

Combination of Bard Scoring System and Transient Elastography or Serum Hyaluronic Acid for Diagnosis of Severe Fibrosis in Patients with NAFLD

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ABSTRACT

Background: Liver fibrosis is the main prognostic factor of liver injury in non alcoholic fatty liver disease (NAFLD), and liver biopsy is the gold standard method to assess the severity of fibrosis. Currently, BARD scoring system is an easily tool for predicting liver fibrosis based on available clinical data. Additionally serum hyaluronic acid (HA) and transient elastography (TE) have also been shown as promising non-invasive tools in detecting severe fibrosis among patients with NAFLD.

Objective: To determine whether the combination of BARD score and serum HA or TE can reliably predict the severity of liver fibrosis in Thai NAFLD patients

Methods: A prospective study was conducted in 39 patients with histological proven NAFLD in King Chulalongkorn Memorial Hospital (KCMH), Thailand during July 2010 and February 2012. Demographic data, metabolic profile, BARD score were collected. Liver fibrosis was graded according to Brunt scoring system. Serum hyaluronic acid level was measured by ELISA. Transient elastography with FibroScan(tm) was performed.

Results: The 43.6% of our patients were female with the mean age at 45.2±14.0 yrs. Twenty patients (51.3%) had ≥ 3 components of metabolic syndrome. According to the histological result, 26 patients (66.7%) had severe liver fibrosis (F2-4). Multivariate logistic analysis showed that higher NAS score and older age were the significant predictors of severe liver fibrosis in NAFLD patients with OR 3.9 and 1.1, respectively ($p<0.05$). The prediction levels expressed by area under the ROC curve (AUC) of serum HA and TE for severe liver fibrosis in NAFLD were 0.81 and 0.83. For predicting severe liver fibrosis in patients with NAFLD, the cut-off value of 76 ng/mL of serum HA provided the sensitivity, specificity, PPV, NPV and accuracy were 91.3%, 60%, 84%, 66.7%, and 77.5%. The cut-off value of 6.5 kPa of TE found 87%, 50%, 87%, 50% and 79.3%, respectively. By using the combination of BARD score and serum HA or TE, at least 50% of our patients can be avoided from liver biopsy.

Conclusion: Combination of BARD score and serum hyaluronic acid or transient elastography is a promising tool with very high positive predictive value (84.6%) for predicting severe liver fibrosis in NAFLD patients.

Key words : Bard scoring system, transient elastography, serum hyaluronic acid, liver fibrosis, NAFLD

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide^(2,3). There is a variation in the disease spectrum, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) with significant fibrosis⁽⁴⁾. The patients with biopsy-proven NASH and especially those with fibrosis are at risk for progressive liver disease^(5,6), requiring close monitoring and follow-up. At present, liver biopsy remains the gold standard for diagnosing NASH and staging the extent of fibrosis⁽⁷⁾. However, there is some concern about the sampling variability, interobserver discordance and most importantly, the risk of the procedure⁽⁸⁾. All of this emphasize the clinical importance of developing non-invasive biomarkers for NASH and NASH-related fibrosis⁽⁹⁾. BARD scoring system⁽¹⁾ (BMI >28=1 point, AST/ALT ratio of >0.8=2 points, DM=1 point), Fibroscan^(tm), serum hyaluronic acid are the promising non invasive test for the liver fibrosis in NAFLD from the previous study⁽¹⁰⁻¹⁷⁾. However, all of them are still not widely used in Thailand. In this study, we sought to determine whether combination of BARD scoring system and transient elastography or serum hyaluronic is a reliable non-invasive marker for hepatic fibrosis (especially severe fibrosis) in patients with NAFLD.

PATIENTS AND METHODS

Patients

From July 2010 to February 2012, the consecutive thirty-nine patients with histologically confirmed NAFLD seen at King Chulalongkorn Memorial Hospital, Thailand were included in our study. The diagnosis of NAFLD was established based on the following criteria: (1) persistently abnormal liver enzymes for more than 6 months, (2) a radiological evidence of NAFLD, and (3) the exclusion of other liver diseases. The patients with clinical evidence of cirrhosis or the one who consumed more than 70 g and 140 g alcohol/week in woman and man, respectively were excluded from this study. For all patients, a complete medical history and physical examination were performed prior to liver biopsy. Laboratory evaluation included liver biochemistries, metabolic profile (fasting glucose, total and HDL-cholesterol, and triglycerides), serum hyaluronic acid at the date of liver biopsy. Demographic and anthropometric data included age, sex, body weight, height, and body mass index, and BARD score were calculated. The study was approved by the institutional review board.

Liver histology

Ultrasound guided liver biopsy was performed via

Table 1. Brunt grading system for NASH.

