Logistic-Regression Model for Assessing Portal Hypertension by Measuring Hyaluronic Acid (Hyaluronan) and Laminin in Serum

Jürgen Kropf,1 Axel M. Greiner,1 and Wolfgang Tittor2

We earlier observed a positive correlation between portal venous pressure (PVP) and the concentration of laminin in serum. Here we investigated whether the diagnostic efficacy could be improved by considering additional analytes and application of multivariate statistical analysis. In 102 patients with fibrotic liver disease of various etiologies we measured PVP as the gradient of the wedged and free hepatic venous pressures and determined the concentrations of hyaluronic acid and laminin in serum. Regression coefficients established by logistic regression in this group were subsequently used to predict portal hypertension (PVP >5 mmHg) in an independent group of 45 patients. By comparison with the known PVP, we obtained a sensitivity of 0.83 (confidence interval: 0.63–0.93) and a specificity of 0.82 (0.61–0.93) for diagnosis of portal hypertension by means of the concentrations of hyaluronic acid and laminin in serum. Application of the model is suggested as a tool for pre-screening and monitoring patients to be subjected to assessment of portal hypertension.

Additional Keyphrases: screening · monitoring disease · fibrotic liver disease · connective tissue

The concentrations of several components of connective tissue in serum have been determined mainly to assess their utility for diagnosis and monitoring of diseases with fibrotic activity (1, 2). In cases of liver fibrosis, the aminoterminal propeptide of type III procollagen (PIIINP) (3), hyaluronan (hyaluronic acid) (4), and laminin (5) have been studied intensively, and increased concentrations of these analytes in serum related to the severity and (or) activity of the disease have generally been found.8

Portal hypertension is a frequent and serious life-threatening complication of chronic liver diseases. It currently cannot be diagnosed and monitored by clinical chemical tests. Previously we reported a positive correlation between the concentration of laminin in serum and portal venous pressure (PVP) in patients with fibrotic and cirrhotic liver diseases (6). In an attempt to optimize diagnostic efficacy, we applied logistic-regression analysis (7, 8) to the combined results obtained for hyaluronic acid, laminin, PIIINP, and some analytes commonly used for the diagnosis of fibrotic liver disease. Portal hypertension, established by measurement of the gradient of the wedged and free hepatic venous pressures (WHVPG = WHVP – FHVP) (9), served as an independent criterion for evaluating the severity of liver disease.

The likelihood ratio, estimated by logistic regression as proposed by Albert (10), has the advantage that one can quantify the diagnostic ability of multiple tests as a single numerical value. The predictive value of a positive test result is easily computed from the likelihood ratio and the pre-test probability of the disorder being considered (see Methods of Computation).

A preliminary investigation (11) suggested that the prediction of portal hypertension might be improved if values for laminin and hyaluronic acid were used in combination. This result was, however, confirmed within the same population from which it had been derived and therefore was suspected of being too optimistic (12). The aim of the present study was to validate the diagnostic efficacy of the serum concentrations of these connective-tissue components for portal hypertension in a new, i.e., independent, population of patients.

Materials and Methods

Subjects

We investigated two independent populations of patients with chronic liver diseases, recruited from the Stoffwechselklinik der LVA Bad Mergentheim, F.R.G.

Group A comprised 102 patients (82 men and 20 women, ages 24–71 years) with histologically verified liver fibrosis of anamnestically explored etiologies (Table 1). Serum sampled from these patients, who had been already evaluated in a previous study (6), was used as the training set for the computation of the coefficients of the logistic regression.

Group B: One of us (W.T.) collected sera from 45 consecutive patients (29 men and 16 women, ages 23–69 years) with liver fibrosis who were being clinically assessed for portal hypertension. The investigators performed the chemical and statistical analyses of these sera had no patient-related information.

