Original Article

Diagnostic Performance of Fibrotest/Actitest for Staging Significant Liver Fibrosis in Thai Chronic Hepatitis C Patients

Kawin Tangvoraphonkchai, MD[1], Tanita Suttichaimongkol, MD[1], Churairat Kularbkaew, PhD[2], Prakasit Sangaimwibool, MD[2], Wattana Sukeepaisarnjaroen, MD[1]

[1] Division of Gastroenterology, Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand
[2] Department of Pathology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand

Background: Chronic hepatitis C viral (HCV) infection remains a major critical challenge in Thailand.Clinical management requires diagnostic significant liver fibrosis. Fibrotest/Actitest is a novel non-invasive testing for alternative liver biopsy. There are limited studies of the testing in Thailand.

Objective: To demonstrate the diagnostic performance to predict significant fibrosis (METAVIR stage ≥F2) using Fibrotest/Actitest compared to liver biopsy in HCV patients.

Materials and Methods: The present study prospectively enrolled 100 HCV patients, who underwent liver biopsy. Fibrotest/Actitest was done in the same day. Liver histology was evaluated using the METAVIR scoring system. Diagnostic stat was calculated and evaluated for the best cut-off values of patients with METAVIR fibrosis F≥2.

Results: The AUROC for Fibrotest/Actitest was 0.74 (0.64 to 0.83) and the best cut-off was ≥0.44 for prediction significant fibrosis (F≥2) in Thai Chronic hepatitis C viral infection patients with sensitivity, specificity, PPV, and NPV as 75.4% (63.1 to 85.2), 71.4 (53.7 to 85.4), 83.1 (71.0 to 91.6) and 61 (44.5 to 75.8), respectively. This cut-off was more accuracy than the international cut-off (≥0.49) and improved sensitivity, PPV and NPV for prediction significant fibrosis in Thai chronic HCV infection.

Conclusion: Fibrotest/Actitest addresses a critical need for management of chronic HCV infection. Cut-off ≥0.44 was a predictor of the significant fibrosis (F2) and acceptable diagnostic performance.

Keywords: Chronic hepatitis C virus, Fibrotest/Actitest, Liver biopsy, METAVIR fibrosis

J Med Assoc Thai 2020;103(Suppl.8): S68-72
Website: http://www.jmatonline.com

Chronic hepatitis C viral (HCV) infection is the impact health problem, with an estimated 185 million patients infected worldwide[1,2]. In Thailand, there are 758,940 HCV antibody-positive individuals, 356,670 of whom have HCV viremia[3]. The chronic HCV patients could develop cirrhosis, decompensated cirrhosis and cancer[4]. HCV eradication is the only way to reduce liver cirrhosis and cancer. Presently, the THASL guideline has suggested direct-acting antiviral drugs or pegylated-interferon were the main treatment of choice in Thailand for satisfactory results. However, the patients must have significant fibrosis[5,6]. In the past, this was determined through liver biopsy and interpreted based on the METAVIR staging fibrosis score (it must be greater than or equal to 2)[7]. However, there are limitations of liver biopsy such as severe complications, sampling errors and requiring expert pathologist[7].

Recently, Fibrotest/Actitest has become available and has gained acceptance around the world. The component of Fibrotest/Actitest are age and gender combined with surrogate blood biomarkers for fibrosis (alpha 2-macroglobulin, haptoglobin, apolipoprotein A1, gamma glutamyl transpeptidase (GGT) and total bilirubin) and alanine aminotransferase (ALT, only for activity score). Studies from overseas have found a Fibrotest/Actitest cut-off score of ≥0.49 to be equal to a fibrosis METAVIR score of ≥F2 in HCV patients, indicating significant fibrosis[8-10]. The performance of Fibrotest/Actitest could vary according to country of the study because the prevalence of fibrosis and activity in patients on the different cohorts is not the same[8-10].

