Efficacy of Serum Laminin Measurement for Diagnosis of Fibrotic Liver Diseases

J. Kropf, A. M. Greaser, and A. Negwer

We examined the efficacy of laminin assay in serum for diagnosis of fibrotic liver diseases. Values for subjects with liver disease significantly (P<0.05) exceeded those for healthy subjects and patients with nonhepatic diseases. At a cutoff value of 1.45 kimo-units (arb.)/L (0.33 mg/L) and an assumed prevalence of fibrotic liver diseases of 0.5, positive and negative predictive values of the test were 0.97 and 0.83, respectively, for the comparison with a healthy reference population and 0.81 and 0.80 for nonhepatic diseased patients. Increases in laminin concentration were positively correlated with the extent of fibrotic transition of the liver. Discrimination between fibrotic and cirrhotic stages of chronic liver diseases by means of laminin assay was better than with the amino-terminal propeptide of type III procollagen.

According to the criteria of diagnostic efficacy, we conclude that determination of laminin in serum improves the possibilities of clinical-chemical diagnosis of liver fibrosis and cirrhosis. However, as commonly true for other biochemical tests, determination of laminin cannot replace conventional diagnostic methods.

Additional Keyphrases: nonhepatic diseases compared - cirrhosis - fibrosis - type III procollagen - data handling - cutoff value - screening

Fibrosis, a serious and frequent complication of chronic active liver diseases, is characterized by excessive deposition of various normal components of connective tissue in liver, including several species of collagen and proteoglycans, hyaluronic acid, elastin, fibronectin, and laminin (1-3). At present, liver biopsy is the only reliable method for detecting the fibrotic reaction of inflamed liver tissue, but the technique has obvious limitations, the most prominent being (a) the high degree of sampling error, (b) the invasive nature of the method, and (c) the fact that histology does not necessarily reflect ongoing fibrogenic activity.

Several approaches were made to circumvent these problems, aimed at assessing liver fibrosis by noninvasive tests. Measurements in serum of some metabolites and enzymes involved in collagen and proteoglycan/glycoprotein metabolism—such as hydroxyproline, proline, proteoglycans, hyaluronic acid, procollagen peptides, and prolyl hydroxylase activity—were carried out (4-9). Among these analytes, radioimmunological determination of the concentration of the aminoterminal propeptide of type III procollagen appears to be most widely used (10), but the limitations to the clinical value of this test have been stressed (11-13).

Laminin, a major noncollagenous, high-molecular-mass glycoprotein of basement membranes (14, 15), increases in fibrotic livers mainly via the development of a continuous basal lamina beneath the endothelial cell layer in the space of Disse (16). The concentration of this glycoprotein is increased also in sera of patients with fibrotic liver diseases (5, 17-19) but not necessarily specifically in liver fibrosis (20-23). The clinical value of measuring laminin in serum as a screening test for this kind of hepatic disease is not known. Therefore, we tried to quantify the diagnostic power of serum laminin for fibroproliferative liver disorders.

Patients and Methods

Patients. For the reference population of clinically and biochemically apparently healthy persons, we used samples from blood donors (n = 146, 90 men, ages 19-56 y).

Sera of patients with nonhepatic diseases (n = 112, 69 men, ages 18-99 y) were collected from the routine service of the Central Laboratory. Only patients having no anamnesis, clinical, or clinical-chemical signs of acute or chronic liver diseases were included. According to their primary clinical diagnosis, the patients were assigned to one of the categories shown in Figure 1.

The group of patients with fibrotic liver diseases (n = 79, 60 men, ages 21-83 y) was subdivided, on the basis of histological examination of liver-biopsy specimens, into patients with liver fibrosis (n = 39, 27 men, ages 21-83 y) and those with liver cirrhosis (n = 47, 33 men, ages 28-65 y). The etiologies of fibrosis and cirrhosis explored anamnestically are shown in Figure 2. The patients with histologically verified fibrosis or cirrhosis were recruited from the Department of Gastroenterology of the University of Marburg (kindly provided by Prof. K. Weigand) and from the Stoffwechselklinik der LVA Bad Mergentheim (kindly provided by Prof. W. Tittor).

![Fig. 1. Box plots showing the distribution of laminin concentrations in our study populations of healthy subjects, individuals with nonhepatic disease, and patients with fibrotic liver diseases](image-url)
Specimen collection. Venous blood was sampled in the morning from subjects who had fasted for 12 h. Serum was prepared and stored at 70 °C until assay (no longer than three to four weeks).