For all patients the portal venous pressure was determined indirectly by the wedged hepatic vein pressure gradient (WHVPG) (9). Pressure >5 mmHg was considered to be pathological. The fractions of patients with
Table 1. Mean Concentrations of Extracellular Matrix Proteins and Portal Venous Pressure in Sera from Patients with Liver Disease

<table>
<thead>
<tr>
<th></th>
<th>Mean concentration ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
</tr>
<tr>
<td>Ref. values</td>
<td>23</td>
</tr>
<tr>
<td>Group A</td>
<td>102</td>
</tr>
<tr>
<td>Ethanol toxic</td>
<td>47</td>
</tr>
<tr>
<td>Other</td>
<td>55</td>
</tr>
<tr>
<td>Group B</td>
<td>45</td>
</tr>
<tr>
<td>Ethanol toxic</td>
<td>39</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
</tr>
</tbody>
</table>

* Significantly greater than the reference limits (P < 0.05). b Not done.

Methods of Computation

The likelihood ratio (LR) is defined as the ratio of two probabilities:

\[ LR(X) = \frac{P(X|D)}{P(X|\neg D)} \]  

i.e., the probability of finding value(s) X, given the presence of disease D, divided by the probability of finding X in the absence of disease D. [Although disease (D) is considered here as a binary variable, it could take several values (8).] Given n diagnostic tests, \( X^T = (x_1, \ldots, x_n) \) is a vector of n results \( x_1, \ldots, x_n \), and the likelihood ratio can be expressed as

\[ LR(X) = \exp(a_0 + a_1x_1 + \ldots + a_n x_n) \]

The unknown coefficients \( a_0, \ldots, a_n \) are estimated by a logistic-regression procedure.

By application of Bayes's theorem, the posterior probability (predictive value) of disease, given the test result(s) X, can be easily calculated from the likelihood ratio and the pre-test probability (prevalence) (p) of disease D:

\[ P(D|X) = \frac{P LR(X)}{(1-p) + p LR(X)} \]

With the procedure outlined, the number that the clinician is actually interested in, P(D|X), is obtained by a simple mathematical operation on the test results and some previously calculated coefficients.

We used the CATMOD procedure of the SAS statistic package (SAS PC V6.03; SAS Institute, Cary, NC) for computation of likelihood ratios. SAS procedure REG was used for computing multiple linear regressions between WHVP and the analytes.

We tested the association between variables by using Spearman rank correlation coefficients (SAS procedure CORR). For testing differences between groups we used the Mann–Whitney U test (SAS procedure NPARYWAY). No corrections for multiple comparisons were made.

Results

Mostly above-normal concentrations of hyaluronic acid, laminin, and PIIINP were found in both groups as

high values for WHVPG were 0.43 for group A and 0.51 for group B.

Specimen Collection

Venous blood was sampled in the morning from subjects who had fasted overnight. The serum was stored at −70 °C for no more than two months until assay. The interval between sampling of the sera of group A and acquisition of group B was three years; the duration of both sampling periods was about two months.

Determination of Laminin and Hyaluronic Acid

Laminin was measured in serum by a competitive radioimmunoassay (Behring-Hoechst, Frankfurt, F.R.G.) with antibodies directed against antigenic determinants of fragment P1 (13, 14). Analytical details have been described before (5, 14). The concentrations of laminin in serum are given in arbitrary units, based on the median value of the concentrations in a healthy population, because no international standard of laminin is available. Because of changes in reagent composition and kit design during the three years between both sampling intervals, the standard values for the analyses for serum group B were 1.33 times higher than those for group A. To maintain the comparability of absolute values, which is important for our study, we multiplied the original (group A) concentrations by 1.33 before statistical analysis.

Hyaluronic acid was measured by a previously described radiometric assay (Pharmacia, Uppsala, Sweden) (15). The intra- and interassay CVs (at \( \bar{x} = 182 \) µg/L) were 6.9% and 7.3%, respectively.

PIIINP was determined in serum by radioimmunoassay (Behring-Hoechst) as described previously (16), of which the analytical criteria are reported elsewhere (17).