To date, no study has been conducted on the diagnostic performance of Fibrotest/Actitest to indicate significant fibrosis in HCV patients in Thailand. The present study aims to demonstrate the diagnostic performance of

Correspondence to:
Tangvoraphonkchai K.
Srinagarind Hospital, Khon Kaen University, Khon Kaen 40000, Thailand.
Phone: +66-81-7082108
Email: Kawin_tang@hotmail.com

How to cite this article:
Tangvoraphonkchai K, Suttichaimongkol T, Kularbkaew C, Sangaimwibool P, Sukeepaisarnjaroen W. Diagnostic Performance of Fibrotest/Actitest for Staging Significant Liver Fibrosis in Thai Chronic Hepatitis C Patients. J Med Assoc Thai 2020;103 (Suppl.8): S68-72.
doi.org/10.35755/jmedassocthai.2020.S08.12027

Fibrotest/Actitest to predict significant fibrosis compared with liver biopsy in Thai patients with HCV.

Materials and Methods

Study design & population

The present study is a prospective, single center diagnostic study with approval by the Khon Kaen University Ethics committee for Human Research based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (HE591548).

The authors prospectively enrolled chronic HCV patients who underwent liver biopsy before treatment at Khon Kaen University Faculty of Medicine's Srinagarind hospital from April 2017 to November 2019. Inclusion criteria were age 18 to 65 years, detectable serum anti-HCV and HCV RNA, ECOG performance status of 0 and no treatment contraindications. Exclusion criteria were contraindication for liver biopsy, coinfection of chronic hepatitis B and/or human immune deficiency virus (HIV), Child-Pugh score >6, previously treatment with interferon, peginterferon alfa 2a or 2b and ribavirin, consumption of alcohol within 6 months or use of addictive drugs, hepatocellular carcinoma or other active cancer, comorbid disease that could not be adequately controlled such as hypertension, diabetes, coronary artery disease, emphysema, hyperthyroidism, alcoholism, major uncontrollable depressive disorder, pregnancy or unwillingness to use contraception, and history of kidney, heart, or lung transplant, patients with acute or chronic hemolysis or extra hepatic cholestasis or Gilbert's syndrome. Patients were enrolled after giving their written informed consent.

The authors collected data on the following parameters: patient characteristics, history of alcohol consumption, co-morbidity, complete blood count with platelet count, prothrombin time with INR, liver tests (serum albumin, total bilirubin, ALT, aspartate aminotransferase (AST), and alkaline phosphatase (ALP)), viral hepatitis studies, HCV RNA, and genotypes. The sample size was calculated by the biometric statistician with Buderer's formula for specificity. Estimated total sample size is 100 patients (Figure 1).

Fibrotest/Actitest measurement

Blood samples (amount 2 cc) were collected after fasting at least 8 hours, in the same day of liver biopsy. Blood samples were centrifuged within 1 hours after collected blood and were sent to Thai standardized laboratory by freezing -25°C (Nhealth and Brialab are the standard lab that makes determination of components of Fibrotest/Actitest according to the technical recommendations that are required by the proprietary of the test in Bangkok, Thailand) to estimate value of Fibrotest/Actitest.

