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## GENERAL REVIEWS AND PROSPECTIVE ANALYSES

# Automated quantification of serum hyaluronic acid for non-invasive assessment of liver fibrosis in chronic hepatic diseases

*Apport d'un test automatisé de dosage de l'acide hyaluronique pour une évaluation non invasive de la fibrose des hépatopathies chroniques*

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Hyaluronic acid;  
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### MOTS CLÉS

Acide hyaluronique ;

**Summary** To assess liver fibrosis, we evaluated automated serum hyaluronic acid (HA) measurement alone or included in the Hepascore in 130 patients with different chronic liver diseases (CLD). We confronted HA with Fibrotest, and, when available, with transient elastography (Fibroscan) and liver biopsy used for liver fibrosis diagnosis. HA was the only biomarker showing difference between "advanced fibrosis" and "cirrhosis", Hepascore and Fibrotest being significantly different only between the groups "cirrhosis" and "lack of fibrosis" defined by Fibroscan or by biopsy. For cirrhosis, HA less than 65 ng/mL correctly identified non-cirrhotic patients in 96% of the cases while HA greater than 175 ng/mL correctly identified cirrhotic patients in 81% of the cases. For fibrosis, the cut-off of 115 ng/mL showed a positive predictive value of 90%. Here we demonstrate that HA alone or included in Hepascore reveals a good ability to detect all stages of CLD, especially to exclude cirrhosis from advanced fibrosis. HA assay might be used to evaluate liver fibrosis in complement to other non-invasive diagnostic markers.

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**Résumé** Afin d'évaluer le degré de fibrose hépatique, nous avons testé la détermination automatisée quantitative de l'acide hyaluronique (AH) seul ou inclus dans l'Hépascore chez 130 patients atteints de différentes hépatopathies chroniques. Nous avons confronté les données obtenues avec les résultats du Fibrotest, du Fibroscan et de la biopsie hépatique

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Hépascore ;  
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disponibles. L'AH était le seul biomarqueur qui montrait une différence significative entre le groupe « cirrhose » et celui de « fibrose avancée » ; les autres biomarqueurs donnaient une différence significative uniquement entre les groupes « cirrhose » et « sans fibrose », définies par le Fibroscan ou la biopsie. Pour la cirrhose, une détermination d'AH inférieur à 65 ng/mL identifie correctement les patients non cirrhotiques dans 96 % des cas. En revanche, l'AH supérieur à 175 ng/mL identifie correctement les patients cirrhotiques dans 81 % des cas. Pour la fibrose, le cut-off de 115 ng/mL donnait une valeur prédictive positive de 90 %. Dans cette étude nous montrons que l'AH seul ou inclus dans l'Hépascore possède de bonnes performances diagnostiques pour les différents stades des maladies hépatiques chroniques, spécialement pour différencier la cirrhose de la fibrose avancée. Le dosage de l'AH a sa place comme un test non invasif d'évaluation de la fibrose en complément d'autres tests biologiques diagnostiques des hépatopathies chroniques.

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## Introduction

Chronic liver diseases (CLDs) represent a major factor of morbidity and mortality worldwide. These diseases are most frequently caused by chronic viral infection (HCV, hepatitis C virus, and HBV, hepatitis B virus), alcoholic abuse, and metabolic disturbances (NAFLD, non-alcoholic fatty liver disease) [1,2]. Life-threatening complication of most CLD is the hepatic fibrosis. The end stage consequence of fibrosis is cirrhosis with symptoms like portal hypertension, liver failure and a final evolution to hepatocellular carcinoma.

Liver fibrosis is defined by excessive non-specific accumulation of altered extracellular matrix (ECM). Fibrosis leading to cirrhosis can accompany any CLD that is characterized by the presence of hepatobiliary inflammation. There are major achievements in the understanding of its pathogenesis, with the hepatic stellate cell activation and proliferation playing a central role in the response to a tissue injury [3], and contributing to the misbalance between fibrogenesis and fibrolysis. This, combined with the decreased clearance from the circulation as a consequence of perisinusoidal fibrosis, leading to blood shunting bypassing the liver, are the major pathways to elevate the blood concentration of ECM components, e.g. collagen fragments, hyaluronan and laminin, in CLD. A clear diagnosis and staging of liver fibrosis is of paramount importance, as it is directly connected to the prognosis and the subsequent management of the CLD.