Other Analyses

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), and bilirubin were determined in serum by routine methods (18).
compared with the mean values for the analytes in healthy subjects (Table 1).

Groups A and B were homogeneous (no significant differences) with respect to the concentrations of laminin and hyaluronic acid, and to WHVPG (Figure 1). There was a significant effect of etiology (alcoholic vs nonalcoholic) on the concentration of laminin ($P = 0.0016$ and $0.0017$ for the combined groups and group A, respectively) and on WHVPG ($P > 0.0001$ both for the combined groups and group A), but not on hyaluronic acid ($P > 0.05$). The mean values for all analytes were highest within the group of ethanol-toxic etiologies (Table 1). No sex-specific differences could be detected for any analyte investigated. The correlations between the analytes and WHVPG, shown in Table 2, were nearly identical within both groups. Correlations between laminin and hyaluronic acid in group B were 0.76 ($P < 0.001$) and 0.59 ($P < 0.05$) for alcoholic and nonalcoholic etiologies, respectively. A multivariate linear regression of WHVPG with hyaluronic acid and laminin as independent variables gave highly significant ($P < 0.0001$) estimates of the regression parameters for both analytes, but the coefficient of correlation was not greater than for the single variables ($r = 0.73$).

Logistic regression (decision limit: WHVPG >5 mmHg) within group A with models of various combinations of variables gave significant regression coefficients for hyaluronic acid and laminin. Only main effects, i.e., no interactions between single variables, were considered in this step. Because all other variables, including histology (stages: none, fibrosis, cirrhosis), sex, age, and concentrations of PIIINP, AST, ALT, GGT, and bilirubin, were not significant for the discrimination of portal hypertension, we refrained from their determination or consideration in group B. An additional test for a second-order effect in the final model, i.e., an interaction between hyaluronic acid and laminin, was not significant.

The likelihood ratio (LR) for portal hypertension is computed as follows:

$$
LR = \exp(1.974 \cdot \text{laminin[kilo-units/L]} + 0.0407 \cdot \text{hyaluronic acid[\mu g/L]} - 7.119)
$$

To facilitate the interpretation, we computed the more familiar measures of diagnostic sensitivity, specificity, and efficiency from the predictions obtained by logistic regression and the "true" WHVPG. The pre-test probabilities required for the computation of the predictive values from the likelihood ratios were taken from the frequency of diseased patients ($WHVPG > 5$ mmHg) within the respective groups. A predictive value of 0.5 was taken as the decision limit for positive or negative "test" results, respectively. Figure 2 shows the WHVPG within group B in relation to the computed predictive values.

The diagnostic measures for the reclassification within the training set (group A) are compiled in Table 3a. Application of the coefficients attained from the training set on group B gave the results shown in Table 3b. With hyaluronic acid alone, the sensitivity and specificity were 0.77 and 0.86, respectively, whereas with laminin as the single variable these figures were 0.86 and 0.64, respectively. The combination of both analytes gave a sensitivity of 0.83 and a specificity of 0.82.

Because the different distribution of etiologies in both groups could be noticed only after the settlement of the primary analysis (blinded design of the study), we recalculated the logistic regression, considering only

### Table 2. Spearman Rank Correlation Coefficients of WHPVG with Hyaluronic Acid, Laminin, and PIIINP

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid</td>
<td>0.75*</td>
<td>0.72</td>
</tr>
<tr>
<td>Laminin</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>PIIINP</td>
<td>0.15 (n.s.)</td>
<td>0.18 (n.s.)</td>
</tr>
</tbody>
</table>

* $P < 0.01$ except where stated otherwise.

* Six cases only.
cases with alcoholic etiology. In contrast to the results for the complete group, the regression coefficient of laminin was not significant if laminin was put in the model together with hyaluronic acid. The simple model, considering laminin only, had a very high sensitivity: only one case (WHVPG = 6 mmHg) out of 39 was falsely classified as <5 mmHg. On the other hand, there were 11 false-positive classifications, albeit six of them were within the borderline range of 4–5 mmHg, for a very poor specificity. Using hyaluronic acid as the single variable improved sensitivity (0.84) and specificity (0.90) within the ethanol-toxic group in comparison with the complete group.

