Evolutionary Changes in Acute-Phase Proteins in Alcoholic Hepatocellular Diseases

C. Perier, 1 A. Chamson, 1 R. Engler, 2 and J. Frey 1,3

We studied the pattern of acute-phase proteins (orosomucoid, C-reactive protein, and haptoglobin) in hepatocellular deficiency due to chronic alcohol consumption, characterized by a decrease in serum transferrin concentration. We found that their patterns could vary independently of hepatocellular deficiency, but depend on the progression of hepatic disease. The most useful protein for discriminating the stage of inflammatory reaction is orosomucoid. In moderate hepatocellular deficiency, acute-phase proteins are increased independently of the decrease in transferrin, whereas in severe hepatocellular deficiency the acute-phase proteins are also decreased. Thus, it is possible to distinguish the two stages of hepatocellular deficiency by following changes in the concentration of orosomucoid.

Additional Keyphrases: orosomucoid - haptoglobin - C-reactive protein - transferrin - inflammatory reaction - hepatocellular deficiency - cirrhosis - liver disease

During injury to the liver, either as a consequence of moderate and chronic alcohol abuse or a parasitic or viral disease, the functions of this organ can be altered. The reversibility of these alterations depends on the severity of the injury. Many biological exploratory tests are currently used to measure the severity of the functional disorder.

We investigated simultaneously the progression of hepatocellular deficiency and of the inflammatory process as reflected by the concentration of several acute-phase proteins. These proteins signal early and accurately the presence of inflammatory reaction; their concentrations in serum depend on both the severity of the inflammatory process and their biosynthesis by hepatocytes (1).

During hepatocellular deficiency, the production of proteins such as albumin, transferrin, and prothrombin decreased because of either intracellular retention of synthesized proteins or some alteration in their biosynthesis. We also wanted to determine if the acute-phase proteins had the same metabolism as transferrin, or if their production by the hepatocytes varied independently of transferrin production. If the latter case were to be true, this might distinguish the inflammatory phase preceding the fibrotic process and the phase accompanying hepatocellular deficiency.

Materials and Methods

Patients. We investigated serum specimens from 39 patients with hepatocellular deficiency whose transferrin concentration in serum was <2 g/L. The study population consisted of 28 men (range 31–82 years, mean 57 years) and 11 women (range 36–90 years, mean 58 years). These patients had a history of chronic alcohol abuse. In our study, we considered the total population without considering individual differences in the clinical alteration of the liver; we divided the population into several groups by a factor analysis called "principal components analysis." After this statistical analysis, we verified the analogy between the clinical data and the divisions thus obtained. The study population was divided into three groups (Figure 1) and two groups (Figure 2) on the basis of the statistical data. In view of the clinical or histological data, Group 1 consisted predominantly of patients with chronic alcoholism, while Group 2 was mainly patients with either cirrhosis or decompensated cirrhosis (Figure 1 and Figure 2).

Analytes measured. The biological components we selected for evaluation were transferrin, immunoglobulin A fraction, albumin, and three acute-phase proteins (haptoglobin, orosomucoid, and C-reactive protein). Albumin was determined after electrophoresis (Sebia cellogel kit, Issy les Moulineaux, France). Total protein was measured by the biuret reaction (Technicon AutoAnalyzer). Radial immunodiffusion (2) was used to determine transferrin (M. Partigen Kit; Behring, Paris, France), the immunoglobulin A fraction (Tri Partigen Kit, Behring), orosomucoid, and C-reactive protein (Lc. Partigen Kit, Behring). Haptoglobin was assayed by immunonephelometry (Technicon) (3, 4) and by a colorimetric method based on the peroxidase activity of haptoglobin-hemoglobin (5). Results for haptoglobin did not differ significantly by the two different methods.

Fig. 1. Distribution of C-reactive protein, orosomucoid, and haptoglobin and division of the patients after factor analysis ("principal components analysis," \( n = 39 \))

P1 and P2, the principal axes of the principal plane, represent 84% of the total variance in the studied population. The direction and the position of the vectors C-reactive protein, orosomucoid, and haptoglobin were defined by their correlation with the axes P1 and P2.

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**Table 1. Correlation (r-value) between the Biological Variables and the Principal Axis of the Total Variance**

<table>
<thead>
<tr>
<th></th>
<th>Acute-phase proteins only</th>
<th>All measured variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st axis</td>
<td>2nd axis</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.28</td>
<td>0.95</td>
</tr>
<tr>
<td>Orosomucoid</td>
<td>0.87</td>
<td>-0.05</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>0.84</td>
<td>-0.27</td>
</tr>
<tr>
<td>Immunoglobulin A</td>
<td>-0.80</td>
<td>-0.28</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.60</td>
<td>-0.45</td>
</tr>
<tr>
<td>Transferrin</td>
<td>0.67</td>
<td>-0.50</td>
</tr>
</tbody>
</table>