Liver biopsy has long been considered the gold standard for liver fibrosis assessment although its limitations are largely known (potential morbidity and mortality, sampling error, inter-observer variability, and higher cost) [4–8]. Recently, non-invasive serum biomarkers and measurement of liver stiffness by transient elastography [9] have been proposed and investigated as non-invasive assessment methods for liver fibrosis diagnosis. Several groups [10–12] have studied these non-invasive methods for their ability not only to diagnose liver fibrosis but also to differentiate its stages and to monitor the response of the fibrotic changes to treatment.

Serum markers are generally divided into two main groups, i.e. indirect and direct [13,14]. Indirect biomarkers are correlated to the functional alterations of the liver; they are biochemical parameters measurable in the peripheral blood that indirectly reflect liver damage (e.g. clotting factors, platelets, cholesterol, bilirubin, transaminases, triglycerides, haptoglobin). In order to increase their diagnostic performances, a combination of different indirect

biomarkers in sequential algorithms have been developed and validated, among which Fibrotest™ [15–17] is the most popular one.

Direct biomarkers are defined as serum components having a direct relation to the mechanism of fibrogenesis, either as secreted matrix-related components of activated hepatic stellate cells and fibroblasts or as mediators of ECM synthesis or turnover (e.g. hyaluronan, laminin, type IV collagen, matrix metalloproteinase-2, tissue inhibitor of metalloproteinase-1, and amino-terminal peptide of procollagen III) [18–22]. They reflect primarily the metabolism of ECM and the speed of fibrogenesis. Most of them, however, are parameters that are not routinely determined in the laboratory.

One of the most investigated direct biomarker is the hyaluronic acid (HA). Hyaluronan is an unbranched glycosaminoglycan, a single chain of polymers of disaccharide units containing N-acetylhexosamine and hexose, with molecular weight of  $10^4$ – $10^6$  daltons. HA is widely distributed in the ECM of different tissues, and is physiologically degraded by the hepatic sinusoidal endothelial cells; in the liver it is mostly synthesized by the stellate cells [22,23]. HA is one of the direct markers of liver fibrosis along with other glycoproteins, the collagen family, the collagenases and their inhibitors and a number of cytokines. Serum levels of HA are directly linked to the modifications in ECM turnover during fibrogenesis due to higher production by the activated stellate cells, and reduced degradation by the sinusoidal endothelial cells as a consequence of perisinusoidal fibrosis and blood shunting bypassing the liver [22,23].

HA serum determination can be used alone or in combination with other direct or indirect markers of liver fibrosis. It is included in different logarithmic scores like European Liver Fibrosis (ELF) [21], Fibrometer [22] and Hépascore [24]. HA has been extensively studied in viral hepatitis [25–28] while few studies are available in other etiologies. Serum concentration of HA was found consistent with stage of fibrosis and with a response to interferon therapy.

The main disadvantage of the direct fibrosis serum markers is that the methods for their measurement are diverse and all very laborious (radioimmunoassay, RIA or immunoenzyme methods, ELISA) and that it is difficult to investigate the inter-laboratory reproducibility.

Recently, a new HA detection reagent (HA LT detection reagent, Wako, Osaka, Japan) was developed using the latex

agglutination method that made it possible its application to general clinical chemistry analyzers.

For several years now, an algorithm for liver fibrosis evaluation in CLD is established in our hospital; it consists of non-invasive serum biomarkers determination, i.e. the Fibrotest score, and liver stiffness measurement by transient elastography, Fibroscan. Fibroscan is a patented device composed of an ultrasound transducer detecting the transmitted vibrations through the underlying hepatic tissue. The measurement of the wave propagation and its velocity is directly linked to the liver stiffness which reflects the liver fibrosis. Results range from 2.5 to 75 kPa and there are already established cut-off values for presence of significant fibrosis (> 7.6 kPa) and cirrhosis (> 14.1 kPa) [10–12]. When the two non-invasive techniques give a discordant result, a liver biopsy is performed.

