HEPATOBIOLOGY

Transient elastography compared to serum markers to predict liver fibrosis in a cohort of Chinese patients with chronic hepatitis B

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Key words
biomarker, fibrosis, inflammation, noninvasive, transient elastography.

Accepted for publication 15 October 2014.

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The authors have no conflict of interest to declare.

Trial Registration Number: ChiCTR-DS-07000039.

This is study was supported by Wang Bao-en Research Fund for Liver Fibrosis (an unrestricted grant from China Foundation for Hepatitis Prevention and Control and partly by Key Project from Beijing Municipal Science and Technology Commission (Z09050700940902, D121100003912003) and Program for National Science and Technology Major Project (Z2013Z10002004).

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Abstract

Background and Aim: Liver stiffness measurement (LSM) using transient elastography (FibroScan) is a useful tool to assess fibrosis in various chronic liver diseases. However, studies were mainly performed in Western countries and largely focused on chronic hepatitis C (CHC). We therefore carried out a multicenter study to validate the accuracy of LSM in the assessment of liver fibrosis in a large cohort of Chinese patients with chronic hepatitis B (CHB).

Methods: We compared LSM results to histological staging and serum fibrosis markers (five direct markers, APRI and FIB-4) using Spearman correlation analysis and area under receiver operating characteristic (ROC) curves (AUROCs).

Results: Four hundred sixty-nine patients were enrolled and eligible for statistical analysis. LSM in F0 to F4 was 5.5 ± 1.7, 5.8 ± 2.2, 7.6 ± 3.4, 14.5 ± 10.8, and 22.3 ± 13.6 kPa, respectively (correlation with fibrosis stage r = 0.522, P < 0.001). AUROC for LSM to correctly allocate patients to histological fibrosis stage ≥ F2, ≥ F3, and F4 was 0.82, 0.88, and 0.90, respectively. LSM outperformed serum fibrosis markers for detection of fibrosis F ≥ 2 and F4. Patients with ALT levels 1–5x and > 5x the upper limit of normal values had significantly higher stiffness values than stage-matched patients with normal alanine aminotransferase.

Conclusion: Transient elastography is a reliable noninvasive technique to predict significant liver fibrosis in Chinese patients with CHB, being superior to current biomarker panels. However, enhanced inflammatory activity can lead to elevated stiffness values unrelated to histological fibrosis stage.

Introduction

Chronic hepatitis B (CHB) is the primary cause of liver-related morbidity and mortality in China. Based on a comprehensive serological survey of hepatitis B virus (HBV) infection in China in 2006, the weighted prevalence of HBsAg, of anti-HBs and of anti-HBc in the population aged 1–59 years was 7.2%, 50.1%, and 34.1%, respectively.1 Thus, it is estimated that about 93 million Chinese people are chronically infected with HBV.2 Of note, chronic active HBV infection progresses to cirrhosis and/or hepatocellular carcinoma (HCC) in an estimated 15% to 40% of patients, and the five-year survival rate for patients with decompensated cirrhosis or HCC is between 35% to 50% and 15% to 26%,3 respectively. With early diagnosis and the advent of effective antiviral therapies, the prognosis of CHB, even when presenting with histologically advanced fibrosis or cirrhosis, can be
improved significantly, with a concomitant gain in the patients' quality of life. Therefore, early staging and risk assessment in patient with CHB is of utmost importance in China.

Liver biopsy has traditionally been considered the gold standard for the assessment of liver fibrosis. However, it has limitations such as cost, invasiveness, associated risk for complications, and sampling variability. Therefore, noninvasive approaches have been suggested to overcome these limitations and reduce the need for liver biopsies.

Previous studies have already demonstrated a correlation between several direct fibrosis markers such as hyaluronic acid, laminin, N-terminal propeptide of type III procollagen (PⅢNP), tissue inhibitor of metalloproteinases-1 (TIMP-1), and transforming growth factor beta 1 (TGFβ1), or indirect fibrosis markers, and especially of fibrosis marker panels with the extent of fibrosis. For example, the aspartate aminotransferase (AST) to platelet ratio index (APRI) and fibrosis-4 (FIB-4) (based on age, AST, alanine aminotransferase [ALT], and platelets) are scores showing good performance to exclude liver cirrhosis. However, they were mostly studied in patients with chronic hepatitis C (CHC), and only few studies addressed their performance in patients with CHB. Notably, to date, all markers showed less accuracy to identify intermediate stages of fibrosis.