Grade	Steatosis	Ballooning	Inflammation
Mild (grade 1)	1-2 (up to 66%)	Minimal	L= 1-2; P=0-1
Moderate (grade 2)	2-3	Present	L= 1; P=1-2
Severe (grade 3)	2-3	Marked	L= 3; P=1-2

Table 2. Brunt staging system for NASH.

Stage	Zone 3 perisinusoidal fibrosis	Periportal fibrosis	Bridging fibrosis	Cirrhosis
1	Focal or extensive	0	0	0
2	As above	Focal or extensive	0	0
3	±	±	+	0
4	±	±	Extensive	0

Note: Steatosis: grade 1 ≤ 33%; grade 2 = 33-66%; grade 3 ≥ 66%

Ballooning: zone location noted

Lobular Inflammation (L): 0 = none; 1 = < 2 foci/20 x field; 2 = 2-4/20 x field; 3 = > 4/20 x field

Portal inflammation (P): 0 = none; 1 = mild; 2 = moderate; 3 = marked

the percutaneous route in 32 patients (82%). This was performed using 16G disposable biopsy needles (Hepafix, Braun, Germany) via the standard Menghini technique, and during surgery in seven patients (18%). All liver biopsy specimens were stained with the hematoxylin-eosin and Masson's trichrome and assessed by one experienced pathologist (N.K.). The mean length of the included liver biopsies was 2.1 ± 1.1 cm. Features of NAFLD were scored based on scoring system proposed by Brunt et al.⁽¹⁸⁾.

Hyaluronic acid

Hyaluronic acid (HA) was measured in serum samples that were obtained at the day of liver biopsy and stored at -70°C . Hyaluronic acid was measured by

ELISA method (S.M.Chemical Supplies Co.,Ltd, Thailand).

Transient elastography

FibroScan(tm) (Echosens, Paris, France) is a medical device based on transient elastography (TE), performed within 4 weeks prior to liver biopsy. The examination was performed on the right lobe of the liver through the intercostal space with the standard (M) probe. Ten successful acquisitions were performed on each patient. The success rate was automatically calculated by the machine as the ratio of the number of successful acquisitions over the total number of acquisitions. Only TE results obtained with ten valid measurements, with a success rate of at least 60% and an

Table 3. Characteristics of patients with nonalcoholic fatty liver disease, stratified by the severity of liver fibrosis.

Variables	Total cases (n=39)	No/mild fibrosis (F0-1) (n=13)	Severe fibrosis (F2-4) (n=26)	p-value
Gender (%female)	18 (46.2%)	3 (7.7%)	14 (35.9%)	0.05
Age (years)	45.5±14.3	36.7±14.1	49.7±11.8	0.01
BMI (kg/m ²)	33.6±1.2	42.1±15.3	29.4±5.6	0.02
BMI (kg/m ²) (Morbid obese pts. were excluded)	28.4±4.7	27.9±6.1	28.6±4.4	0.74
Subjects with metabolic syndrome, n(%)	20 (51.3%)	6 (15.4%)	14 (35.9%)	0.50
Subjects with morbid obesity, n(%)	7 (17.9%)	6 (15.4%)	1 (2.6%)	<0.01
Subjects with IFG/DM	28 (71.8%)	5/2 (17.9%)	12/9 (53.8%)	0.04
BARD score, n(%)				
0	2 (5.1%)	1 (2.6%)	1 (2.6%)	0.06
1	14 (35.9%)	7 (17.9%)	7 (17.9%)	
2	5 (12.8%)	2 (5.1%)	3 (7.7%)	
3	16 (41.0%)	3 (7.7%)	13 (33.3%)	
4	2 (5.1%)	0 (0%)	2 (5.1%)	
≥2	23 (58.9%)	5 (12.8%)	18 (46.2%)	
BARD score	2.1±1.1	1.6±0.9	2.4±1.1	0.05
AST (IU/L)	50.5±24.0	38.2±22.4	53.2±22.2	0.08
ALT (IU/L)	72.6±39.9	59.5±42.4	76.1±37.6	0.30
FPG (mg/dL)	120.4±41.6	103.6±20.6	132.6±46.7	0.02
Serum hyaluronic acid (ng/mL)	167.8±171.4	90.4±121.0	198.0±180.0	0.06
AST/ALT ratio	0.8±0.3	0.8±0.4	0.8±0.3	0.97
Fibroscan (kPa)	9.4±3.4	6.6±1.4	10.0±3.5	0.04
NAS Score	4.4±1.9	2.9±1.7	5.3±1.2	<0.01
FIB-4*	1.2±0.6	0.9±0.6	1.3±0.6	0.08
APRI*	0.9±0.5	0.7±0.5	1.0±0.5	0.09

FIB-4¹⁹ = age* AST (IU/l)/platelet count (*10⁹/litre)*√ALT(IU/L); APRI¹⁹ = AST (IU/L)/(UNL)/platelet count (*10⁹/litre)*100

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interquartile range (IQR) $\leq 30\%$ of the median, were considered reliable.