Radioimmunoassay of laminin. To measure laminin in serum, we used a competitive radioimmunoassay (Behring-Hoechst, Frankfurt, F.R.G.), of which some analytical characteristics were reported elsewhere (20, 24). The antibody is directed against antigenic determinants of the pepsin-resistant laminin fragment P1 isolated from human placenta (20). The within-run and day-to-day imprecisions (CVs) were 3.1 and 4.9%, respectively, for a mean concentration of 1.8 kilo-units/L. The inaccuracy ranged from −12 to +11%. The concentrations of laminin in serum are given in arbitrary units, as suggested by the manufacturer, because no international standard of laminin is available. One unit is defined by the manufacturer as the mean quantity of laminin per milliliter of serum pooled from a group of obviously healthy persons, and it corresponds to −0.230 µg.

Other analyses. Aminoterminal propeptide of type III procollagen (P-III-P) was determined as described previously (4, 5, 31).1 Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), and bilirubin were determined by well-accepted methods (25).

Statistical methods. The laminin concentrations in sera of healthy subjects showed obvious deviations from the normal (gaussian) distribution, being skewed toward low concentrations. We therefore used nonparametric measures and methods when appropriate. For data input for analysis of variance (ANOVA) and discriminant analysis, we used the logarithms of laminin concentrations. Logarithmic transformation approximated gaussian distribution, as confirmed by the chi-square and Kolmogorov–Smirnov tests (26). ANOVA and discriminant analysis were performed with procedures GLM and DISCRIM of the SAS/PC software package (SAS Institute Inc., Cary, NC). The options Schaeffe and REGOF of the GLM procedure were used for multiple comparisons of means within ANOVA. Distribution fitting and testing, calculation of percentiles, medians, rank correlation coefficients, and summary statistics were obtained by the STATGRAPHICS program (STCS Inc., Rockville, MD). The computations of quantitative diagnostic criteria were facilitated by a program we wrote, using the well-known relationships (27) for calculating sensitivity, specificity, and positive and negative predictive values, respectively. Efficiency was calculated as the fraction of correct classifications among all studied subjects. The likelihood ratio of a given test result, the probability for this result when disease is present divided by the probability for the same result when disease is absent (28, 29), was computed by dichotomizing the measured concentrations at various cutoff values and dividing the true-positive fraction by the false-positive fraction.

Results
Laminin Concentrations in Sera from Various Clinical Groups

The concentrations of laminin in sera of healthy individuals ranged from 0.81 to 1.43 kilo-units/L (2.5th to 97.5th percentile, median 1.04 kilo-units/L), with no significant sex-related differences (males: 0.85–1.53 kilo-units/L, median 1.02 kilo-units/L; females: 0.75–1.30 kilo-units/L, median 1.05 kilo-units/L). The median concentration of laminin in this group is thus very close to the value of 1.00 kilo-unit/L that one would expect for healthy persons. Serum from patients with nonhepatic diseases had slightly higher laminin concentrations, the median value of the total group being 1.10 kilo-units/L. A rough classification of the various diseases into three general categories gave the following medians (kilo-units/L) for the subgroups: tumors 1.05, "surgical diseases" 1.00, and internal diseases 1.20 kilo-units/L. In patients with fibrotic liver diseases, the concentrations of laminin in serum were significantly (P < 0.001) increased, the median value of the overall group being 1.90 kilo-units/L (fibrosis: 1.63, cirrhosis: 2.20 kilo-units/L) (Figure 1). ANOVA of the total population under investigation (n = 337) established three groups with significantly (P < 0.05) different laminin concentrations: cirrhosis, fibrosis, and all other cases (healthy subjects, tumors, surgical, and internal diseases).

The liver diseases were further classified according to etiology. The boxplots of Figure 2 illustrate the distribution of the concentrations of laminin in relation to etiology and histology. The greater concentration of laminin in cirrhosis than in fibrosis was consistent in all etiologies except for primary biliary cirrhosis. The median concentrations of laminin in the ethanol-induced and post-hepatitis cases (2.1 and 2.15 kilo-units/L, respectively, for the combined cirrhosis and fibrosis cases) exceeded those found in primary biliary cirrhosis and unknown etiology (1.65 and 1.70 kilo-units/L, respectively). However, ANOVA gave no indication of a statistically significant effect of etiology on the concentration of laminin.

In the sera of patients with fibrotic liver diseases, we measured several additional clinical chemical analytes. Table 1 lists the mean concentrations and the significant rank correlation coefficients between pairs of these variables.