LSM by transient elastography has been widely studied in Western countries to noninvasively assess the stage of liver fibrosis in patients with chronic liver diseases, especially in CHC. Performance of LSM in CHB has been studied in Western and Asian populations, but further validation in larger cohorts of patients from different regions in China is still needed.

The aim of this multicenter prospective study was to (i) validate the diagnostic accuracy of LSM for fibrosis in a cohort of Chinese patients with CHB, using histology as a reference; (ii) compare LSM with five direct fibrosis markers and with two biomarker panels; (iii) evaluate the effect of ALT levels on liver stiffness cutoffs values and diagnostic performance.

Methods

Patients. Between September 2007 and April 2009, consecutive patients with CHB and an indication for percutaneous liver biopsy were screened for enrollment in this study.

Inclusion criteria were: (i) age between 18 and 65 years; (ii) clinical history of CHB or HBsAg(+) for longer than six months; (iii) a liver biopsy within six months of the FibroScan (Echosens, Paris, France) examination; (iv) off potential transaminase-lowering drugs; (v) a clinical history of CHB or HBsAg(+); (vi) a liver biopsy within six months of the FibroScan (Echosens, Paris, France) examination; (v) off potential transaminase-lowering drugs; (vi) a clinical history of CHB or HBsAg(+); (vii) body mass index (BMI) < 30 kg/m²; (viii) absence of chronic liver cirrhosis; (ix) absence of ascites; (x) pregnancy; (xi) prothrombin activity < 35%; (xii) HCV co-infection; (xiii) presence of at least two weeks prior to biopsy sampling for biochemistries; (x) written informed consent.

Exclusion criteria were: (i) HBV immune tolerant patients or asymptomatic carriers since not candidate for biopsy; (ii) white blood cell count < 3.5×10^9/L or platelet count < 80×10^9/L, or prothrombin activity < 60%; (iii) HCV co-infection; (iv) presence of other causes of chronic liver disease such as overt alcoholic or nonalcoholic fatty liver disease, autoimmune liver disease, hereditary metabolic liver diseases, biliary diseases, or hepatobiliary parasitic infections; (v) decompensated cirrhosis, including patients with ascites; (vi) pregnancy; (vii) body mass index (BMI) > 28 kg/m²; (viii) cardiac pacemaker or defibrillator; (ix) unhealed wound in right upper quadrant.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the institutions' human research committees.

Liver biopsy. Percutaneous liver biopsy was performed using a 16 G needle under ultrasound guidance. Biopsies were fixed in 10% formalin, embedded in paraffin, and stained with HE, Masson’s trichrome and reticulin staining for histological assessment. Only liver biopsies with a minimum length of 1 cm and at least eight complete portal tracts were considered suitable for further analysis. Fibrosis stage and inflammation grade were assessed by two independent pathologists according to the META VIR scoring system. Discordant cases were reviewed by pathologists to reach consensus.

Liver stiffness measurement. LSM were performed using FibroScan equipped with the standard probe (Ultrasound frequency of 3.5 MHz) sampling the liver between 2.5 and 6.5 cm below the skin surface. All operators were trained according to the manufacturer’s recommendations. Only LSM with at least 10 valid measurements, success rate ≥ 60% and interquartile range (IQR) over median ratio ≤ 30% were considered reliable.

Clinical laboratory parameters

Routine laboratory parameters. Blood samples were obtained from fasting patients within one week of LSM. Serum bilirubin, albumin, ALT, AST, gamma-glutamyltranspeptidase (GGT), alkaline phosphatase, platelet count, prothrombin activity, and international normalized ratio were measured in central approved laboratories. Additional serum of 8 mL and 3 mL plasma were stored in refrigerators below −80°C for fibrosis marker analysis.

Fibrosis marker determinations. These were determined in an approved central laboratory (Beijing 302 Military Hospital, China). Five direct fibrosis markers (laminin, hyaluronic acid, PⅢNP, TGFβ1, and TIMP-1) were quantified by ELISA with autoanalyzers (Thermo Mutiskan MK3, Thermo Lab systems, USA). The testing kits for fibrosis biomarkers were supplied by two companies: the kits for PⅢNP, hyaluronic acid, and laminin were supplied by Xiamen Lujia Biotechnology Company (China), whereas the kits for TIMP-1 and TGFβ1 were supplied by R&D Systems (USA).