The formula for the computation of the likelihood ratio for portal hypertension within ethanol-toxic etiologies is as follows:

\[
LR = \exp(0.0658 \cdot \text{hyaluronic acid} \ [\mu g/L] - 3.971)
\] (5)

The number of cases in the group of nonethanol toxic etiologies was too low to justify the evaluation of an analogous formula for this group. In extension of the above models, etiology (coded: nonalcoholic 0, alcoholic 1) was included as an additional independent variable with laminin and hyaluronic acid. In this model significant effects \((P < 0.05)\) emerged for etiology, hyaluronic acid, and for the combinations hyaluronic acid · etiology and laminin · etiology; laminin by itself was not significant \((P = 0.09)\). The positive significance test for laminin · etiology confirms that different relations exist between WHVP and the concentrations of laminin in serum in patients with alcoholic etiology and in those with nonalcoholic etiology.

**Example**

To demonstrate the steps necessary for the application of the proposed procedure, we give a detailed example, using the results for one of the patients (marked with the arrow in Figure 2). The concentrations of laminin and hyaluronic acid in the serum of this patient were 2.38 kilo-units/L and 131 \(\mu g/L\), respectively. From anamnesis an ethanol-toxic etiology of the disease could be inferred.

With this information we compute the likelihood ratio by using equation 5 because of the ethanol-toxic etiology:

\[
LR = \exp(0.0658 \cdot 1.31 - 3.971)
\]
\[
= \exp(4.6)
\]
\[
= 100
\]

The likelihood ratio considerably exceeds 1, so we can strongly suspect that portal hypertension is in effect.

From an inspection of the medical files of previous investigations we determined that in 55% of all patients of this community portal hypertension had been established; i.e., the prevalence \((p)\) of portal hypertension within our clinical setting was 0.55. We could then finally compute an individual predictive value for this patient by use of equation 3:

\[
P(D|X) = \frac{0.55 \cdot 100}{(1 - 0.55) + 0.55 \cdot 100} = 0.99
\]

This result shows that the individual pre-test probability for portal hypertension has increased from 0.55 to 0.99 after logistic-regression analysis. In this example, our result was confirmed by measuring WHVPG, which was 11 mmHg for the patient considered. (If we had assumed non-ethanol-toxic etiology and had used equation 4, additionally considering the laminin concentration, essentially the same evidence would have emerged, but with lower values for the likelihood ratio, 18, and the predictive value, 0.96.)

These computations can be easily performed with the aid of a simple pocket calculator or, even more expeditiously, by use of a small program run on a personal computer.

**Discussion**

In continuation of our previous investigations \((6, 11, 19)\), this study was designed to find a clinical chemical approach for diagnosis of portal hypertension in pa-
tients with chronic liver diseases.

The results show that portal hypertension might be, to a large extent, reliably estimated by use of multivariate statistical methods operating on results for concentrations of hyaluronic acid and laminin in serum. The diagnostic specificity (0.84) and sensitivity (0.90) reached with hyaluronic acid within the group of ethanol-toxic etiologies compare well with those figures obtained by many common laboratory blood tests in other fields, e.g., 0.89 and 0.91 for pancreatic amylase for the diagnosis of acute pancreatitis (20). The results (sensitivity: 0.83, specificity: 0.82) for the whole group, independent of etiology, are only slightly less. We stress, however, that these figures should be considered only as estimates of the “true” values: The small number of samples in group B gives rise to the relatively wide confidence intervals shown in Table 3. In accordance with others (21) and in confirmation of our previous results (17, 22), we found no statistical relation between PIIINP and WHVPG.

