Optimal correlation between different instruments for Fibrotest-Actitest protein measurement in patients with chronic hepatitis C

Maria Alessandra ROSENTHAL-ALLIERI (1), Albert TRAN (2), Philippe HALFON (3), Françoise IMBERT-BISMUT (4), Mona MUNTEANU (5), Djamilia MESSOUS (4), Marie-Line PERITORE (2), Thierry POYNARD (5), Alain BERNARD (1)

Laboratoire central d’immunologie, CHU de Nice, Hôpital de l’Archet, Nice (1); Laboratoire AlphaBio, Marseille (2); Laboratoire de biochimie (3); Laboratoire d’hépatologie-gastroentérologie, Groupe hospitalier Pitié-Salpêtrière, Université Paris VI, Paris (4, 5)

SUMMARY
Objectives — Combination of alpha 2-macroglobulin, haptoglobin, apolipoprotein-A1, \(\gamma\)-glutamyl transpeptidase, total bilirubin and alanine aminotransferase measurements allows to determine the Fibrotest-Actitest score, an alternative to liver biopsy in hepatitis C virus infection. The aims of this study were to evaluate the analytical variability of the Fibrotest-Actitest proteins alpha 2-macroglobulin, haptoglobin and apolipoprotein-A1, and to assess their impact on the Fibrotest-Actitest scores.

Methods — We compared 129 sera from hepatitis C virus infected patients for alpha 2-macroglobulin, haptoglobin and apolipoprotein-A1 levels obtained with the Immage\textsuperscript{®} (Beckman-Coulter) and the BNProspec\textsuperscript{®} (Dade-Berhing) automates. We evaluated Fibrotest-Actitest results obtained with the two nephelometers.

Results — Optimal correlation was found for alpha 2-macroglobulin \(Y=1.05X+0.01\), correlation coefficient: 0.98) and haptoglobin \(Y=1.05X+0.07\), correlation coefficient: 0.98) and apolipoprotein-A1 levels, as determined by Immage\textsuperscript{®}, were slightly lower than those obtained by BNProspec\textsuperscript{®} \(Y=0.86X-0.02\), CC=0.95). When Fibrotest-Actitest scores obtained with the two protein measurements were compared adjusting for apolipoprotein-A1 from Immage\textsuperscript{®}, the concordance rate was 0.903\pm0.06, with only 2/107 patients showing minimal discordance \(<=0.10\) for Fibrotest, and 1.000\pm0.06 for Actitest, with no discordance \(<=0.10\).

Conclusions — Measurement of apolipoprotein-A1, included in the Fibrotest-Actitest score, depends on the equipment used. Such discordance is of little clinical consequence for liver fibrosis evaluation in hepatitis C virus patients.

Introduction
Liver fibrosis is the main complication of all chronic liver disease, with progression to cirrhosis as its end-stage expression. Although liver biopsy is the gold standard for the diagnosis of liver fibrosis, it is an invasive procedure and not without risk \([1, 2]\). The possibility of adverse events \([4]\), sampling errors \([5, 6]\) and inter- and intra-pathologist variability \([7]\) have led to the search for biological markers of fibrosis.

Fibrotest and Actitest are the most described non-invasive tests \([8-14]\). They provide liver fibrosis scores from the combination of 5 biochemical markers: alpha 2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin and gamma-glutamyl-transpeptidase. In the Actitest, alanine aminotransferase is added to assess hepatic necro-inflammatory histological activity \([8]\). Fibrotest and Actitest were validated with specific
recommendations. The research was conducted according to the Helsinki 
trifugation conditions were in conformity with the tube manufacturer’s 
anticoagulant were separated after coagulation by centrifugation. Cen-
ematic parameters alanine aminotransferase and gamma-glutamyl-trans-
several cryotubes (250 µL) were obtained with the BNProspec® analyzer [20]. For both 
ments on Fibrotest and Actitest results.