The aim of this study was to evaluate serum HA level alone or included in Hepascore in different CLD. These biochemical markers were confronted with the Fibrotest, and, when available, with transient elastography (Fibroscan) and liver biopsy.

## Patients and methods

### Patients and study design

Criteria of inclusion: 131 patients with different CLD at different disease stages were included over the period of October 2009 to February 2010. The fibrosis stage was assessed by Fibrotest and Fibroscan; when these two non-invasive techniques gave a discordant result or when liver stiffness measurement failed, a liver biopsy was performed. The patients had a regular follow-up for a CLD in the Clinic of Gastroenterology and Hepatology, Erasme Hospital, Brussels. One patient was excluded because of a multiple organ failure. Sixty-six patients presented a viral hepatitis (59 HCV, 8 HBV and 5 co-infections) and 57 suffered from a non-viral CLD (alcoholic liver disease, non-alcoholic fatty liver disease or mixed pathology). Ninety-eight had a concomitant transient elastography (Fibroscan) performed and 69 had a concomitant liver biopsy.

### Laboratory measurements

Serum HA levels were measured by latex-sensitized immunoturbidimetry (Hyaluronic acid LT, Wako Chemicals GmbH, Neuss, Germany) using a Hitachi 917s.

An analytical study, including evaluation of the imprecision from between-run CV and interference with hemoglobin, free bilirubin and triglycerides were conducted. For the other test performances, the analytical results from the manufacturer were taken into consideration.

The following direct and indirect hepatic biomarkers are included in the scores calculated in this study:

- Hepascore: bilirubin,  $\gamma$  glutamyl transferase ( $\gamma$ GT),  $\alpha$ 2macroglobulin, HA, age, sex;
- Fibrotest: bilirubin,  $\gamma$ GT, haptoglobin,  $\alpha$ 2macroglobulin, apolipoprotein A-I, age, sex.

## Statistical analysis

Graphpad Prism® software was used for data analysis with one-way ANOVA and Bonferroni's Multiple Comparison test and non-parametric correlation (Spearman test). The sensitivity of the test is defined as its ability to identify the patients with cirrhosis, the specificity as the ability of the test to identify the patients without cirrhosis, the positive predictive value (PPV) is the value of a given measurement or higher to indicate cirrhosis, the negative predictive value (NPV) is the value of a measurement or lower than that shown to exclude the presence of cirrhosis.

## Results

### Analytical performances of the hyaluronan measurement by immunoturbidimetry

The analytical performances of the method of latex agglutination that are declared by the manufacturer and those found in our laboratory are summarized in Table 1.

### Correlation between HA, HS and FT

The correlation between HA, HS and FT calculated according to Spearman analysis for every data pair is presented in Table 2. The correlation between HS and FT for all patients ( $n = 130$ ) was better than that between HA and FT ( $r = 0.764$  vs  $r = 0.605$ ). The correlation between HS and FT analyzed separately for viral CLD ( $n = 66$ ) and non-viral CLD ( $n = 57$ ) was not significantly different ( $r = 0.706$  for viral CLD vs  $r = 0.740$  for non-viral CLD).

### Correlation between HA alone or included in HS and FT

Relationship between plasma HA concentration (alone or included in HS) and FT was evaluated. Different cut-offs for Fibrotest were used according to the literature [15–17] to divide the patients in different disease stages that correspond to the histological classification of Metavir (Table 3). The distribution of the obtained results for HA and HS is shown in Fig. 1. There was a significant difference between the results of HA in patients with "cirrhosis" ( $FT > 0.75$ ) and "mild fibrosis" ( $0.15 < FT < 0.5$ ) or "no fibrosis" ( $FT < 0.15$ ). When data obtained with Hepascore were analyzed, there was a statistically significant difference between all pairs of groups.