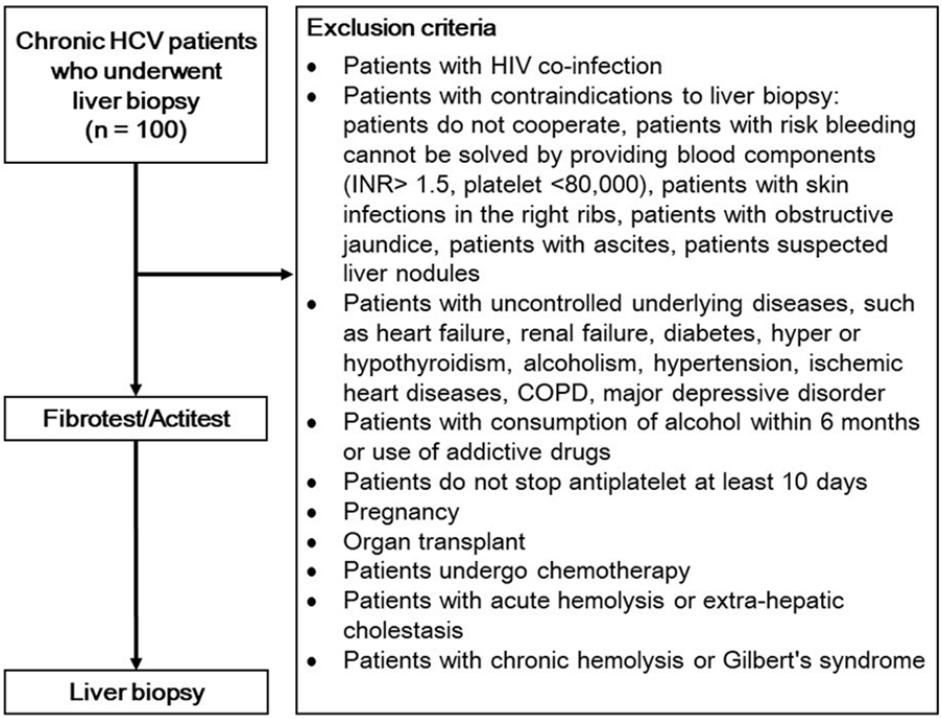

Figure 1. Study flow.

Liver biopsy and interpretation

Percutaneous liver biopsy was performed with a 1.6-mm diameter Trucut needle with a length of at least 2.5 cm. All specimens were fixed in formalin, embedded in paraffin, cut, and stained with hematoxylin and eosin. All liver histology interpretations were performed by two pathologists (K. Churairat and S. Prakasit) with experience in reading biopsied liver samples and blinded to the Fibrotest/Actitest results. Disagreements between pathologists were resolved through discussion and mutual decision about the results. Reports were based on the METAVIR scoring system, which specifies a fibrosis score from 0 to 4 (F0: no fibrosis; F1: stellate enlargement of portal tract without septa formation; F2: enlargement of the portal tract with rare septa formation; F3: numerous septa without cirrhosis; F4: cirrhosis).

Statistical analysis

Baseline demographic and clinical characteristics of patients were summarized using descriptive statistics. For categorical variables, numbers for all categories were presented with percentages. For continuous variables, mean, standard deviation, median, minimum, and maximum were presented. The diagnostic performance of Fibrotest/Actitest was assessed by using receiver operating characteristic (ROC) curves. The ROC curve was used to identify the best cut-off values for detection of patients with METAVIR fibrosis ≥F2. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the ROC curve (AUROC) were also calculated to obtain diagnostic accuracy. Statistical analyses were performed with STATA® 10.1 software.

Results

The 100 patients were included in the study, 67 were male (67%) and 33 were female (33%). The average age was 51.0±8.3 years. The genotypes were genotype 1a, 1b, 3 and 6 in 15 (15%), 17 (17%), 40 (40%) and 28 (28%) patients, respectively. The liver fibrosis from biopsy were F0, F1, F2, F3 and F4 in 7 (7%), 28 (28%), 33 (33%), 17 (17%) and 15 (15%), respectively (Table 1).

The findings indicated that the Fibrotest/Actitest values for each fibrosis stage are F0 = 0.26±0.20, F1 = 0.37±0.21, F2 = 0.58±0.25, F3 = 0.71±0.28 and F4 = 0.64±0.27 (Table 2). The AUROC of Fibrotest/Actitest values and fibrosis at stage 2 or higher is showed in Figure 2.

The most reliable cut-off for diagnosis of significant fibrosis (F≥2) using Fibrotest/Actitest obtained from the population in the present study was ≥0.44 with sensitivity 75.4% (63.1 to 85.2), specificity 71.4% (53.7 to 85.4), positive predictive value 83.1% (71 to 91.6), negative predictive value 61% (44.5 to 75.8), likelihood ratios for positive test 2.64 (1.53 to 4.54), likelihood ratios for negative test 0.35 (0.21 to 0.55) and AUROC 0.74 (0.64 to 0.83). Using the international cut-off ≥0.49, the results showed sensitivity 72.3% (59.8 to 82.7), specificity 71.4% (53.7 to 85.4), positive predictive value 82.5 (70.1 to 91.3), negative predictive value 58.1 (42.1 to 73.0), likelihood ratios for positive test 2.53 (1.47 to 4.36), likelihood ratios for negative test 0.39 (0.25 to 0.61) and AUROC 0.72 (0.63 to 0.81) (Table 3).