Materials and methods

Patients and blood samples

Blood samples were collected by venipuncture from 129 patients 
chronic hepatitis C included in the Fibropanel Study [14]. All patients 
had untreated chronic hepatitis C virus infection, documented by positi-

Fibrotest and Actitest parameters

We measured the Fibrotest-Actitest proteins by the Immage analyzer 
the same thawed sera already analyzed in the previous study [20], 
and stored at 4°C up to 10 months. New reagents were used for alpha 2-macroglobulin and new batches for haptoglobin and apolipoprotein-
A1 (Beckman-Coulter manufacturer). We compared these new results to 
those previously obtained with the BNProspec® analyzer [20]. For both 
analyzers, reagents are standardized against the International Certified 
Material 401 for alpha 2-macroglobulin and haptoglobin [21, 22] and against the World Health Organization International Federation of 
Clinical Chemistry SP-01 for apolipoprotein-A1 [23, 24].

Apolipoprotein A1 measurement accuracy

In this work, we re-evaluated the previously studied sera [20] in 
in order a) to verify transferability of the results of the three Fibro-
test-Actitest proteins with new reagents available for the Immage system; b) to evaluate the impact of different protein measurements on Fibrotest and Actitest results.

Determination of fibrosis and activity scores

Fibrotest and Actitest were correlated to the degree of liver 
fibrosis, as determined by the Metavir score in a liver biopsy [8]. Fibrosis 
Fibrotest and Actitest scores were correlated to the degree of liver 
combined variability, for both methods.

Determination of fibrosis and activity scores

Fibrotest and Actitest scores were correlated to the degree of liver 
fibrosis, as determined by the Metavir score in a liver biopsy [8]. Fibrosis 
according to the Metavir score is measured on a 5-point scale: F0, no 
fibrosis; F1, portal fibrosis; F2, portal fibrosis with rare septae, F3 portal 
fibrosis and F4 is termed “significant fibrosis”, whereas the term “advanced hemo-

clinical analysis

Data were expressed as means ± SD. Comparison between measur-
ments of Fibrotest proteins was analyzed according to the evaluation 
procedure for technique accuracy put forward by the Société Française 
de Biologie Clinique [29, 30]. Regression analysis was performed accor-
ding to the procedures described by Passing and Bablok [31, 32] and 
Deming [30]. Comparison of assay results of Fibrotest and Actitest was 
assessed by linear regression using Pearson’s test. The kappa concor-
dance test was used to assess agreement between Fibrotest and 
Actitest [32]. A difference <0.10 in Fibrotest was defined as minimal, 
representing a discrepancy of less than one stage of liver fibrosis, as 
compared to the Metavir score in a liver biopsy [30]. Two groups were 
studied for their clinical and therapeutic impact: patients with no (F0) or 
minimal (F1) fibrosis, that do not receive treatment, and patients with 
significant fibrosis (F2 F3 F4) requiring treatment and closer follow-up. 

Results

Analytical variability for alpha 2-macroglobulin

We studied 129 samples to assess the correlation between 
BNProspec® and Immage® with new reagents. Under these 
conditions, levels of alpha 2-macroglobulin measured with the 
Immage® analyzer correlated with those obtained with 
BNProspec®, according to both Deming and Passing-Bablok methods. Comparisons were assessed at low (1 g/L), medium 
and high levels (4 g/L), on the basis of criteria put forward by 
the Société Française de Biologie Clinique [29, 30] (figure 1a). 
Correlations were of type Y = 0.04 X + 0.05 and Y = 0.01 
for Deming and Passing-Bablok statistical methods, respectively, 
with a correlation coefficient of 0.98 for both methods.
Analytical variability of the Fibrotest proteins

### Acceptability criteria

<table>
<thead>
<tr>
<th>Level*</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>X = a</td>
<td>1.00</td>
<td>2.00</td>
<td>4.00</td>
</tr>
</tbody>
</table>

| Calculated Y - a | 1.06 | 2.12 | 4.22 |
| Tolerable accuracy | 0.11 | 0.25 | 0.55 |
| Observed accuracy | 0.06 | 0.12 | 0.22 |
| Conclusions | accepted | accepted | accepted |

**Diagram of differences (Y - X) = f(X)**

**Diagram of ratios (Y/X) = f(X)**

Very little scattering was observed in the difference analysis (figure 1b), with fewer than 10% of values outside acceptable boundaries. However, wider scattering was noted in the ratio analysis (figure 1c), with 42.6% of values outside the defined limits.