### Correlation between HA serum concentration (alone or included in HS), FT, and the results obtained by Fibroscan, used as reference method for fibrosis assessment

A Fibroscan was realized for 98 patients and allowed us to divide those patients in four categories according to established cut-off values [10–12]: less than 7.6 kPa for "without fibrosis", between 7.7 and 9.5 kPa for "mild fibrosis", between 9.6 et 14.1 kPa for "advanced fibrosis", and

**Table 1** Analytical performances of the automated quantitative latex method measurement of HA.

|  | Wako Chemicals                       | Erasmé  |
|--|--------------------------------------|---|
| Expected normal values   | 23 ± 17 ng/mL                        | 21.2 ± 12.1   |
| Quality control set ready-for-use  | Should produce ± 20% of target value | Produced ± 2% of target value                               |
| Inferior detection limit   | 5.8 ng/mL                            | NA  |
| Linearity  | Up to 1000 ng/mL                     | NA  |
| Within-run precision   | < 4.3%                               | ND  |
| Between-run precision  | < 5.3% (at three levels)             | 0.56 < CV < 2.66% (n = 10)                                  |
| Interference study in presence of Hemoglobin   | 97.9%                                | 97% (Hemolysis index = 174, n = 1)                          |
| Bilirubin  | 95.5%                                | 99.4% (Icterus index = 27, n = 1 bilirubin 24 mg/dl)        |
| Triglycerides  | ND                                   | 98.6% (Lipemia index = 467, n = 1 triglycerides 1031 mg/dl) |
| Other endogenous substances (ascorbic acid, EDTA-2Na, sodium citrate, ammonium oxalate, NaF) | 93–105%                              | ND  |
| Correlation between latex method and ELISA, society 'A' (n = 133)                            | r = 0.995<br>y = 1.004x + 1.979      | ND  |
| Correlation and comparison between serum and plasma samples (n = 36)                         | r = 0.998<br>y = 1.013x + 0.275      | ND  |

NA: not applicable; ND: not done.

greater than 14.1 for "cirrhosis". The distribution of the results obtained for HA, HS, and FT is shown in Fig. 2. When transient elastography was considered as the method of reference, HA was the only biomarker that showed difference between "advanced fibrosis" and "cirrhosis", Hepascore and Fibrotest being significantly different only between the lowest, "without fibrosis", and the highest, "cirrhosis", Fibroscan score group.

### Correlation between HA serum concentration, alone or included in HS, FT, and the results obtained by liver biopsy, used as reference method for fibrosis assessment

The distribution of the results obtained for HA, HS, and FT in different fibrosis groups defined by the histology result is shown in Fig. 3. When results were analyzed using the biopsy as the reference method, a significant difference was demonstrated for HA for the group of cirrhosis compared with each of the other groups while the results of HS and FT showed differences only between "cirrhosis" (F4) and "lack of fibrosis" (F1) (Fig. 3A). When patients were merged into two main groups: "no/mild fibrosis"

**Table 2** Correlation between HS and FT.

|    | HA                             | HS                             |
|----|--------------------------------|--------------------------------|
| HS | 0.8 [95% CI (0.8–0.9)]         |                                |
| FT | 0.605 [95% CI (0.4791–0.7064)] | 0.764 [95% CI (0.6791–0.8292)] |

(F0-F1) and "advanced fibrosis/cirrhosis" (F2-F3-F4), significant difference was observed with each of the three methods studied, i.e. HA, HS, and FT. Nevertheless, the results of HS and FT appeared more significantly different ( $P < 0.001$ ) than HA alone ( $P < 0.05$ ) (Fig. 3B).

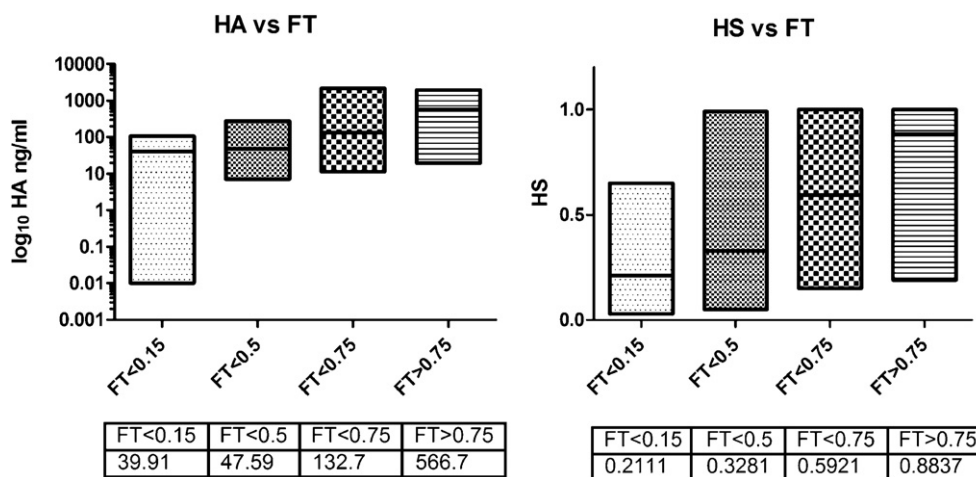
The results of the measurement of HA, HS and FT in the patients with NAFLD (Fig. 3C) showed the same trend as in the patients with alcoholic or viral CLD.

### Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)

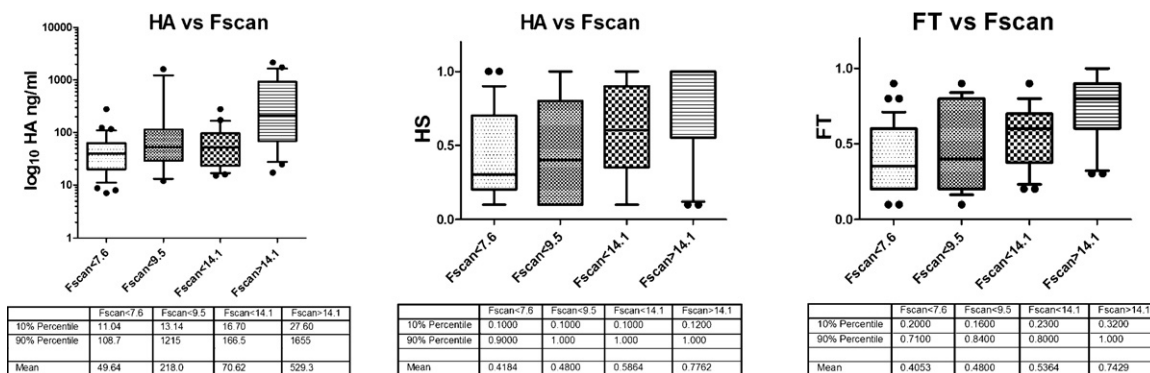
In order to evaluate if HA level was able to predict the presence or the absence of cirrhosis we applied three cut-offs of

**Table 3** Patients distribution according to Fibrotest using Metavir classification.

|                    | Without fibrosis | Mild fibrosis | Significant fibrosis (few septa: F2, septal fibrosis: F3) | Cirrhosis |
|--------------------|------------------|---------------|---|-----------|
| Metavir            | F0               | F1            | F2-F3   | F4        |
| Fibrotest          | < 0.15           | < 0.5         | < 0.75  | > 0.75    |
| Number of patients | 18               | 48            | 34  | 30        |



**Figure 1** Results for HA and HS in function of fibrosis stages defined by FT values. The graphs represent the minimum, the maximum and the line of mean. The tables below the graph show the mean per group.



**Figure 2** HA, HS and FT results in function of Fibroscan results and following fibrosis stages. The graphs show "Box & Whisker plot" with [10–90] percentile and the line of median. The points outside the boxes represent the outliers.

values between 65 and 175 ng/mL according to other published data [20,26] and determined the predictive values for these various HA levels (Table 4). For example, each value of HA greater than 65 ng/mL was considered as 'positive' (presence of cirrhosis) and each value of HA less than 65 ng/mL was considered as 'negative' (absence of cirrhosis). As seen in Table 4, a negative determination for serum HA (<65 ng/mL) correctly identified non-cirrhotic patients 96% of the time. In contrast, a positive determination of HA greater than 175 ng/mL correctly identified cirrhotic patients in 81% of the cases, the other assessed cut-offs giving an insufficient VPP. The best sensitivity (90%), defined as the ability of the test to correctly identify patients with cirrhosis, was obtained when the cut-off of 65 ng/mL was applied. The best specificity (96%), defined as the ability of the test to correctly identify patients without cirrhosis, was found with the cut-off of 175 ng/mL. When the cut-off of 115 ng/mL was applied, the specificity was 92%. Finally, the accuracy of HA measurement was calculated to be 77% for the cut-off of 65 ng/mL, and 88% for both of the other two cut-offs.

