Serum concentrations of laminin in cirrhosis of the liver

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Abstract

Laminin, a glycoprotein synthesised by Ito cells, has been considered a marker of fibrogenesis. The behaviour of laminin and clinical and laboratory data in 83 patients with cirrhosis were studied to find the factors associated with increases in this glycoprotein. There were increased concentrations of laminin in 62.7% of the patients (40% of the Child's A, 64.5% of the Child's B, and 75% of the Child's C categories). Significant differences in laminin concentrations were found between the Child's grades ($p=0.009$) and between patients and controls ($p<0.0001$). Correlations were found between laminin concentrations and mean corpuscular volume, aspartate aminotransferase, aspartate aminotransferase: alanine aminotransferase ratio, alkaline phosphatase activity, bilirubin and glycocholic acid concentrations, and hypoalbuminaemia – that is, variables related to liver insufficiency and alcohol intake. Moreover, patients with an alcohol intake higher than 100 g/day had higher laminin concentrations than those with a lower intake ($p=0.03$). Conversely, there was no significant association with portal hypertension. Multivariate analysis showed that mean corpuscular volume, bilirubin concentrations, and hypoalbuminaemia were independently associated with laminin concentrations. Poor degradation associated with liver insufficiency seems to play an important part in the increase in serum laminin concentrations in these patients.

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Laminin is a glycoprotein with a molecular weight of about 850 kdaltons, mainly synthesised by Ito cells or lipocytes located in the hepatic space of Disse.¹ It constitutes a part of the extracellular matrix along with other proteins (collagen and elastin), glycoconjugates (structural proteins and proteoglycans), and glycosaminoglycans.^{2,3} Under some inflammatory stimuli Ito cells can transform into myofibroblast-like cells, thus increasing the synthesis of the different components of the extracellular matrix.^{2,4} Accordingly, laminin has been considered to be a marker of fibrogenesis.^{5,6}

In liver disease, laminin concentration has been related to the degree of portal hypertension⁷⁻⁹ and some authors¹⁰⁻¹² have suggested that laminin concentration can be used to evaluate the portal venous pressure. The meaning of the serum concentrations of laminin is difficult to interpret, however, because they are influenced by some poorly known factors.¹³ Also, laminin has been related to alcohol abuse and alcoholic liver disease.¹⁴⁻¹⁶

This study was carried out to compare the serum concentrations of laminin in patients with cirrhosis with comprehensive clinical and laboratory tests in each patient to identify the factors associated with the increase of this glycoprotein.

Methods

Sixty two men and twenty one women patients with cirrhosis confirmed by biopsy were included in the study. Ages ranged between 25 and 83 (mean 56.5) years. All patients were grouped according to the classification of Child and Turcotte¹⁷ with the modifications of Pugh *et al*¹⁸ and Christensen *et al*,¹⁹ which are closely related. There were 20 patients in the Child's A, 31 in the Child's B, and 32 in the Child's C categories.

All patients were evaluated within 24 hours of admission. Blood and urine were collected simultaneously for assessments in the laboratory. The samples that were not processed immediately were frozen at -20°C until analysis. All patients underwent ultrasonographic evaluation and portal hypertension was assessed according to established criteria,²⁰ including an enlarged portal vein, absence of variations with respiratory movements, splenomegaly, portal venous collaterals, recanalisation of the umbilical vein, and detection of hepatofugal flow with a real time/Doppler system. Laminin was measured by radioimmunoassay (Hoechst-Behring, Frankfurt). The upper limit of normality was established as 1.8 U/ml, which corresponded to the mean +2 SD of a control group composed of 81 HBsAg negative healthy subjects with ages ranging between 18 and 64 (mean 40.9) years.