**Analytical variability for haptoglobin**

Samples from 107 patients were available for evaluation. Identical levels of haptoglobin were obtained with both analyzers. The correlation was of type Y = 1.05X - 0.08 and Y = 1.05X - 0.07 respectively, with a correlation coefficient of 0.95 and 0.93 for Deming and Passing-Bablok methods respectively. Less than 10% of values lying outside of previously established boundaries in the difference analysis (figure 2b). Nevertheless, some scattering of results was noted in the ratio analysis (figure 2c).

**Analytical variability for Apolipoprotein A1**

One hundred and seven sera were studied. Levels of apolipoprotein A1 measured with the Immage® analyzer were slightly lower than those obtained with BNProspec®. Linear regression analysis showed a correlation coefficient of 0.95 and 0.93 for Deming and Passing-Bablok methods, respectively (Y=0.92X - 0.09 and 0.86X - 0.02). Results were accepted for the low (0.50 g/L), medium (1.50 g/L) and high (2.00 g/L) levels (figure 3a). The differences observed between the two nephelometric assays were homogeneous (figure 3b). Major scattering of results (77.5%) was noted on the ratio analysis (figure 3c).

We verified the accuracy of apolipoprotein A1 measurement with the two nephelometers by measuring the International Reference Material SP1-01 and three serum pools with previously determined target values [24]. Values obtained with the Immage instrument were lower than the Reference Materials. Values obtained with the BNProspec instrument were slightly higher than the target values (table I). This confirmed the discordance observed between the two measurements in our group of patients.

Since Fibrotest-Actitest scores were validated with BNProspect values, apolipoprotein-A1 values obtained with the Immage instrument (X) were corrected by introducing the following correction factor: X x 1.2147 - 0.1222. When the correction factor was applied, linear regression was improved (Y=1.070X - 0.038) with a correlation coefficient of 0.95.

**Variations in Fibrotest and Actitest results using protein measurements with two different nephelometric equipments**

Regression analysis was performed by comparing Fibrotest and Actitest scores obtained with protein results from the two analyzers (table II). The mean ± SD of Fibrotest values calculated...
with the Immage® proteins was higher than that obtained with the BNProspec® protein measurements. No significant difference was observed in Actitest calculated with the two nephelometric systems. When the adjustment was applied for apolipoprotein-A1 results obtained with the Immage® system for Fibrotest, linear regression was improved (Y=1.070X - 0.038) and the means ± SD of Fibrotest values became identical. Moreover, the number of patients with an Fibrotest difference >0.10 decreased significantly to 2/107 (1.8%), while it was 18/107 (16.6%) before adjustment for apolipoprotein-A1. Nevertheless, difference in Fibrotest obtained by the two nephelometers without the adjustment were minimal without major clinical consequences, leading to 1 Metavir stage difference in 14/107 patients (13.1%), <1 Metavir stage in 4/107 patients (3.7%) and no difference > 2 Metavir stages. For Actitest results, the correlation was optimal without the correction factor, with no changes following adjustment. Concordance rates (kappa statistics) were calculated for grouped stages F0 vs F2F3F4. For Fibrotest values, k value was improved when we applied the correction factor for apolipoprotein-A1 (k=0.903±0.096) and k=0.803±0.09 for the adjusted versus the non-adjusted Fibrotest, respectively. Values were acceptable also without the adjustment: 8.4% of patients (9/107) changed the group: 1/56 (0.9%) was F0F1 with the Immage measurement and F2F3F4 with the BNProspec tests and 8/51 patients (7.5%) were F2F3F4 with the BNProspec and F0F1 with the Immage measurements. Results for Actitest were excellent with k=1.000±0.096 for grouped grades A0A1 vs A2A3. No discordance >0.1, i.e. no difference in grade of activity was found (data not shown), without any changes following the application of the correction factor. We also looked for differences in concordance rate for each stage of liver fibrosis, i.e. F0 to F4. This analysis did not show major differences compared to global analysis (data not shown).

**Discussion**

Fibrotest and Actitest are important tools for non-invasive evaluation of liver fibrosis [8, 9, 11-13, 35-37]. However, the use of Fibrotest-Actitest as a real alternative to liver biopsy requires the standardization of measurement methods. In the present study, we found that apolipoprotein-A1 measurement, one of the major proteins included in the Fibrotest-Actitest score, is dependent on the instrument used. We also found that the impact of such discordance on the Fibrotest-Actitest score may be avoided by introducing a correction factor.