Table 1. Baseline characteristics of the 100 patients

Characteristics	Values
Male: Female	67: 33
Age (years)	51.0 (8.3)
Underlying disease	
None	76 (76)
Diabetes mellitus	11 (11)
Hypertension	12 (12)
Other	2 (2)
Hemoglobin (g/dl)	13.8 (1.4)
Platelet count (10^3/mm^3)	192.5 (56.5)
Creatinine (mg/dl)	0.9 (0.2)
Albumin (g/dl)	4.4 (0.4)
Total bilirubin (mg/dl)	0.7 (0.3)
ALT (U/L)	73.2 (54.8)
AST (U/L)	63.6 (43.3)
ALP (U/L)	90.0 (36.8)
INR	1.0 (0.1)
HCV viral load (IU/ml)	4,032,315 (5,919,394)
HCV genotypes	
1A	15 (15)
1B	17 (17)
3	40 (40)
6	28 (28)
Fibrosis score (METAVIR)	
F0	7 (7)
F1	28 (28)
F2	33 (33)
F3	17 (17)
F4	15 (15)

Data are expressed as mean (standard deviation) or number (%). ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase, INR = international normalized ratio

Discussion

In the present study, the authors demonstrated that Fibrotest/Actitest test had a good AUROC and correlated well with METAVIR score from liver biopsy. The authors also demonstrated that the cut-off value of Fibrotest/Actitest that yielded the highest AUROC was ≥0.44 (sensitivity 75.4%, specificity 71.4%, PPV 83.1%, NPV 61% and AUROC 0.74). These findings differed

substantially from those of some previous studies. For instance, Leroy et al performed a study on chronic HCV patients, demonstrated a cut-off for significant fibrosis of ≥0.40 (sensitivity 66%, specificity 82%, PPV 78% and NPV 73%)[8]. Zarski et al obtained a cut-off for significant fibrosis of ≥0.48 (sensitivity 75.8%, specificity 66.2%, PPV 66.2% and NPV 75.8%)[9].

Since the present study found that the cut-off ≥0.44 was more accurate than international cut-off of ≥0.49, as well as the higher sensitivity, PPV, NPV and AUROC, it might support the use the lower cut-off of ≥0.44 for chronic HCV infection in Thailand.

There are many possible reasons that results of this study differed from previous studies. First, most of the population in the research had high proportion F3 and F4 METAVIR score (32%). Second, most of the genotypes were genotype 3, which differed from previous studies[8,9]. The severity of inflammation in the genotype 3 might affect the result of the test. Further study on the effects of inflammation on the test results may be required. Nevertheless, Fibrotest/Actitest can be a good option to replace liver biopsy because there is no risk of complications, no complicated tools required and it is more convenient for examination in many places.

Table 2. Values of Fibrotest/Actitest at each stage of liver fibrosis by liver biopsy

Fibrosis score (liver biopsy)	Number	Fibrotest/Actitest, mean (SD)
F0	7	0.26 (0.20)
F1	28	0.37 (0.21)
F2	33	0.58 (0.25)
F3	17	0.71 (0.28)
F4	15	0.64 (0.27)

SD = standard deviation

The strengths of the present study were the thorough planning of the clinical data analysis and the authors rechecked the liver fibrosis scores by 2 pathologists. The present study, however, has some limitations. It is a single center study in the northeastern part of Thailand. However, information from the Thai Ministry of Public Health showed that majority of chronic HCV patients were from northeastern Thailand. In addition, this study should include a much larger population for all genotypes as a multicenter trial, as it will lead to the more accurate results. The correlation of inflammation and the results of the test should also be further studied.

Conclusion

Fibrotest/Actitest addresses a critical need for management of chronic HCV infection. Cut-off ≥0.44 was a predictor of the significant fibrosis (F2) and acceptable diagnostic performance.

