for the quantification of serum laminin (control group).

An additional group of 53 patients (18 to 74 years old), mainly Caucasians (93%), was included in the study and divided into:

Group 1. Thirty-three patients with liver cirrhosis and ascites detectable on physical examination. The diagnosis of cirrhosis was proven by liver biopsy or based on clinical-biochemical criteria (17). This group consisted of 21 males and 12 females with a mean age of 47 ± 12 years. The cirrhosis etiology for this group was alcoholism (21 cases), viral infection (8 cases), non-alcoholic steatohepatitis (2), α1 antitrypsin deficiency (1), and cryptogenic cirrhosis (1).

Group 2. Twenty patients with neoplastic ascites established by the presence of neoplastic cells in the ascitic fluid and confirmed by the presence of peritoneal implants during surgery or laparoscopy. This group consisted of 9 men and 11 women with a mean age of 60 ± 14 years. The sites of primary tumor origin (all adenocarcinomas) were: ovary (8), colon (4), stomach (4), bladder (2), pancreas (1), and uterus (1).

Exclusion criteria were ascitic fluid with cellularity above 250 PMN/mm³ or positive ascitic fluid culture, systemic infectious processes at present or in the last 3 months before inclusion in the study, patients who had been previously treated or were currently being treated with chemotherapy or radiotherapy, macroscopically hemorrhagic ascitic fluid, suspicion of peritoneal tuberculosis based on laparoscopic findings or ascites cellularity.

All patients were informed of the procedures that they would be subjected to and gave their written informed consent prior to inclusion in the study. The study protocol was approved by the Medical Ethics Committee of São Paulo Hospital, Universidade Federal de São Paulo-Escola Paulista de Medicina, São Paulo, SP, Brazil.

After selection, the patients were submitted to abdominal paracentesis and blood was collected from a peripheral vein for serum evaluation. The samples were centrifuged and divided into aliquots and a part was stored frozen at -80°C for laminin determination. Laboratory analysis of the ascitic fluid included total and differential cell counts, total protein and amylase determination, bacterial culture, and oncotic cytology.

Laminin was isolated from human placenta as described by Schechter and Lopes (18). The isolated protein was identified by 5% SDS-PAGE under reducing conditions and protein concentration was estimated by the method of Bradford (19-21). Total protein extraction from approximately 500 g of fresh placental tissue yielded 31 mg laminin.

Antiserum was produced in female New Zealand rabbits using monthly intramuscular injections of 100 µg purified laminin in complete and incomplete Freund’s adjuvants (22). Antilaminin titer was measured by enzyme-linked immunosorbent assay (ELISA) using anti-rabbit IgG conjugated with peroxidase and o-phenylenediamine (23). For the purification of the antibody obtained, 10 mg laminin was coupled to a CNBr-acti-
vated Sepharose affinity column (Phar- 
ma- 
cia, Uppsala, Sweden) and eluted with 0.1 M 
glycine-HCl buffer, pH 3.0, containing 0.15 
M NaCl. The final protein concentration 
obtained was 852 µg antilaminin antibody/ 
ml. The conjugation of the secondary anti- 
body with horseradish peroxidase was per- 
formed by the method of Avrameas et al. 
(24). The purified antibodies were used in an 
immunohistochemical test with fresh sec- 
tions of rat liver (25).

Laminin concentrations in serum and as- 
citic fluid of subjects with peritoneal carci-
nomatosis and with liver cirrhosis, and in the 
serum of healthy individuals were tested in 
triplicate by ELISA. The serum-ascites lam-
inin gradient was calculated by subtracting 
the ascites values from the serum concentra-
tion, while the serum/ascites ratio was ob-
tained by dividing the serum value by the 
ascites concentration. The receiver operat-
ing characteristic (ROC) curve was used to 
determined the relationship between serum 
laminin levels and the diagnosis of neoplas-
tic ascites (26).

Statistical analysis was carried out by 
Kruskal-Wallis analysis of variance comple-
mented by Dunn’s test and Pearson’s linear 
correlation coefficient (27).

Results

The isolated human laminin was charac-
terized by SDS-PAGE under reducing con-
ditions (Figure 1) and by rat liver immuno-
histochemistry (Figure 2). The electropho-
retic profile, purity grade and immunohisto-
chemical results agreed with those reported 
in the literature (25-29).