Statistical analysis. Statistical analysis was performed using NCSS and PASS statistical software (Kaysville, UT, USA). Patients’ characteristics were summarized as means and standard deviation, or numbers of cases and percentages, as appropriate. Correlations were evaluated by Spearman’s correlation coefficient for continuous variables and by Kendall’s correlation coefficient for categorical variables. A P-value ≤ 0.05 was considered significant. Predictive factors of LSM were determined by performing univariate analysis. Since LSM do not follow a normal distribution, multivariate analysis was performed with a Box–Cox transformation of LSM values versus the parameters significantly correlated.
correlated in the univariate analysis for identification of independent predictors. The diagnostic performance of all noninvasive markers evaluated was assessed by receiver operating characteristic (ROC) curves using histology as a reference. The area under ROC curves (AUROCs) were compared using the DeLong method and software version 12.2.1.0 (Medcalc, Mariakerke, Belgium). Sensitivity (Se), specificity (Sp), positive predictive value, negative predictive value, positive likelihood ratio, and diagnostic accuracy (DA) were calculated.

In addition, the Obuchowski measure was assessed,\(^2\) taking into account the distribution of fibrosis stages in the study population.

Optimal cutoff values for the noninvasive markers were obtained using the following criteria: maximal DA (MA, percentage of patients correctly classified); maximal sum of SE and SP (MS), at least 95% of SP (95 SP) and at least 95% of SE (95 SE). Comparison of DAs was performed using Chi-square tests of proportions from independent samples.

### Results

**Clinical characteristics of patients.** From September 2007 to April 2009, 555 CHB patients who fulfilled the study criteria were enrolled from seven centers in China. Forty-two patients were excluded because of inappropriate biopsy samples, and 44 (7%) because of unreliable LSM. Therefore, 469 patients were eligible for statistical analysis. Patient’s characteristics are given in Table 1.

**Liver stiffness measurement and histology.** Forty patients had F0 (8.5%), 142 F1 (30.3%), 124 F2 (26.4%), 106 F3 (22.6%), and 57 F4 (12.2%) fibrosis. LSM values were significantly correlated with fibrosis stage (Kendall \(\tau = 0.522, P < 0.001\), Fig. 1), with mean values of 5.5 kPa, 5.8 kPa, 7.6 kPa, 14.5 kPa, and 22.3 kPa for F0, F1, F2, F3, and F4, respectively.

**Predictive factors of LSM.** By univariate analysis, age, METAVIR fibrosis stage and inflammatory activity, BMI, ALT, AST, albumin, GGT and platelets were significantly correlated with LSM (p < 0.05). By multivariate analysis, only fibrosis stage, inflammatory activity, AST/ALT ratio, albumin, GGT, and platelets were independent predictors of LSM (p < 0.05).

**Diagnostic accuracy of LSM and biomarkers**

**Liver stiffness measurements.** Diagnostic performances of LSM (AUROCs) for diagnosing \(\geq F2\), \(\geq F3\), and F4 were 0.82, 0.88, and 0.90, respectively. Adjusted AUROC using the Obuchowski method (0.94 ± 0.0049) confirmed this excellent performance.

Cutoffs calculated according to different criteria are given in Table 2. By choosing MS as primary criterion, cutoffs for diagnosing \(\geq F2\), \(\geq F3\), and F4 were 7.3, 9.7, and 10.7 kPa, respectively.

**Biomarkers.** Because biomarker results were missing for 82 patients, performances of the five studied biomarkers and the two biochemical scores (APRI, FIB-4) were assessed on 387 patients. AUROCs of APRI, FIB-4, hyaluronic acid, PIIINP, laminin,
Application of preexisting cutoffs to our study population. Table 4 shows analysis of the DA of LSM to stage fibrosis and cirrhosis in our cohort according to two sets of pre-existing cutoffs. Here, for diagnosing cirrhosis in our cohort, ALT-related cutoffs revealed a DA of 94% for patients with normal ALT, and 82% for patients with ALT one to five times ULN. This compares to 97% and 89%, in the original cohort of Chan et al. ($P = 0.67$ and $P = 0.15$, respectively, NS). The cutoff of Marcellin et al.$^{14}$ showed a DA of 72.7% for diagnosing $F \geq 2$ and of 83% for diagnosing F4 when applied to our population. This compares to 76% ($P = 0.47$ vs the original cohort, ns) and 87% ($P = 0.26$ vs original cohort, NS), respectively.