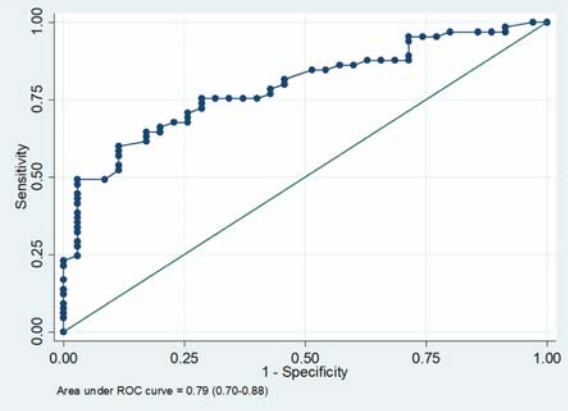

Figure 2. Receiver operating characteristic curve for significant fibrosis (>F2) measurement using Fibrotest/Actitest.

Table 3. Cutoff value of Fibrotest/Actitest for diagnosis significant fibrosis (METAVIR ≥F2) and 95% confidence interval

Cut-off	≥0.44	≥0.49
Sensitivity (%)	75.4 (63.1 to 85.2)	72.3 (59.8 to 82.7)
Specificity (%)	71.4 (53.7 to 85.4)	71.4 (53.7 to 85.4)
AUROC	0.74 (0.64 to 0.83)	0.72 (0.63 to 0.81)
Positive predictive value (%)	83.1 (71.0 to 91.6)	82.5 (70.1 to 91.3)
Negative predictive value (%)	61.0 (44.5 to 75.8)	58.1 (42.1 to 73.0)
Positive likelihood ratio	2.64 (1.53 to 4.54)	2.53 (1.47 to 4.36)
Negative likelihood ratio	0.35 (0.21 to 0.55)	0.39 (0.25 to 0.61)

AUROC = area under the receiver operating characteristic curve

What is already known on this topic?

Fibrotest/Actitest has a good diagnostic performance for prediction significant fibrosis in chronic HCV patients. It is an acceptable replacement liver biopsy because there is no risk of complication, no complicated tools required it is more convenient.

What this study adds?

The best cut-off of Fibrotest/Actitest is ≥0.44 for Thai chronic HCV infection patients to predict significant fibrosis (≥F2 METAVIR score). This cut-off is more accurate than international cut-off (≥0.49) because it improves sensitivity, PPV, NPV and AUROC.

Acknowledgement

The authors thank the patients for their participation, the Faculty of Medicine, Khon Kaen University, for their support, The Gastroenterological Association of Thailand and Digital Diagnostic Asia Pte. Ltd. for sponsor Fibrotest/Actitest test.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Kohli A, Shaffer A, Sherman A, Kottilil S. Treatment of hepatitis C: a systematic review. JAMA 2014;312: 631-40.
2. Lim SG, Aghemo A, Chen PJ, Dan YY, Gane E, Gani R, et al. Management of hepatitis C virus infection in the Asia-Pacific region: an update. Lancet Gastroenterol Hepatol 2017;2:52-62.
3. Wasitthankasem R, Posuwan N, Vichaiwattana P, Theamboonlers A, Klinfueng S, Vuthitanachot V, et al. Decreasing hepatitis C virus infection in Thailand in the past decade: evidence from the 2014 national survey. PLoS One 2016;11:e0149362.
4. Wedemeyer H. Hepatitis C. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease: Pathophysiology, diagnosis, management. 10th ed. Philadelphia: Elsevier Saunders; 2016. p. 1332-52.
5. Thai Association for the Study of the Liver (THASL). Assessment of the severity of fibrosis in chronic hepatitis C. In: Thailand practice guideline for management of chronic hepatitis C 2016. Bangkok: THASL; 2016. p. 7-9.
6. Thai Association for the Study of the Liver (THASL). Assessment of the severity of fibrosis in chronic hepatitis C. In: Thailand practice guideline for management of chronic hepatitis C 2018. Bangkok: THASL; 2018. p. 9-10.
7. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. Hepatology 2009;49:1017-44.
8. Leroy V, Sturm N, Faure P, Trocme C, Marlu A, Hilleret MN, et al. Prospective evaluation of FibroTest®, FibroMeter®, and HepaScore® for staging liver fibrosis in chronic hepatitis B: comparison with hepatitis C. J Hepatol 2014;61:28-34.
9. Zarski JP, Sturm N, Guechot J, Paris A, Zafrani ES, Asselah T, et al. Comparison of nine blood tests and transient elastography for liver fibrosis in chronic hepatitis C: the ANRS HCEP-23 study. J Hepatol 2012;56:55-62.
10. Boursier J, Brochard C, Bertrais S, Michalak S, Gallois Y, Fouchard-Hubert I, et al. Combination of blood tests for significant fibrosis and cirrhosis improves the assessment of liver-prognosis in chronic hepatitis C. Aliment Pharmacol Ther 2014;40:178-88.