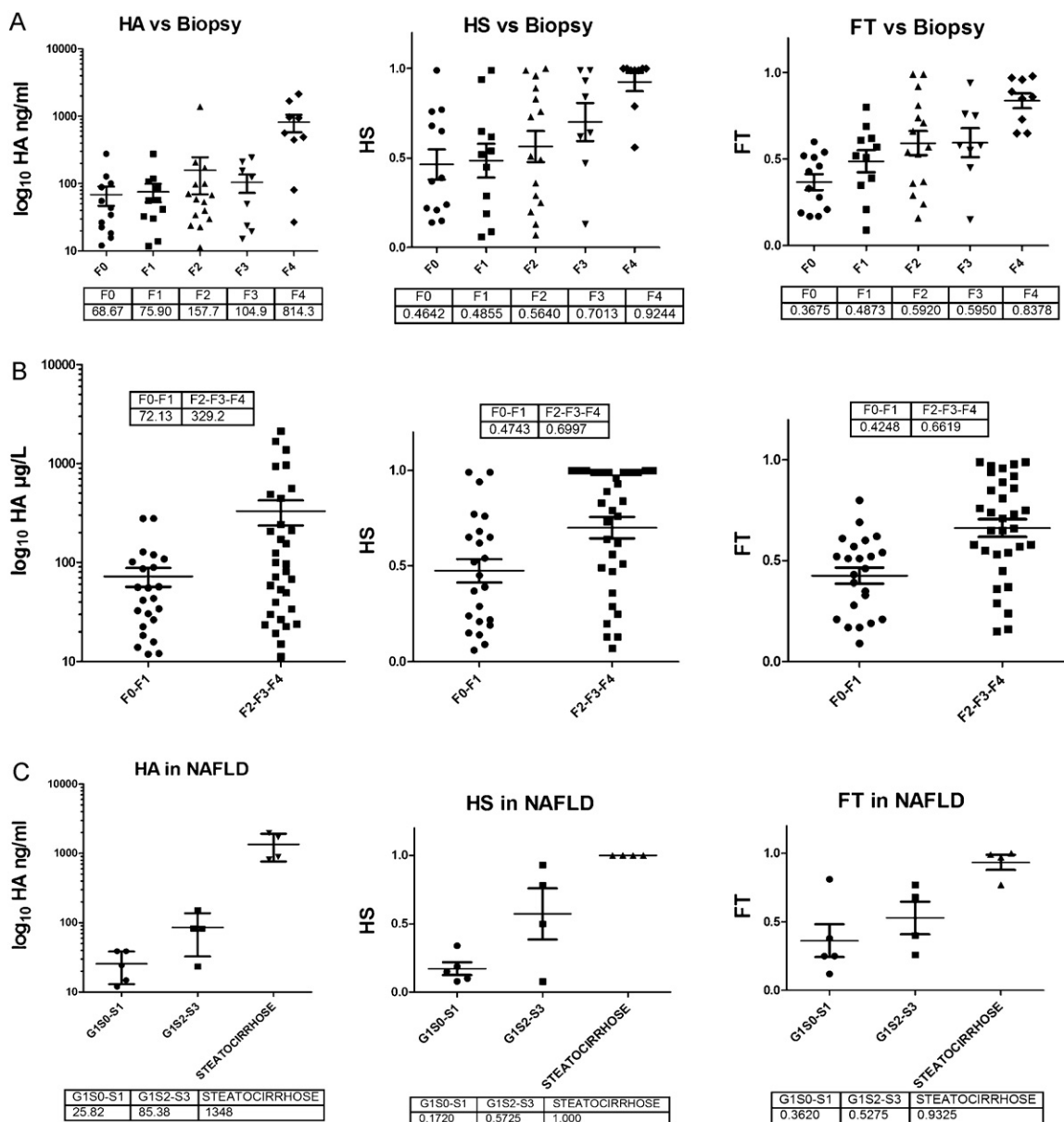
We also aimed at finding out what is the capacity of hyaluronan measurement to predict the presence or the absence of significant fibrosis. We merged the results into

two groups, F0-F1 and F2-F3-F4, following Metavir classification and Fibrotest distribution that have already been validated [15–17]. The cut-off of 115 ng/mL showed better performances to correctly identify the absence of fibrosis than its presence; 90% of the patients with HA value greater than 115 ng/mL had significant fibrosis but within the fibrotic patients only 43% showed HA level greater than 115 ng/mL.