Quantitative Diagnostic Criteria of Laminin for Liver Fibrosis and Cirrhosis

The numerical values of the most commonly used measures of test efficacy are compiled in Table 2. Dichotomiza-

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1 Nonstandard abbreviations: P-III-P, amino-terminal propeptide of type III procollagen; ALT, alanine aminotransferase (EC 2.6.1.2); AST, aspartate aminotransferase (EC 2.6.1.1); GGT, γ-glutamyltransferase (EC 2.3.2.2); ANOVA, analysis of variance.
Table 1. Concentrations of Some Analytes in Sera from Patients with Liver Disease, and Correlations between All Pairs of Analytes and Laminin

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Mean concn</th>
<th>SD</th>
<th>Spearman rank correlation coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ALT</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>42</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>AST, U/L</td>
<td>40</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Bilirubin, µmol/L</td>
<td>41</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>GGT, U/L</td>
<td>120</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>P-III-P, µg/L</td>
<td>26</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

n.s., not significant (P > 0.05).

Table 2. Diagnostic Value of a Serum Laminin Concentration of 1.45 kilo-units/L (Upper Reference Limit) for Detection of Liver Fibrosis and Cirrhosis

<table>
<thead>
<tr>
<th>Type of liver disease</th>
<th>Reference group</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Efficiency</th>
<th>Predictive values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosis</td>
<td>Blood donors</td>
<td>0.98</td>
<td>0.63</td>
<td>0.92</td>
<td>Positive result: 0.97, Negative result: 0.72, Likelihood ratio: 30</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Nonhepatic diseases</td>
<td>0.81</td>
<td>0.63</td>
<td>0.77</td>
<td>Positive result: 0.77, Negative result: 0.68, Likelihood ratio: 3.3</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Blood donors</td>
<td>0.98</td>
<td>0.91</td>
<td>0.96</td>
<td>Positive result: 0.98, Negative result: 0.92, Likelihood ratio: 45</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Nonhepatic diseases</td>
<td>0.81</td>
<td>0.91</td>
<td>0.84</td>
<td>Positive result: 0.83, Negative result: 0.91, Likelihood ratio: 4.9</td>
</tr>
<tr>
<td>All</td>
<td>Blood donors</td>
<td>0.98</td>
<td>0.80</td>
<td>0.92</td>
<td>Positive result: 0.97, Negative result: 0.83, Likelihood ratio: 39</td>
</tr>
<tr>
<td>All</td>
<td>Nonhepatic diseases</td>
<td>0.81</td>
<td>0.80</td>
<td>0.81</td>
<td>Positive result: 0.81, Negative result: 0.80, Likelihood ratio: 4.3</td>
</tr>
</tbody>
</table>

*Calculations are based on a prevalence of fibrotic liver disease of 0.5.

The predictive values of positive and negative test results and the likelihood ratio were calculated both with healthy subjects (blood donors) and with patients with nonhepatic diseases as reference groups (Table 2). As expected, the best results were obtained for the comparison of liver-disease patients with healthy subjects, as indicated by the positive and negative predictive values and the likelihood ratio of 0.97, 0.83, and 39, respectively. The corresponding results for comparison with a reference group of patients with nonhepatic diseases were weaker, the values being 0.81, 0.80, and 4.3, respectively. The prevalence of fibrotic liver diseases was arbitrarily chosen to be 0.5 in all calculations, to facilitate the comparison of the results. The effective prevalence of fibrotic liver diseases in the (synthetic) population of this investigation was 0.35 in the healthy vs the liver-disease group and 0.41 in the nonhepatic vs the liver-disease group. Figure 3 shows the positive and negative predictive values at two cutoff values as a function of disease prevalence.

In order to evaluate the effect of case mix (34), we repeated the calculations for fibrosis and cirrhosis separately. The results (Table 2) show that generally higher values were obtained for patients with cirrhosis than for patients with fibrosis.

With regard to the different histology-dependent mean concentrations of laminin, we tried to use the serum laminin concentration of patients with histologically verified liver diseases to discriminate between cases with fibrosis and cirrhosis. Linear discriminant analysis (32, 33), based on logarithms of the laminin concentrations alone, with histology as the classification factor, resulted in the correct allocation of 78% of all patients (Table 3); the misclassified cases were proportionately distributed among fibrosis and cirrhosis. The extension of the discriminant model with other liver-related analytes in serum (P-III-P, AST, ALT,
GOT, and bilirubin) slightly increased, in some combinations, the proportion of correct classifications. The best set of analytes (laminin and bilirubin) yielded 82% correct classifications (Table 3). With P-III-P alone the percentage of correct classifications was only 53%; i.e., the discriminating efficiency was not better than random allocation. Similar results were obtained with all other single tests except laminin.