Discussion

In the present study, we evaluated and compared the performance of LSM with a spectrum of five serum fibrosis markers and two marker combinations, using histology as reference. Ours is the first study to evaluate such a broad panel of noninvasive tools in a large population of Chinese patients with CHB.

We confirmed the good performance of LSM to stage significant fibrosis ($F \geq 2$), with an AUROC of 0.82 at a cutoff of 7.3 kPa. Performance was very good for detecting cirrhosis ($F4$) with an AUROC of 0.90 at a cutoff of 10.7 kPa. These results are consistent with previous studies.$^{11,14,15,22}$ In the two larger studies that included 125 or 202 European patients with CHB, AUROCs of FibroScan for $F \geq 2$ ranged from 0.81 to 0.85 and for F4 from 0.90 to 0.93.$^{14,15}$ In another Asian study from Korea performed on 170 patients, performances were even better than ours, with AUROCs of 0.94 and 0.96 for $F \geq 2$ and F4, respectively.$^{23}$

The optimal cutoffs in our study of patients from China are also similar to the cut-offs reported by Marcellin et al. that is, 7.3 versus 7.2 kPa for $F \geq 2$ and 10.7 kPa versus 11 kPa for $F4$. In addition to this, Chon et al. performed a meta-analysis on 2772 HBV-infected patients and also reported equivalent cutoffs of 7.9 kPa for $F \geq 2$ and 11.7 kPa for F4, respectively.$^{22}$

In comparison with LSM, performances of the five direct biomarkers showed significantly lower AUROCs for both diagnosis of $F \geq 2$ and F4, indicating that serum levels of single direct biomarkers do not sufficiently mirror the amount of accumulated fibrous tissue. In fact, these markers may rather reflect the dynamics of extracellular matrix (ECM) turnover or even fibrogenesis, as suggested by recent data using the enhanced liver fibrosis (ELF) panel, which consists of hyaluronic acid, PIIINP, and TIMP-1, or certain individual direct markers.$^{22,24}$

We found that indirect (or mixed) marker panels performed slightly better than the single direct serum markers. Thus, APRI exhibited AUROCs of 0.71 (confidence interval [CI] 0.66–0.75) for diagnosing $F \geq 2$ and 0.66 (CI 0.61–0.70) for detecting F4, similar to previous prior studies.$^{11-14}$ The higher performance of APRI found by Zhu et al.$^{11}$ who studied 177 patients with CHB, might be explained by a cohort from which patients with ALT $> 2$ times ULN were excluded. Our finding of a lower performance of APRI for predicting cirrhosis can be explained by higher transaminase levels in our population of CHB patients compared to previous studies. Equally, in the present study, the performance of FIB-4 was relatively poor for prediction of both $F \geq 2$ and F4, with AUROCs of 0.69 (CI 0.64–0.73) and 0.75 (CI 0.70–0.80).
not improve the DA of LSM. This result could be explained by the relatively small number of patients with moderately elevated ALT (three to five times ULN) and remarkably elevated ALT (>5 times ULN), in our cohort (11.3% and 9.9% of the cohort, respectively).

However, the AUROCs of LSM for cirrhosis assessment was still markedly decreased for patients with ALT levels >5 ULN in comparison with other patients with lower ALT levels. We therefore confirm the results previously reported26 which indicate the need to interpret with caution LSM on patients with acute exacerbation of CHB, and thus to wait for normalization of ALT to ensure optimal diagnosis.