Serum and ascites laminin concentrations 
found in the different groups studied are 
shown in Table 1. The mean serum laminin 
values were significantly higher in patients 
with carcinomatosis and cirrhosis than in 
individuals from the control group. Further-
more, the values obtained for the ascitic 
fluid were statistically higher in patients with 
carcinomatosis than in patients with cirrhosis.

The values of the serum-ascites gradient 
(0.5 ± 0.34 in the cirrhotic group vs 0.5 ± 
0.12 in the neoplastic group, means ± SD, P 
= 1.00) and the serum/ascites laminin ratio 
(1.35 ± 0.29 vs 1.21 ± 0.20, respectively, P = 
0.065) did not differ between the two groups 
(Figure 3). A highly significant correlation 
was observed between laminin concentra-
tions in serum and in ascites of the patients 
studied (r = 0.93, P < 0.0001; Figure 4).

ROC curve analysis showed that the area 
under the curve of serum laminin was 0.967 
(0.927-1.008 95% CI, P < 0.01). For serum

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Table 1. Serum and ascites laminin concentrations in patients with cirrhosis and neoplastic ascites and in control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (N = 25)</th>
<th>Cirrhotic patients (N = 33)</th>
<th>Neoplastic patients (N = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin in serum</td>
<td>0.183 ± 0.06</td>
<td>2.1 ± 0.4</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>Laminin in ascites</td>
<td>1.6 ± 0.4</td>
<td>2.8 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as means ± SD.
laminin values higher than 2.25 µg/ml, the diagnostic sensitivity was 100% and the specificity 73%.

**Discussion**

We describe here an uptake assay that utilizes a peroxidase-coupled double antibody system. This test is simple, quick and reproducible and is able to measure laminin levels in serum and ascites. Contrary to what was observed by Smith and Bentsen (16), the response of the test was linear for laminin determination.

Increased serum laminin concentration in patients with chronic liver diseases was first reported by Gressner and Tittor (4), who attributed this increase to capillarization of hepatic sinusoids, since laminin levels were correlated with the portal pressure values of their patients. These findings were confirmed by others (6,7) and were observed in non-cirrhotic patients with portal hypertension (4,5). As mentioned before, increases in serum laminin levels are due to the formation of basement membranes in the hepatic sinusoids. However, an additional effect due to a lack of degradation of this protein by liver endothelial cells cannot be ruled out (30). On the other hand, the increased serum laminin concentration has been related to hepatic fibrosis and liver function in both human and experimental studies (4,7,31-34).

Significantly higher serum laminin levels were observed in patients with peritoneal carcinomatosis (12-15,35,36). This elevated serum concentration could be due to the higher turnover of this glycoprotein in patients with advanced tumor lesions (12-15), but could also be related to a greater release of laminin molecule fragments, as reported in neoplasms (37,38). Results obtained in studies on neoplastic diseases have shown that the growth of the tumor matrix and the metastasizing process lead to an increase in serum levels of basement membrane constituents with tumor progression (13).

The mean laminin values in ascites of patients with neoplasms were significantly higher than in the cirrhosis group. However, when the serum-ascites gradient and the serum/ascites ratio of the studied groups were analyzed, no significant differences could be observed between them, suggesting that these values were interdependent. This finding is contrary to that reported by Byers et al. (39), who found increases in laminin values in ascites, but not in blood of patients with ovarian tumors. In the present study, the serum laminin values were higher than those found in the ascitic fluid in every patient. Furthermore, the lack of any significant differences in the serum-ascites gradient and in
the serum/ascites ratio, as well as the highly significant correlation between laminin levels in ascites and serum, clearly suggest that the circulating levels of this glycoprotein are the main determinant factor for the laminin levels in ascites.

On the other hand, a high correlation between serum and ascites laminin values provides further evidence of the specificity of the isolated antibody. Using the ROC curves, we could define a cut-off value for serum laminin to differentiate malignant from benign (cirrhotic) ascites. The diagnostic performance of serum laminin showed a sensitivity of 100% and a specificity of 73% for the diagnosis of neoplastic ascites.

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References