The same analysis was performed for Hepascore. Following recently published data [29], three cut-offs were applied. Hepascore less than 0.75 could exclude cirrhosis with a sensitivity of 86% and a NPV of 96%. For significant fibrosis diagnostic, the optimal cut-off was 0.5, with a sensitivity of 78%, a specificity of 82%, a PPV and a NPV of 80%. Furthermore, when Hepascore was less than 0.25 significant fibrosis could be excluded with a sensitivity of 95% and a NPV of 93%.

### Discussion

With the present study, our objective was to evaluate the analytical and diagnostic performances of the HA measurement by the automated latex method of Wako Chemicals in different chronic hepatic diseases.



**Figure 3** HA, HS and FT in viral or alcoholic liver disease patients ( $n=55$ ) and in patients with NAFLD ( $n=13$ ) with biopsy. The graphs show the mean  $\pm$  SEM for every group. The table shows the mean per group. A. Mean hyaluronic acid (HA) concentration (ng/mL) in  $\log_{10}$ , Hepascore, HS and Fibrotest, FT for the five groups of patients according to the stage of fibrosis (F0 "no fibrosis", F1 "mild fibrosis", F2 "portal fibrosis", F3 "bridging fibrosis", and F4 "cirrhosis"). B. The same patients as in (A) merged into two main groups, "no/mild fibrosis" (F0-F1) and "advanced fibrosis/cirrhosis" (F2-F3-F4). C. HA, HS and FT according to fibrosis stages of Brunt's histological classification: G1S0-S1 (focal fibrosis), G1S2-S3 (focal and periportal fibrosis with or without bridging), S4 (steatocirrhosis).

Our investigation of the analytical performances of Wako immunoturbidimetry assay was not exhaustive; nevertheless, we agree with the declared performances by the manufacturer and with a recent publication over a greater patients' cohort [30].

There are several studies investigating the diagnostic value and clinical utility of serum HA [20–29,31–33]. These studies, focusing on different pathologies, showed interesting perspectives; however, some of them gave no precision over the prospective or retrospective character of the study, the result of the hepatic biopsy, or the patients cohort

(consecutive or not). Furthermore, these studies used different non-automated methods for hyaluronan measurement, various cut-offs, and the diagnostic performances found were variable, which could be explained by a inter-laboratory variability. A recent multicentric study [29] in 512 chronic HCV patients based on automated HA measurement and Hepascore calculation has demonstrated that from one serum sample, a non-invasive index of liver fibrosis that accurately predicts liver fibrosis can be totally automated using a single analyzer. We also confirm, even if our cohort is heterogeneous and smaller, that this immunoturbidimetric

**Table 4** Predictive values for cirrhosis and fibrosis for different values of HA and HS applying FT ( $n = 130$ ) or biopsy ( $n = 55$ ) as reference methods.

| Cut-off                 | PPV                 |                     | NPV    |        | Sensitivity |        | Specificity |        | Accuracy |        |
|-------------------------|---------------------|---------------------|--------|--------|-------------|--------|-------------|--------|----------|--------|
|                         | FT <sup>a</sup> (%) | LB <sup>a</sup> (%) | FT (%) | LB (%) | FT (%)      | LB (%) | FT (%)      | LB (%) | FT (%)   | LB (%) |
| Hyaluronic acid (ng/mL) |                     |                     |        |        |             |        |             |        |          |        |
| Cirrhosis               |                     |                     |        |        |             |        |             |        |          |        |
| 65                      | 50                  | 30                  | 96     | 96     | 90          | 89     | 73          | 59     | 77       | 64     |
| 115                     | 72                  | 41                  | 93     | 95     | 75          | 78     | 92          | 78     | 88       | 78     |
| 175                     | 81                  | 54                  | 90     | 95     | 61          | 78     | 96          | 87     | 88       | 85     |
| Fibrosis <sup>b</sup>   |                     |                     |        |        |             |        |             |        |          |        |
| 65                      | 74                  | 70                  | 70     | 53     | 64          | 59     | 79          | 65     | 72       | 62     |
| 115                     | 90                  | 78                  | 64     | 51     | 43          | 44     | 96          | 83     | 70       | 60     |
| Hepascore               |                     |                     |        |        |             |        |             |        |          |        |
| Cirrhosis               |                     |                     |        |        |             |        |             |        |          |        |
| 0.75                    | 61                  | 35                  | 96     | 97     | 86          | 89     | 84          | 67     | 85       | 71     |
| Fibrosis <sup>b</sup>   |                     |                     |        |        |             |        |             |        |          |        |
| 0.25                    | 70                  | 65                  | 93     | 67     | 95          | 87     | 61          | 35     | 77       | 65     |
| 0.50                    | 80                  | 67                  | 80     | 57     | 78          | 72     | 82          | 52     | 80       | 63     |