We also evaluated whether previously defined cutoffs would exhibit the same accuracy in our study population compared to the original populations. Cutoffs in the study of Marcellin et al.14 exhibited slightly lower DAs both for diagnosing F4 in patients with ALT between one to five times ULN (82% vs 86%, 97% vs 99%) and, somewhat counter-intuitively, an even lower AUROC of 0.77 vs 0.82 (0.79–0.86) MS 7.3 0.65 0.84 86 62 3.99 72

Table 2 Diagnostic performances of LSM for staging grades of fibrosis, and optimal cutoffs according to different criteria (n = 469)

| AUROC (95% CI) | Criteria | Cut off (kPa) | Se  | Sp  | PPV (%) | NPV (%) | LR+ | DA (%) |
|---------------|---------|--------------|-----|-----|---------|---------|-----|--------|
| F ≥ 2         | 0.82 (0.79–0.86) | MS 7.3 | 0.65 | 0.84 | 86  | 62  | 3.99 | 72 |
|               |         | 95 SP 9.1   | 0.32 | 0.95 | 69  | 82  | 1.40 | 71 |
|               |         | 95 SE 4.7   | 0.95 | 0.51 | 86  | 61  | 10.3 | 68 |
| F ≥ 3         | 0.88 (0.84–0.91) | MS 9.7 | 0.73 | 0.90 | 79  | 86  | 7.46 | 84 |
|               |         | 95 SP 12.4  | 0.58 | 0.95 | 86  | 81  | 11.84 | 82 |
|               |         | 95 SE 5.8   | 0.95 | 0.48 | 49  | 95  | 1.84 | 64 |
| F ≥ 4         | 0.90 (0.87–0.93) | MS 10.7 | 0.90 | 0.82 | 39  | 97  | 4.85 | 83 |
|               |         | 95 SP 21.3  | 0.40 | 0.95 | 52  | 93  | 8.45 | 89 |
|               |         | 95 SE 8.2   | 0.95 | 0.69 | 26  | 99  | 3.03 | 72 |

AUROC, area under ROC curve; DA, diagnostic accuracy; kPa, kilopascal; LR+, positive likelihood ratio; LSM, liver stiffness management; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

0.70–0.79), respectively. This again contrasts with the study of Zhu et al., in which FIB-4 yielded AUROCs of 0.86 for F ≥ 2 and, somewhat counter-intuitively an even lower AUROC of 0.77 for F4. In line with the data from other cohorts, we found that FIB-4 showed a modest performance for detecting cirrhosis (0.75, CI 0.70–0.79) and a significantly lower performance to diagnose significant fibrosis (0.69, CI 0.64–0.73). As before, the discrepant results of the study by Zhu et al., could be explained by selection bias, since they excluded patients with ALT levels higher than two times the ULN which affects the overall diagnostic performance of FIB-4.

Therefore, although APRI and FIB-4 had previously been shown to be useful to stage liver fibrosis in CHC patients, our results suggest that in Chinese patients with CHB both panels are significantly inferior to LSM measurement. Notably, in CHB elevated ALT levels seem to affect the diagnostic performance of these markers for intermediate and advanced stages of fibrosis.

Numerous studies have demonstrated the influence of necroinflammatory activity on LSM, with increased stiffness values being associated with elevated transaminase levels.25 In the present study, as expected, patients with elevated ALT levels showed higher LSM values. However, performances of LSM was not significantly compromised with these ALT elevations, and predictive value remained identical for diagnosis of F ≥ 2, while there was a decrease, yet insignificant, of diagnostic performance for predicting cirrhosis. Notably, applying a specific algorithm with adjusted cutoffs taking into account stratified ALT levels did not improve the DA of LSM. This result could be explained by the relatively small number of patients with moderately elevated ALT (three to five times ULN) and remarkably elevated ALT (>5 times ULN), in our cohort (11.3% and 9.9% of the cohort, respectively).

However, the AUROCs of LSM for cirrhosis assessment was still markedly decreased for patients with ALT levels >5 ULN in comparison with other patients with lower ALT levels. We therefore confirm the results previously reported26 which indicate the need to interpret with caution LSM on patients with acute exacerbation of CHB, and thus to wait for normalization of ALT to ensure optimal diagnosis.

We also evaluated whether previously defined cutoffs would exhibit the same accuracy in our study population compared to the original populations. Cutoffs in the study of Marcellin et al.14 exhibited slightly lower DAs both for diagnosing F4 in patients with ALT between one to five times ULN (82% vs 86%, 97% vs 99%) and, somewhat counter-intuitively, an even lower AUROC of 0.77 vs 0.82 (0.79–0.86) MS 7.3 0.65 0.84 86 62 3.99 72

Figure 2 Area under ROC curves (AUROCs) of liver stiffness measurement (LSM), FIB-4 and aspartate aminotransferase (AST) Platelet Ratio Index (APRI) for the diagnosis of F ≥ 2 (a) and F4 (b) META/IVR stages. ——, LSM; ———, APRI; ——, FIB4.

