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

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SUMMARY

Objectives — Combination of alpha 2-macroglobulin, haptoglobin, apolipoprotein-A1, γ-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® (Beckman-Coulter) and the BNProspec® (Dade-Berhing) automates. We evaluated Fibrotest-Actitest results obtained with the two nephelometers.

Results — Optimal correlation was found for alpha 2-macroglobulin (r=1.05 X -0.07, correlation coefficient 0.98) and haptoglobin (r=1.05 X -0.07, correlation coefficient 0.98). Apolipoprotein-A1 levels, as determined by Immage®, were slightly lower than those obtained by BNProspec® (r=0.86 X -0.02, CC=0.95). When Fibrotest-Actitest scores obtained with the two protein measurements were compared, acceptable discordance >0.10 for Fibrotest and 1.00±0.06 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 a 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-pathological 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...
The research was conducted according to the Helsinki recommendations. The anticoagulant were separated after coagulation by centrifugation. Cerebrospinal fluid (CSF) samples from 250 tubes with separation gel, recovered after centrifugation, were transferred into several cryotubes (250 µL/tube). For the enzymatic tests, total bilirubin, gamma-glutamyl-transpeptidase and alanine aminotransferase, transferability of results were excellent when the same standardized method and same calibrator were used [16-19]. However, few data were available for proteins [16-18].

We have previously shown great analytical variability between two nephelometers for the Fibrotest protein measurement, namely BNPreact® (Dade-Berhing), used for the initial validation, and Immage® (Beckman-Coulter), which is a widely distributed nephelometric equipment [20]. We have shown optimal correlation for haptoglobin, but there were important differences between alpha 2-macroglobulin and apolipoprotein-A1, with mean differences of 40% (Immage > BNPreact) and of 12% (Immage < BNPreact), respectively.

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

Materials and methods

Patients and blood samples

Blood samples were collected by venipuncture from 129 patients with chronic hepatitis C included in the Fibropaca Study [14]. All patients had untreated chronic hepatitis C virus infection, documented by positivity of hepatitis C virus-RNA in the serum, without liver complications such as ascites. For the Fibrotest proteins, sera were collected in 7 mL tubes with separation gel, recovered after centrifugation, transferred into several cryotubes (250 µL/tube) and stored at -80 °C. For the enzymatic tests, total bilirubin, gamma-glutamyl-transpeptidase and alanine aminotransferase were separated after coagulation by centrifugation and stored at -80 °C.

In this work, we re-evaluated the previously studied sera [20] for their clinical and therapeutic impact: patients with no (F0) or minimal fibrosis (F1), portal fibrosis (F2) and 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 BNPreact® (X) and Immage® (Y) with new reagents. Under these conditions, levels of alpha 2-macroglobulin measured with the Immage® analyzer correlated with those obtained with BNPreact®, according to both Deming and Passing-Bablok methods. Correlations were acceptable for low (1 g/L), medium (2 g/L) 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=1.05X + 0.01 and Y=1.05X + 0.01 for Deming and Passing-Bablok statistical methods, respectively, with a correlation coefficient of 0.98 for both methods.
Very little scattering was observed in the difference analysis (figure 1a), with fewer than 10% of values outside accepted 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 for both Deming and Passing-Bablok methods respectively, with a correlation coefficient of 0.95 and 0.93 for both regression methods. The correlation was acceptable for low, medium and high haptoglobin levels (3 g/L) (figure 2a). The distribution of results (77.5%) was noted on 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 2b). The differences observed between the two nephelometric assays were homogenous (figure 2b). 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-Acistest scores were validated with BNProspec® 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 Acistest results using protein measurements with two different nephelometric equipments

Regression analysis was performed by comparing Fibrotest and Acistest scores obtained with protein results from the two analyzers (table II). The mean ± SD of Fibrotest values calculated...
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].
Analytical variability of the Fibrotest proteins

<table>
<thead>
<tr>
<th>Level</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
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<tbody>
<tr>
<td>$X^{a,b}$</td>
<td>0.50</td>
<td>1.50</td>
<td>2.00</td>
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<tr>
<td>Calculated $Y^{a,c}$</td>
<td>0.41</td>
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<td>1.71</td>
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<tr>
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<tr>
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<td>-0.22</td>
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<tr>
<td>Conclusions</td>
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Fig. 3 – Linear regression for Apolipoprotein A1 in 107 sera analyzed with the BNProspec® (Dade-Behring) and the Immage® (Beckman-Coulter) analyzers according to the Valtec procedure.
A. Observed and acceptable accuracy for low, medium and high levels of Apolipoprotein A1 and results of comparison between the two techniques. B. Diagram of differences. C. Diagram of ratios.

transferability 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 levels 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 manufacturers even after protein standardization [45]. Several factors could potentially cause variations 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. On the other hand, other factors may cause variations, such as the calibrator and the type of calibration, antigen excess, matrix differences, molecular weight and/or the functional conformation of the protein, characteristics of the lipid fraction for lipoproteins, length of the immunological reaction and, finally, individual genetic heterogeneity.

Fibrotest-Actitest scores were validated using laboratory results obtained with defined apparatus. For Fibrotest-Actitest scores, BNProspec was initially used. We consider this apparatus as the Fibrotest-Actitest reference, even if apolipoprotein-A1 values were slightly higher than expected targets. Immage and BNProspec were both linear and without matrix effect with respect to the SP1 01 Reference Material (data not shown). However, lower and higher values comparing to international controls were found for Immage and BNProspec, respectively.

In order to obtain comparable results for patient follow-up with the Immage® system, we propose to add a correction factor to values obtained with this instrument. Further controls are necessary to verify eventual variations in time of the correction factor. We are aware that this solution is not satisfactory from an
analytical point of view, but it is the best we can do, now, to obtain Fibrotest-Actitest results with excellent concordance and negligible impact on patient classification. The Immage nephelometer is widely used in about 40% of clinical laboratories all over the world and biological markers of liver fibrosis are becoming essential for patients with hepatitis C virus infection and other liver pathologies leading to end-stage fibrosis. Moreover, minimal clinical consequences in Fibrotest results without the application of the CF were seen.

In conclusion, our data suggest that alpha 2-macroglobulin, haptoglobin and apolipoprotein-A1 can be routinely computed from the Immage nephelometer for Fibrotest-Actitest. Fibrotest-Actitest has led to great improvement in the accuracy of several liver pathologies leading to end-stage fibrosis. Moreover, minimal clinical consequences in Fibrotest results without the application of the CF were seen.

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