**Statistical analysis.** In considering changes in the measured analytes and the possibility of their interdependence, we did a statistical analysis of the results by the method of factor analysis called "principal component analysis" (6), which allowed us to follow the changes in concentration of each, to determine their correlation, and to establish the division of the patients. By allowing a simple interpretation of the given body of data, the method offers a fundamental description of the set of the analyzed variables. Data for all the patients, each characterized by the same number of variables (n), were put in a space with n dimensions, corresponding to their n variables. Then a group of points was obtained (one point for each patient, in the space with n dimensions). From this space, a principal plane was determined so that, in the representation of points so obtained, the initial distances between the points were not too much modified. The center of gravity of this plane was the same as the center of gravity of the original group of points. Then we determined the two principal axes of inertia (P1 and P2), i.e., the two straight lines intersecting the center of gravity. These two axes are perpendicular and represent the highest percentage of the total variance in the population studied. On the other hand, the analyzed variables could be defined in a space with n dimensions, with regard to the n patients. The analyzed variables could be projected on the same principal plane, and their positions were defined by their correlation with the axes P1 and P2. Thus, the variable that had the highest coefficient of correlation with the axis P1 was the most discriminant. In addition, we were able to determine the correlation between the variables and the patients to distinguish several groups of patients and to determine which variables characterize each of the previously defined groups. In addition, to confirm the discrimination obtained for orosomucoid and haptoglobin after factor analysis with principal components analysis, we applied Student's t-test to the measured mean values in each group.

**Results**

The interpretation of the data obtained by principal components analysis permits the following observations (Figure 1). First, in our study, the most discriminating acute-phase protein was orosomucoid, its correlation coefficient with the first axis being greater than that for haptoglobin with the same axis (Table 1). The correlation coefficient was 0.51 for the two components. Second, it was possible to distinguish three groups. Groups 1 and 2 can be individualized on the orosomucoid vector and haptoglobin vector, because the projection of these two groups on the orosomucoid vector and haptoglobin vector were separate, whereas Group 3 was characterized by C-reactive protein. In addition, C-reactive protein was not correlated with haptoglobin and orosomucoid. Group 2 was mainly composed of patients with decompensated cirrhosis or those in the process of decompensation, with a severe decrease in orosomucoid and haptoglobin. For Groups 1 and 3, this phenomenon was not observed. For all the measured components, a similar analysis was performed (Figure 2), and the data previously observed were confirmed. Nevertheless, there was a negative correlation (Table 2) between orosomucoid and immunoglobulin A (r = -0.67) and between hapto-

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**Table 2. Correlation (r-value) between the Biological Variables**

<table>
<thead>
<tr>
<th></th>
<th>Acute-phase proteins only</th>
<th>All measured variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C-reactive protein</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td>Orosomucoid</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>0.03</td>
<td>1</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulin A</td>
<td></td>
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</tr>
</tbody>
</table>

* n = 39, p = 0.001 for r = 0.50. ** n = 33, p = 0.001 for r = 0.55.

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Fig. 2. Distribution of immunoglobulin A fraction, transferrin, albumin, orosomucoid, haptoglobin, and C-reactive protein and division of the patients after factor analysis ("principal components analysis," n = 33) P1 and P2 represent 69% of the total variance in the population studied. The vectors immunoglobulin A, transferrin, albumin, orosomucoid, haptoglobin, and C-reactive protein were defined by their correlation with the axes P1 and P2.

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globin and immunoglobulin A ($r = -0.57$). In addition, there was a correlation between albumin and transferrin ($r = 0.50$).

This observation confirmed the hepatocellular deficiency characterized by a simultaneous decrease in the production of these two proteins. Moreover, transferrin was not correlated with orosomucoid, haptoglobin, or C-reactive protein. In the second investigation, the patients were divided into two groups, and the results (Table 4) show the changes in immunoglobulin A fraction, orosomucoid, and haptoglobin.

**Discussion**

Taken together, the results presented here allow us to conclude that the acute-phase proteins can vary independently of any hepatocellular deficiency in hepatic injury. In addition, they depend on the severity in hepatic disease. We found the most useful protein for discriminating the stage of inflammatory reaction to be orosomucoid. Thus, our study confirmed that orosomucoid, like haptoglobin, should be considered as a protein undergoing two pathophysiological phenomena: an increase in production during the inflammatory reaction and a decrease in production during severe hepatocellular deficiency. Indeed, the population selected for this work, characterized by chronic alcohol abuse and a decrease in serum transferrin concentration, could be divided into two groups: Group 1 (Figures 1 and 2, Tables 3 and 4), characterized by patients with a slight increase in acute-phase proteins, and Group 2 (Figures 1 and 2, Tables 3 and 4), characterized by a decrease in acute-phase proteins.

In addition, distinguishing Groups 1 and 2 on the vector orosomucoid (Figures 1 and 2) was confirmed after statistical comparison of the measured average values (Tables 3 and 4) for orosomucoid in each group, according to Student's $t$-test. These two groups were significantly different ($p < 0.001$) in both investigations previously described (for orosomucoid, theoretical $F = 3.67$, calculated $F = 2.25$, theoretical $t = 3.65$, calculated $t = 7.53$, Figure 1; theoretical $F = 2.96$, calculated $F = 2.45$, theoretical $t = 3.69$, calculated $t = 6.50$, Figure 2).

As for haptoglobin, Student’s $t$-test could not be applied in considering the dispersion of the experimental results. In Group 1, the inflammatory process could precede the severe hepatocellular deficiency observed in Group 2. In the latter, the hepatocellular deficiency could be characterized by a marked decrease in orosomucoid and haptoglobin concentrations, due to either a failure in their biosynthesis or in their secretion by hepatocytes (7, 8). Thus, it was possible, simply by determining orosomucoid, to distinguish the two successive levels in hepatic injury due to chronic alcohol abuse, the first level being characterized by an inflammatory reaction only and the second by a severe hepatocellular deficiency with cirrhosis.

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**References**