STATISTICAL ANALYSIS

Spearman's rank correlation coefficient was used for correlations between continuous variables. The comparison between two categorical variables was made with the χ^2 test. The Kruskal-Wallis test and the Mann-Whitney U test were used to compare groups as appropriate. A $p<0.05$ level for a two sided test was considered statistically significant. To assess the independent effect of predictor variables on the laminin concentration (dependent variable), we used a multivariate linear regression modelling technique with forward stepwise addition of variables and a probability of <0.05 for the F test used as a criterion for the addition of a predictor variable. Laminin was used as a continuous variable for most statistical tests. It was used as a categorical variable whenever the definition of abnormal values – that is, greater than the upper

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TABLE I Serum concentrations of laminin according to Child's classes

Patients	Total No	Increased No (%)	p Value	Laminin values (U/ml)			
				Mean	SD	Range	Median
Child's A	20	8 (40.0)	0.00007	1.76	1.15	0.6-5.1	1.5
Child's B	31	20 (64.5)	<0.00001	2.18	1.07	0.6-4.8	2.0
Child's C	32	24 (75.0)	<0.00001	2.88	1.50	0.8-6.8	2.7
All	83	52 (62.7)	<0.00001	2.35	1.34	0.6-6.8	2.1
Controls	81	3 (3.7)	-	1.39	0.21	1.0-1.9	1.4

p Value, χ^2 v controls.

limit of normality – was necessary to calculate sensitivity, specificity, and predictive values.

Results

The mean laminin concentration of all patients with cirrhosis was 2.35 U/ml, with the highest concentrations in patients in the Child's C class. We found no differences between the sexes, and age did not significantly influence the concentrations of laminin. Table I shows the laminin values according to the Child's classes.

The severity of liver disease as measured by Child's classes was significantly associated with laminin both in serum concentrations ($p=0.009$) and in the percentage of above normal values ($p=0.038$). Also patients with cirrhosis had significantly higher concentrations of laminin in serum than controls ($p<0.0001$). Table II shows the correlation between laminin concentrations and other laboratory tests.

No obvious relation was found between the laminin concentrations and viral markers, cholelithiasis, ultrasonographically and clinically evaluated portal hypertension, oesophageal varices, drug addition, smoking habits, treatment, previous icteric, encephalopathic, ascitic or gastrointestinal haemorrhages, aetiology, reason for admission, urinary bilirubin and urobilinogen concentration, hepatomegaly, splenomegaly, or quantity of ascites. On the contrary, relations were found with telangiectasis ($p=0.01$), spider naevae ($p=0.02$), and malnutrition ($p=0.02$). Significantly higher concentrations of laminin were found in patients whose alcohol intake was higher than 100 g/day compared with those with a lower intake ($p=0.03$),

although there was no significant correlation between laminin concentrations and the amount ingested or with the years of ingestion.

Laminin had a sensitivity of 66.6%, specificity 60%, efficiency 65.9%, positive predictive value 92.3%, and negative predictive value 20% with a prevalence of 87.8% to detect portal hypertension. In a crossed comparison, Child's B and C class patients without oesophageal varices had substantially higher laminin concentrations than Child's A class patients with such varices with a p value close to the level of significance ($p=0.068$) despite the low number of patients that fulfilled these requirements.

Finally, the multivariate analysis showed that mean corpuscular volume ($p=0.01$), hypoalbuminaemia ($p=0.01$), and bilirubin concentrations ($p=0.005$) were independently associated with serum laminin concentrations, whereas aspartate aminotransferase activity and portal hypertension were not. When alkaline phosphatase was included in the analysis instead of bilirubin, only mean corpuscular volume and hypoalbuminaemia showed a significant association with laminin.

Discussion

We found abnormal laminin concentrations in most of our patients with cirrhosis, and these were significantly different from controls. Other authors have also found such differences.^{5,6} Increases in laminin concentration have been closely related to portal hypertension.⁷⁻¹² Some of the authors reporting this, however, have compared laminin concentrations in non-homogeneous liver diseases of different severity, and none has evaluated liver function simultaneously. We did not find a significant association between laminin concentrations and portal hypertension in our patients with cirrhosis, and laminin concentration had insufficient sensitivity, specificity, efficiency, and predictability to be clinically reliable in the detection of portal hypertension. Conversely, we found a highly significant association with the severity of liver disease as measured by the Child's classes. There was also a significant association with clinical signs of severe liver dysfunction and with laboratory variables that reflect impaired liver function, such as bilirubin and glycocholic acid concentrations and hypoalbuminaemia.²¹ Moreover, patients with the most severe cirrhosis without oesophageal varices had substantially higher laminin concentrations than the least severe ones with varices, suggesting that it is liver dysfunction and not portal hypertension that is associated with the increased laminin concentrations. Other authors¹⁴ have also found a correlation with severity in alcoholic liver diseases as classified by other indices. Probably the relation found by some authors⁷⁻¹² with portal hypertension is an indirect one, and could be explained by the higher rate and degree of portal hypertension in patients with the most advanced liver disease who also have an impaired liver function. We do not believe that cholestasis is an important factor in the increase in laminin despite the significant correlation found with bilirubin and alkaline phosphatase, because