Discussion

We conclude that

(a) the concentration of laminin in serum is positively related to the severity of the fibrotic liver disease, being significantly higher in cirrhosis than in mere fibrosis;

(b) this increase is relatively independent of the etiology of the disease. Our six cases of primary biliary cirrhosis are too few to allow a decision as to whether this finding is typical for primary biliary cirrhosis or is only a coincidence; and

(c) the diagnostic power of laminin will be too weak to use results of this assay as a reliable substitute for liver biopsy.

Taking the upper reference limit of serum laminin (1.45 kilo-units/L) as the cutoff value between negative and positive test results, predictive values of 0.81 (positive) and 0.80 (negative) are obtained. These values are valid for the comparison with a nonhepatic diseased collective and an assumed prevalence of fibrotic liver diseases of 0.5. In a realistic clinical situation the prevalence of fibrotic liver disease will often be much lower, thus producing lower predictive values, too. With regard to screening for fibrotic liver diseases, recognition of the onset of the disease (mild fibrosis) is much more important than is the case for cirrhosis—which consequently further reduces the useful diagnostic power of the test (Table 2). At small prevalences of disease the increasing rate of falsely positive results becomes important and thus necessitates a high diagnostic specificity. The correlation between the severity of the fibrotic liver disease and the increase in the laminin concentration supports the use of the likelihood ratio. Unlike most other quantitative measures of diagnostic validity, the likelihood ratio takes into account the actual concentration of the variable under consideration. As calculated from the data set in this report, it is about four times more likely to find a laminin concentration of 1.45 kilo-units/L in a patient with liver fibrosis than in a patient suffering from any other disease. This ratio is increased to more than 30 if the measured concentration of laminin is 2.45 kilo-units/L.

The results of discriminant analysis (Table 3) were shown principally for illustrative reasons. The discrimination between the two histological states of the liver by means of one variable only (laminin concentration) is fairly good. Additionally, one must consider that the fraction of 0.75 correct classifications is based on histological verification, which itself is not free of error. The subsequent dichotomization of the histological results, the continuous process of development of cirrhosis, and subjective judgments of the investigator will inevitably introduce some verification bias (34). Linear discriminant analysis presupposes normal distributions and equal dispersion (covariance) matrices of all groups. Both assumptions were not tested for the separated fibrosis and cirrhosis groups, but linear discriminant analysis is generally believed to be rather robust to deviations in this respect (32). It is well known that the rate of correct classifications is overestimated when obtained from the learning class (33), as is the case for the results shown in Table 3. Application of the computed discriminant functions to independent observations might result, therefore, in a higher fraction of misclassifications. Despite the reservations we have discussed, the measurement of serum laminin was more potent in assessing the state of fibrotic organ changes of the liver than any of the other clinical chemical analytes considered. In particular, compared with P-III-P, an analyte frequently used for the diagnosis and follow-up of fibrotic liver diseases (6, 7), laminin proved to be substantially better.

Our results for laminin are in many aspects similar to those obtained by Engström-Laurent et al. (8) for serum hyaluronic acid, a high-molecular-mass polysaccharide component of connective tissues with a quite different metabolic pathway (35). The concentrations of hyaluronic acid in serum of patients with various liver diseases and serum of healthy persons show essentially the same pattern as the concentrations of laminin in this study (Figures 1 and 2). However, there seems to be more variation in the concentrations of hyaluronic acid than we observed for the concentrations of laminin. The numerical values for diagnostic test efficacy of hyaluronic acid reported in ref. 8 are almost identical to those we obtained for laminin (Table 2). If both analytes are not strongly correlated—an assumption which may be unlikely—the combined determination of laminin and hyaluronic acid will be clinically very useful.

Subsequent investigations with independent data are

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**Table 3. Results of Linear Discriminant Analysis of Patients with Liver Disease According to Histology**

<table>
<thead>
<tr>
<th>Histological classification</th>
<th>Laminin</th>
<th>Laminin + bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cirrhosis</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>35</td>
<td>12</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>
necessary to clarify the points of interest that remain open. Multivariate models, using additional analytes and perhaps alternative statistical methods (logistic regression), might eventually make it possible to get reliable diagnostic information concerning fibrotic diseases of the liver simply by measurement of clinical chemical analytes.

References