Evaluation of the first automated hyaluronic acid assay

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INTRODUCTION

Hyaluronic acid (HA, hyaluronan), ubiquitously distributed in the extracellular spaces, is a linear polysaccharide built from repeating disaccharide units (D-glucuronic acid (1→3)-N-acetyl-D-glucosamine (1→4))-n of molecular weights 105-107 daltons. In the liver, HA is mostly synthesized by the hepatic stellate cells and degraded by the sinusoidal endothelial cells. In chronic liver diseases, increases in serum HA levels occur together with the development of liver fibrosis. First, enhancement of HA production by the activated stellate cells may contribute to the increase in serum HA levels observed in patients with chronic liver disease. Later, when cirrhosis is constituted, reduced degradation by sinusoidal endothelial cells may cause greater HA increases. In patients with cirrhosis, high serum HA levels were associated with the occurrence of complications or death and correlated with clinical severity.

HA serum determination is used alone or in association with other tests for non invasive assessment of liver fibrosis in patients with chronic liver disease. Currently, only an enzyme-linked binding protein assay proposed as a self contained kit in the Wako ELISA format (HA Test Kit, Corgenix, Westminster, USA) is commercially available. This assay needs duplicate determinations (standards and samples), must be preceded in batches and, unless the laboratory possesses an automated ELISA plate processor, is time-consuming.

We evaluated a new reagent for the quantitative determination of HA using the latex agglutination method that can be applied to clinical chemistry analyzers (hyaluronic acid detection reagent, Latex method, Wako, Osaka, Japan).

MATERIALS AND METHODS

Principle: A sample is mixed with recombinant hyaluronic acid binding protein (rHABP), and HA in the sample combines specifically with rHABP.

In order to make an inelastic aggregate, latex particles coated with anti-HABP antibody are added, and the latex binds to the above complex. As a result, the inelastic aggregate increases turbidity in the solution. The degree of turbidity of the solution can be measured optically and is proportional to the HA concentration in the serum.

Reagents: The reagents are designed to be used in a commercially available automated analyzer. Reagent 1: Recombinant hyaluronic acid binding protein (rHABP): 52 mL, ready to use and stored at 2-8°C. After opening the bottle, it is stable for 30 days at 2-8°C. Reagent 2: Latex sensitized with anti-HABP antibody (mouse, monoclonal): 12 mL, ready to use and stored at 2-8°C. After opening the bottle, it is stable for 30 days at 2-8°C.

Calibrators: Solutions containing 50, 100, 200, 500 and 1000 µg/L HA, ready to use and stored at 2-10°C.

Test procedure: An OLYMPUS AU640 (Tokyo, Japan) analyzer was used. One calibration per week was made with double measurements of saline blank (0 µg/L) and calibrators. Autodilution of the sample (1/5) was programmed for specimens with HA concentration above 1000 µg/L.

Study protocol: An analytical study, including evaluation of the calibration, the accuracy of the method and the limit of detection, was conducted. HA concentrations measured with the Wako reagents were compared with those obtained routinely (HA Test Kit, Corgenix, Westminster, USA). This test uses HABP coated on the microtiter surface to capture HA. An enzyme (horseradish peroxidase) conjugated with HABP is used to detect the bound HA with a chromogenic reaction (o-toluidine hydrochloride and hydrogen peroxide).

For a comparison of sera vs. heparinized plasma, plasma samples (n=37) came from blood samples for other tests requested at the same time as HA determination. Three pools of sera at three levels of concentration were prepared for within-run and between-run assays. They were fractioned and kept at -20°C until assays.

RESULTS

Comparison between serum and heparinized plasma determinations (n=37)

Comparison between LT Auto Wako Hyaluronic acid (using OLYMPUS AU640) and Corgenix HA-Test in 138 samples: T-test showed no significant differences between the results (p=0.449).

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Inaccuracy 2

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Inaccuracy 2

REFERENCES

2. Wong VW et al. Serum hyaluronic acid is a useful marker of liver fibrosis in chronic hepatitis C virus infection. J Viral Hepatol 2004; 11: 100-7.

DISCUSSION

This study shows that the reagents for the automated determination of HA in serum or heparinized plasma developed by Wako method (hyaluronic acid detection reagent, Latex method) showed analytical characteristics that make it appropriate for clinical practice. Sensitivity and range of measured concentrations are adapted to clinical analysis, particularly in chronic liver diseases. Imprecision is improved when compared to methods previously commercialized. No inaccuracy problem was detected. According to the data presented by the Wako company, no effect of common interferents like lipids, haemoglobin or bilirubin was observed. Correlation with the most used commercialized product is excellent. Consequently, the reference values previously determined could be used and the index for predicting liver fibrosis using combined serum markers including HA levels could be calculated using the described algorithms.

Statistical analysis: GraphPad Prism® software was used for data analysis.

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