PPV: positive predictive value of a given HA value or higher to indicate cirrhosis or fibrosis; NPV: negative predictive value of a given HA value lower than the cut-off to exclude the presence of cirrhosis or fibrosis.

<sup>a</sup> Performances calculated separately using FT ( $n = 130$ ) or liver biopsy, LB ( $n = 55$ ) method as reference.

<sup>b</sup> Patients were separated into two groups: "no/mild fibrosis" (F0-F1) and "advanced fibrosis/cirrhosis" (F2-F3-F4) and the predictive values were recalculated.

method of HA measurement is easily applicable to a general chemistry analyzer.

The correlation between the Fibrotest score and the histological classification of Metavir has already been demonstrated in various studies [15–17]. With the present study we show that HA alone or included in Hepascore has very good diagnostic performances for different stages of CLD. In our patients' group, the correlation between HS and FT was satisfying, i.e. 0.764 [95% CI (0.6791–0.8292)].

There is already an established cut-off for FT to exclude fibrosis, that is FT less than 0.15. With our preliminary data it seems that with HA measurement it would be possible to discriminate "cirrhosis" (F4) from "advanced fibrosis" (F2-F3) which is not applicable either for FT nor for HS. A cut-off of 65 ng/mL could exclude cirrhosis, a cut-off of 175 ng/mL could confirm it. We agree [29] that the respective cut-off for HS is 0.25 to exclude cirrhosis, and 0.75 to confirm it. To differentiate "no/mild fibrosis" from "advanced fibrosis/cirrhosis", all three methods, i.e. HA, HS and FT, were very efficient.

When transient elastography was realized and used as a reference method, HA was the only biomarker that showed differences between advanced fibrosis and cirrhosis, Hepascore and Fibrotest being different only between the lowest and the highest Fibroscan score group.

This is a preliminary study that aimed at testing the feasibility and the diagnostic capacities of automated hyaluronan measurement. Our patient cohort was very heterogeneous with chronic liver disease of different etiologies or combinations of them. In order to validate what we demonstrated with this one-center study, our cohort should be enlarged in view of separate analysis of the various pathologies. The NAFLD group of patients is of special

interest as metabolic diseases increase in their incidence and only few studies on liver fibrosis have investigated these pathologies.

## Conclusions and perspectives

We confirm that the latex immunoturbidimetry method for HA measurement is precise and applicable to general clinical chemistry analyzer. HA alone or included in Hepascore showed a good ability to detect all stages in chronic liver disease. The correlation between HA, HS and FT was good. With these preliminary data for HA it appears that it could exclude cirrhosis from advanced fibrosis which is not the case with either FT or HS. Therefore, the developed HA assay can be used in clinical laboratories to evaluate liver fibrosis in complement to other non-invasive diagnostic markers.

As novel therapies for liver fibrosis evolve, non-invasive measurement of liver fibrosis will be required to help to manage patients with chronic liver disease. Although liver biopsy is the current and time-honored gold standard for measurement of liver fibrosis, it is poorly suited to frequent monitoring because of its expense and morbidity, and its accuracy suffers from sampling variation. At the current writing, serum markers and imaging methods are available and increasingly in use as alternatives to biopsy. However, many questions remain about their indications, accuracy, and cost-effectiveness, and more investigation is required before they are put into widespread use. The development of safe, inexpensive, and reliable non-invasive fibrosis measurement tools remains a research priority in clinical hepatology.

## Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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