ประสิทธิภาพของการตรวจ Fibrotest/Actitest สำหรับการประเมินระดับพังผืดในตับที่มีนัยสำคัญของผู้ป่วยไวรัสตับอักเสบซีเรื้อรังในไทย

กวิน ตั้งวรพงศ์ชัย, ธนิตา สุทธิชัยมงคล, จุไรรัตน์ กุหลาบแก้ว, ประกาศิต เสงี่ยมวิบูล, วัฒนา สุขีไพศาลเจริญ

ภูมิหลัง: การติดเชื้อไวรัสตับอักเสบชนิดซีเรื้อรังเป็นปัญหาสำคัญในประเทศไทย ในทางการรักษาจำเป็นต้องอาศัยการประเมินระดับพังผืดในตับที่มีนัยสำคัญเพื่อให้การรักษา ซึ่ง Fibrotest/Actitest เป็นการตรวจวินิจฉัยใหม่ที่สามารถทดแทนการเจาะชิ้นเนื้อตับได้ แต่ยังไม่มีการศึกษาข้อมูลในประเทศไทยมาก่อน

วัตถุประสงค์: เพื่อศึกษาประสิทธิภาพของการตรวจ Fibrotest/Actitest เมื่อเทียบกับการเจาะชิ้นเนื้อตับ โดยใช้การประเมินระดับพังผืดในตับที่มีนัยสำคัญตามคะแนน METAVIR ที่ ≥F2

วัสดุและวิธีการ: การศึกษาได้คัดเลือกผู้ป่วยไวรัสตับอักเสบชนิดซีเรื้อรัง 100 ราย ที่ต้องได้รับการเจาะชิ้นเนื้อตับ และตรวจ Fibrotest/Actitest ในวันเดียวกับการเจาะชิ้นเนื้อตับ โดยประเมินพยาธิสภาพตับตามคะแนน METAVIR มาเปรียบเทียบกับค่า Fibrotest/Actitest ที่ให้ผลเทียบเท่ากับระดับพังผืดในตับที่มีนัยสำคัญ METAVIR ที่ ≥F2

ผลการศึกษา: ค่า AUROC ของการตรวจ Fibrotest/Actitest คือ 0.74 (0.64 ถึง 0.83) โดยมีค่าจุดตัดอยู่ที่มากกว่าเท่ากับ 0.44 เพื่อใช้ในการประเมินระดับพังผืดในตับที่มีนัยสำคัญคะแนน METAVIR ที่ ≥F2 สำหรับผู้ป่วยไทยที่ติดเชื้อไวรัสตับอักเสบซีเรื้อรังซึ่งให้ความไว, ความจำเพาะ, ค่าพยากรณ์เชิงบวกและค่าพยากรณ์เชิงลบร้อยละ 75.4 (63.1 ถึง 85.2), 71.4 (53.7 ถึง 85.4), 83.1 (71.0 ถึง 91.6) และ 61 (44.5 ถึง 75.8) ตามลำดับ ซึ่งค่าจุดตัดนี้ทำให้การตรวจนี้มีความแม่นยำมากยิ่งขึ้นเมื่อเทียบกับค่าจุดตัดที่กำหนดสากล (≥0.49) และยังช่วยเพิ่มความไว ค่าพยากรณ์เชิงบวก และค่าพยากรณ์เชิงลบสำหรับผู้ป่วยไทยที่ติดเชื้อไวรัสตับอักเสบซีเรื้อรังได้

สรุป: Fibrotest/Actitest สามารถใช้ตรวจประเมินระดับพังผืดในตับที่มีนัยสำคัญ (F2) ของผู้ป่วยไวรัสตับอักเสบชนิดซีเรื้อรัง โดยมีจุดตัดที่ ≥0.44