The design of the study, with independent groups of patients for establishment and verification of the discrimination criteria, avoids common pitfalls concerning the overestimation of the diagnostic criteria when they are tested with the same data set as they are derived from (12). We cannot postulate that our results are representative for every clinical setting, because they were obtained from one (specialized) hospital, but the statistical method used can compensate for different frequencies of liver-disease patients by adjustment of the final predictive values. As can be seen from Figure 1 and Tables 2 and 3, the data from groups A and B show a high degree of similarity. For further validation of the results by use of a larger sample size and for evaluation of such effects as different etiologies or stages of disease and differences in the manometric determination of portal pressure [measurements of WHVPG are known to vary with diseases (23–26)], a multicenter trial is projected. In this context it would be interesting to prospectively investigate the relationship between the concentrations of connective-tissue components in serum and common complications of portal hypertension such as esophageal varices (27–29). The association between the concentrations of laminin and PIIINP in serum and the expression of esophageal varices seems to be weaker than what we report here for PVP (17, 27). However, this finding accords with other studies reporting that a certain degree of portal hypertension is necessary for the development of esophageal varices but that their size is independent of the degree of portal hypertension (30, 31).

A problem generally associated with the assessment of diagnostic criteria is the availability of an objective reference against which the results of the investigated tests can be judged (22). The classification of the severity of liver fibrosis on the basis of histological findings in biopsy specimens suffers from high sampling error, subjective judgements, and a coarse ordinal scale. The frequently used classification scheme of Child and Turcotte (32, 33) overcomes some of these drawbacks by combining physiological indices with laboratory variables but is still based on an ordinal, in part subjective, scale. Nevertheless, investigating the Child–Turcotte ratings in connection with the concentrations of connective-tissue components in serum would be interesting. Unfortunately, for most of the patients in group A, appropriate data were not available.

Although the manometric measurement of PVP is certainly not a method having very high precision, it has the advantage of providing a rational and objective scale. In contrast to histological findings and the Child–Turcotte classification, PVP represents a real physical quantity. On the other hand, the determination of WHVPG is a specialized, expensive, and invasive method that imposes some burden on the patient. Its substitution by the assay of a few analytes from serum would therefore be of major significance for the diagnosis and monitoring of fibrotic liver diseases. We certainly don’t “measure” PVP by serum laminin and hyaluronic acid; rather, we exploit a common cause for the increase in these variables within a discrete state of liver disease, e.g., cirrhosis. It is outside the scope of this study to look for the pathobiochemical mechanisms underlying the association of portal hypertension with the concentrations of certain components of the extracellular matrix in serum. Excessive synthesis and deposition of these proteins and complex carbohydrates during peri-sinusoidal fibrogenesis, contributing by hindrance of sinusoidal blood flow to an increase of portal pressure, might be accompanied by an enhanced escape rate of matrix components into the circulation, leading to an increase in their concentrations in serum (34). Another explanation is based on the fact that laminin (35) and hyaluronic acid (36, 37) are very short-lived serum components, because they are actively degraded in the liver endothelial cells. A reduced clearance function in conjunction with portal hypertension might also cause or contribute to increased concentrations in serum. This is further supported by the recent observation of the dependency of the clearance kinetics or hyaluronic acid on hepatic blood flow (38). However, the striking failure of PIIINP to correlate with WHVPG, despite the fact that both mechanisms are also valid for this analyte (39, 40), indicates that the biochemical relations are obviously much more complex.

We think that measuring hyaluronic acid and laminin in serum is not suited as a general screening test for fibrotic liver disease in an unselected population, i.e., without prior knowledge of the prevalence of the disease. Concentrations of one or both analytes are increased in various other diseases, e.g., diabetes (41), rheumatoid arthritis (42), tumors (13), and hyperthyroidism (43), which will result in a low diagnostic specificity. The assessment of early stages of the disease (e.g., fatty liver) is of course most desirable (21), but one has to deal with lowered sensitivities. However, with given or strongly suspected diagnosis of chronic liver disease, especially in the case of ethanol-toxic etiologies,
the determination of both of these analyses could serve at least as a tool for pre-screening those patients for whom portal venous pressure must be measured.

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References

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