Optimal concordance was found for haptoglobin with new reagent batches, thus confirming previous results [20]. Result
transference with new reagents for alpha 2-macroglobulin is now excellent between the Immage® and the BNProspec® analyzers, showing significant improvement compared to previous methods [20]. This new reagent is now available in laboratory routine analysis. In a rat model of liver fibrosis, alpha 2-macroglobulin is produced at the sites of inflammation and fibrosis by hepatocytes, stellate cells and granuloma cells. Its increased synthesis can inhibit the catabolism of matrix proteins and enhances fibrosis processes in the rat liver [38-40]. In humans, few fundamental data exist, but multivariate analysis from several independent clinical trials shows a relationship between alpha 2-macroglobulin and liver fibrosis and inflammatory activity [41, 42].

In contrast, a difference >10% was constantly present for apolipoprotein-A1 between the two instruments. Apolipoprotein-A1 is trapped on extracellular matrices [43, 44] during the fibrotic process and leads to falsely low serum level measurements. Although standardization has been proposed by the World Health Organization International Federation of Clinical Chemistry (SP1-01, WHO-IFCC SP1-01) for apolipoprotein-A1 [23, 24], some discrepancies still exist. Variations in values could persist according to the different manufactures even after protein standardization [45]. Several factors could potentially cause variation in protein measurements. Among them, the nature and the specificity of the antibody used in the immunological reaction are fundamental. In our study, dramatic improvement of correlation for alpha 2-macroglobulin was seen between the two techniques with a new antibody used in the Beckman-Coulter reagent. Other factors may cause variations, such as the calibrator and the type of calibration, antigen excess, matrix differences, molecular weight and/or functional conformation of the protein, characteristics of the lipid fraction for lipoproteins, length of the immunological reaction and, finally, individual genetic heterogeneity.
Table I – Measured levels of Apolipoprotein A1 with Immage® and BNProspec® instruments in comparison with target values of International Reference Material.

<table>
<thead>
<tr>
<th>Material</th>
<th>Target values (g/L)</th>
<th>Measured values with Immage® (g/L)</th>
<th>Recovery %</th>
<th>Measured values with BNProspec® (g/L)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control pool</td>
<td>High</td>
<td>1.70</td>
<td>1.51</td>
<td>-1.27</td>
<td>1.82</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.40</td>
<td>1.27</td>
<td>-0.47</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>1.14</td>
<td>0.99</td>
<td>-1.12</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Table II – Global results of Fibrotest-Actitest values with and without adjustment for apoliprotein-A1.

<table>
<thead>
<tr>
<th>Material</th>
<th>FT</th>
<th>FT with CF*</th>
<th>AT</th>
<th>AT with CF**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immage® M ± SD</td>
<td>0.47±0.28</td>
<td>—</td>
<td>0.46±0.27</td>
<td>—</td>
</tr>
<tr>
<td>BNProspec® M ± SD</td>
<td>0.41±0.28</td>
<td>0.41±0.28</td>
<td>0.46±0.26</td>
<td>0.46±0.26</td>
</tr>
<tr>
<td>Correlation</td>
<td>0.987</td>
<td>0.990</td>
<td>0.995</td>
<td>0.998</td>
</tr>
<tr>
<td>Estimated regression model</td>
<td>Y=aX+b</td>
<td>Y=0.986X-0.063</td>
<td>Y=0.987X-0.007</td>
<td>Y=0.998X-0.01</td>
</tr>
<tr>
<td>Outliers (a)</td>
<td>7 (6.5)</td>
<td>5 (4.7)</td>
<td>7 (6.3)</td>
<td>6 (6.4)</td>
</tr>
<tr>
<td>Differences (b)</td>
<td>18 (16.83)</td>
<td>2 (1.85)</td>
<td>0 (0.16)</td>
<td>0 (0.16)</td>
</tr>
<tr>
<td>CF recovery (%)</td>
<td>1069 (6.5%</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* = correction factor; † = BNProspec, ‡ = Immage; †† = No. of patients with R. Student’s t.


34. Landis RJ, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977;33:159-64.