TABLE II Correlation of laminin with laboratory tests

Variable	r	p Value
Packed cell volume	-0.141	NS
Mean corpuscular volume	0.416	0.00004
Prothrombin activity (%)	-0.176	NS
Partial thromboplastin time	0.202	NS
Fibrinogen	-0.133	NS
Aspartate aminotransferase (ASAT)	0.383	0.0002
Alanine aminotransferase (ALAT)	0.192	NS
ASAT:ALAT ratio	0.276	0.01
Alkaline phosphatase	0.320	0.002
γ -Glutamyltranspeptidase	0.212	NS
Lactic dehydrogenase	0.188	NS
Total bilirubin	0.338	0.001
Direct bilirubin	0.415	0.00004
Cholesterol	-0.100	NS
Glycocholic acid	0.222	0.04
Total protein	-0.162	NS
Albumin	-0.370	0.0003
γ -Globulin	0.150	NS
IgG	0.163	NS
IgA	0.213	NS
IgM	0.067	NS

r=Spearman's rank correlation coefficient; p Value=significance of correlation coefficient.

alkaline phosphatase activity was not significantly associated with laminin in the multivariate analysis. On the other hand, bilirubin concentration increases with the severity of liver cirrhosis and, in fact, is one of the variables used to evaluate this severity.¹⁷⁻¹⁹

The increase in laminin concentrations in these patients could be due to increased synthesis by Ito cells.^{1,24} Also the liver plays an important part in the clearance and excretion of glycoproteins,²² and the functional state of the liver, particularly the liver endothelial cells,¹³ can lead to a poor metabolism of laminin. Finally, laminin could escape to its degradative process in the liver via portosystemic shunts.

Laminin has been related to alcohol intake.¹⁴ We have found a significant association with alcohol intake and with alcohol markers such as mean corpuscular volume and the aspartate aminotransferase:alanine aminotransferase ratio,²³ but not with the duration of the alcohol intake. Also van Zanten *et al.*²⁴ found significantly increased laminin concentrations in patients with alcohol abuse without cirrhosis and Nouchi *et al.*¹⁶ reported decreasing laminin concentrations in alcoholic subjects after one week of abstinence.

We conclude that patients with cirrhosis have raised serum laminin concentrations independent of portal hypertension, and that liver dysfunction seems to have a pathogenetic role in the increase of this glycoprotein. Laminin concentration is also related to alcohol intake. This glycoprotein could be used as another marker of severity in these patients.