The AUROCs of FIB-4 and APRI were 0.82 (0.79–0.86) and 0.87 (0.84–0.91), respectively, with adjusted cutoffs taking into account stratified ALT levels did not significantly compromise with these ALT elevations, and also for patients with elevated ALT levels seem to affect the diagnostic performance of FIB-4.

In conclusion, our study validated the accuracy of LSM to diagnose significant fibrosis and cirrhosis in a large cohort of Chinese patients with CHB. LSM showed significantly higher diagnostic performances compared to individual direct fibrosis markers or the biochemical marker panels APRI and FIB-4. ALT levels up to five times the ULN did not significantly affect the diagnostic power of LSM.

Acknowledgments

We thank the following contributors to the study for their active support: Xiaojuan Ou and Hong Ma (Liver Research Center,
Table 3  Diagnostic performances (AUROCs) and optimal cut-offs of LSM (chosen for maximizing the sum of Sensitivity and Specificity) according to ALT levels. Impact of the use of stratified ALT related cut-offs on the diagnostic accuracy (DA)

| ALT level | n | AUROC (95% CI) | Cut off (kPa) | Se (%) | Sp (%) | DA (%) | P-value |
|-----------|---|----------------|--------------|--------|--------|--------|---------|
| F ≥ 2     | 200 | 0.78 (0.71–0.83) | 5.1          | 83     | 61     | 72.9   | 0.98 (ns) |
| 1–3 ULN   | 166 | 0.82 (0.76–0.88) | 7.3          | 72     | 83     |        |         |
| 3–5 ULN   | 53  | 0.77 (0.64–0.88) | 9.9          | 69     | 93     |        |         |
| > 5 ULN   | 46  | 0.78 (0.58–0.89) | 12.8         | 63     | 100    |        |         |
| All       | 465 | 0.82 (0.78–0.86) | 7.3          | 65     | 84     | 72.6   |         |

F ≥ 4

| ALT level | n | AUROC (95% CI) | Cut off (kPa) | Se (%) | Sp (%) | DA (%) | P-value |
|-----------|---|----------------|--------------|--------|--------|--------|---------|
| 1–3 ULN   | 166 | 0.92 (0.80–0.97) | 8.1          | 86     | 87     | 76     | 0.58 (ns) |
| 3–5 ULN   | 53  | 0.90 (0.78–0.96) | 13.8         | 100    | 76     |        |         |
| > 5 ULN   | 46  | 0.77 (0.51–0.90) | 14           | 100    | 54     |        |         |
| All       | 465 | 0.90 (0.87–0.93) | 10.7         | 90     | 82     | 83     |         |

n = 465 out of 469 patients with available ALT levels.

Table 4  Comparison of LSM diagnostic accuracies in predicting significant fibrosis (F ≥ 2) and cirrhosis (F4) using already published cut-offs between the reference population and our study population

| ALT level | Reference population | Study population | P-value* |
|-----------|----------------------|------------------|---------|
| F ≥ 2     |                       |                  |         |
| ≤ ULN     | Chan et al.           | 6.0              | 58      | 200    | 63     | 63     | 69.5   | —      |
| 1–5 ULN   | 7.5                   |                  | 98      | 212    | 68     | 77     | 71     | —      |
| All cases | Chan et al.           | Channell et al.  | 7.2     | 202    | 76     | 66     | 83     | 72.7   | 0.47 (ns) |
| F4 ≤ ULN  | Chan et al.           | 12               | 58      | 97     | 200    | 64     | 96     | 94     | 0.67 (ns) |
| 1–5 ULN   | 13.4                  |                  | 98      | 89     | 212    | 83     | 82     | 82     | 0.15 (ns) |
| All cases | Chan et al.           | Channell et al.  | 9       | 156    | 81     | 465    | 93     | 75     | 77     | 0.38 (ns) |

*P-values were calculated for statistical difference between DAs of the reference population versus the study population.

ALT, alanine aminotransferase; DA, diagnostic accuracy; kPa, kilopascal; NPV, negative predictive value; ns, non-significant; PPV, positive predictive value; Se, sensitivity; Sp, specificity; ULN, upper limit of normal.

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