- 1 Maher JJ, Friedman SL, Roll FJ, Bissell DM. Immunolocalization of laminin in normal rat liver and biosynthesis of laminin by hepatic lipocytes in primary culture. *Gastroenterology* 1988; 94: 1053-62.
- 2 Gressner AM. Liver fibrosis: perspectives in pathobiochemical research and clinical outlook. *J Clin Chem Clin Biochem* 1991; 29: 293-311.
- 3 Rohde H, Wick G, Timpl R. Immunochemical characterization of the basement membrane glycoprotein laminin. *Eur J Biochem* 1979; 102: 195-201.
- 4 Plebani M, Burlina A. Biochemical markers of hepatic fibrosis. *Clin Biochem* 1991; 24: 219-39.
- 5 Schneider M, Voss B, Hogeman B, Eberhardt G, Gerlach U. Evaluation of serum laminin P-1, procollagen-III peptides, and N-acetyl-beta-glucosamidase for monitoring the activity of liver fibrosis. *Hepatogastroenterology* 1989; 36: 506-10.
- 6 Kropf J, Gressner AM, Negwer A. Efficacy of serum laminin measurement for diagnosis of fibrotic liver diseases. *Clin Chem* 1988; 34: 2026-30.
- 7 Robert P, Champigneulle B, Kreher I, Gueant JL, Foliguet B, Dollet JM, *et al.* Evaluation of fibrosis in the Disse space in noncirrhotic alcoholic liver disease. *Alcohol Clin Exp Res* 1989; 13: 176-80.
- 8 Gressner AM, Tittor W, Negwer A. Serum concentrations of N-terminal propeptide of type III procollagen and laminin in the outflow of fibrotic livers compared with liver-distal regions. *Hepatogastroenterology* 1986; 33: 191-5.
- 9 Gressner AM, Tittor W. Serum laminin - its concentration increases with portal hypertension in cirrhotic liver disease. *Klin Wochenschr* 1986; 64: 1240-8.
- 10 Gressner AM, Tittor W, Kropf J. The predictive value of serum laminin for portal hypertension in chronic liver diseases. *Hepatogastroenterology* 1988; 35: 95-100.
- 11 Gressner AM, Tittor W, Negwer A, Pick-Kober KH. Serum concentrations of laminin and aminoterminal propeptide of type III procollagen in relation to the portal venous pressure of fibrotic liver diseases. *Clin Chim Acta* 1986; 161: 249-58.
- 12 Mal F, Hartmann DJ, Trinchet JC, Lacombe F, Ville G, Beaugrand M. Laminine serique et pression portale au cours de la cirrose alcoolique. Une étude chez 39 malades. *Gastroenterol Clin Biol* 1988; 12: 841-4.
- 13 Smedsrød B, Paulsson M, Johansson S. Uptake and degradation in vivo and in vitro of laminin and nidogen by rat liver cells. *Biochem J* 1989; 261: 37-42.
- 14 Niemelä O, Risteli J, Blake JE, Risteli L, Compton KV, Orrego H. Markers of fibrogenesis and basement membrane formation in alcoholic liver disease. Relation to severity, presence of hepatitis, and alcohol intake. *Gastroenterology* 1990; 98: 1612-9.
- 15 Annoni G, Colombo M, Cantaluppi MC, Khlai B, Lampertico P, Rojkind M. Serum type III procollagen peptide and laminin (Lam-P1) detect alcoholic hepatitis in chronic alcohol abusers. *Hepatology* 1989; 9: 693-7.
- 16 Nochi T, Worner TM, Sato S, Lieber CS. Serum procollagen type III N-terminal peptides and laminin P-1 peptide in alcoholic liver disease. *Alcohol Clin Exp Res* 1987; 11: 287-91.
- 17 Child CG, Turcotte JG. Surgery and portal hypertension. In: Child CG, ed. *The liver and portal hypertension*. Philadelphia: WB Saunders, 1964: 50-72.
- 18 Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; 60: 646-9.
- 19 Christensen E, Schlichting P, Fauerholdt L, Gluud C, Andersen PK, Juhl E, *et al.* Prognostic value of Child-Turcotte criteria in medically treated cirrhosis. *Hepatology* 1984; 4: 430-5.
- 20 Dubbins PA, Goldberg BB. Abdominal vasculature. In: Goldberg BB, ed. *Abdominal ultrasonography*. New York: John Wiley & Sons, 1984: 19-80.
- 21 Festi D, Labate AMM, Roda A, Bazzoli F, Frabboni R, Rucci P, *et al.* Diagnostic effectiveness of serum bile acids in liver diseases as evaluated by multivariate statistical methods. *Hepatology* 1983; 3: 707-13.
- 22 Thomas P, Zamcheck N. Role of the liver in clearance and excretion of circulating carcinoembryonic antigen (CEA). *Dig Dis Sci* 1983; 28: 216-24.
- 23 Chopra S, Griffin PH. Laboratory tests and diagnostic procedures in evaluation of liver disease. *Am J Med* 1985; 79: 221-30.
- 24 van Zanten RA, van Leeuwen RE, Wilson JH. Serum procollagen III N-terminal peptide and laminin P1 fragment concentrations in alcoholic liver disease and primary biliary cirrhosis. *Clin Chim Acta* 1988; 177: 141-6.