Statistical analysis

All statistical analyses were performed using SPSS software (version 16; SPSS Inc., Chicago, IL, USA). Clinical and laboratory characteristics of patients were expressed as the mean \pm SD. The Chi-square test was used to evaluate the association between categorical variables and liver fibrosis. Comparison between groups was analyzed by a two tail independent t-test. A *p*-value less than 0.05 was judged to be statistically significant. The diagnostic performance to detect liver fibrosis of TE and serum HA was assessed by receiver-operating-characteristic (ROC) curves. The sensitivity, specificity, positive and negative predictive values were calculated for the best cut-off value to differentiate the cases of NAFLD with severe fibrosis (F2-4) from those without severe fibrosis.

RESULTS

The clinical and laboratory data of 39 patients was

summarized in Table 3, stratified by the severity of liver fibrosis. There were 18 females (46.2%) and 21 males (53.8%) with mean age of 45.2 \pm 14.0 yrs. Seven patients (17.9%) were diagnosed as morbid obesity, i.e. a BMI ≤ 40 kg/m².

Multivariate logistic regression found that older age and higher NAS SCORE were significant predictors of severe liver fibrosis in NAFLD patient with odd ratios of 1.1 and 3.9, respectively.

Despite the significant higher BMI, the patients with morbid obesity were younger with lower NAS score and serum HA level. (*p*<0.01)

Diagnostic performance of serum HA and TE in predicting liver fibrosis in NAFLD

The prediction levels expressed by area under the ROC curve (AUC) of serum HA and TE for severe liver fibrosis (F2-4) in NAFLD were 0.81 and 0.83, respectively. For the presence of severe fibrosis, the cut-off value of 76 ng/mL of serum HA was associated with the maximum AUC with sensitivity of 91.3%, specificity of 60%, PPV and NPV of 84% and 66.7%, respectively. When using transient elastography, the

Table 4. Multivariate logistic analysis between mild fibrosis and severe fibrosis.

Variable	Odd Ratio	95%CI	<i>p</i> -value
Age (yrs)	1.1	1.01-1.21	0.02
NAS SCORE	3.6	1.5-9.2	<0.01

Table 5. Characteristics of patients with nonalcoholic fatty liver disease with and without morbid obesity.

Variables	Pts. without morbid obesity (n=32)	Pts. with morbid obesity (n=7)	<i>p</i> -value
Gender (% female)	17 (53.1%)	2 (28.6%)	0.37
Age (yrs.)	48.7 \pm 13.0	31.0 \pm 10.5	<0.01
BMI (kg/m ²)	29.4 \pm 5.4	55.7 \pm 7.5	<0.01
Subjects with severe fibrosis, n(%)	25 (78%)	1 (14.3%)	<0.01
BARD score	1.6 \pm 0.9	2.4 \pm 1.1	0.90
AST (IU/L)	54.2 \pm 23.4	24.9 \pm 6.0	<0.01
ALT (IU/L)	79.9 \pm 37.8	29.1 \pm 9.2	<0.01
AST/ALT ratio	0.7 \pm 0.3	1.0 \pm 0.5	0.12
Albumin (mg/dL)	4.5 \pm 0.4	4.2 \pm 0.2	0.02
FPG (mg/dL)	127.9 \pm 42.9	92.9 \pm 8.5	<0.01
Serum hyaluronic acid (ng/mL)	199.3 \pm 178.5	51.0 \pm 44.9	<0.01
NAS score	4.8 \pm 1.6	2.3 \pm 1.6	<0.01

Table 6. Histopathological results of liver biopsy specimens.

Variables	Total cases (n=39)	No/mild fibrosis (F0-1) (n=13)	Severe fibrosis (F2-4) (n=26)	p-value
Liver biopsy specimens length (cm.)	2.2±1.0 (1-5 cm)	1.6±0.6 (1-2.7 cm)	2.2±1.0 (1-5 cm)	
NAS Score	4.4±1.9	2.9±1.7	5.3±1.2	<0.01
Ballooning				
-minimal	23(58.9%)	11(28.2%)	12(30.8%)	
-present	15(38.5%)	2(5.1%)	13(33.3%)	
-marked	1(2.6%)	0	1(2.6%)	
Steatosis				
-Up to 33%	17(43.6%)	10(25.6%)	7(17.9%)	
-33 to 66%	7(17.9%)	1(2.6%)	6(15.4%)	
-more than 66%	15(38.5%)	2(5.1%)	13(33.3%)	
Inflammation				
-mild	2(5.1%)	2(5.1%)	0	
-moderate	26(65%)	11(28.2%)	15(38.5%)	
-severe	11(27.5%)	0	11(28.2%)	
Fibrosis				
F0	2 (5.1%)	2 (5.1%)	0	
F1	11 (27.5%)	11 (27.5%)	0	
F2	21 (53.8%)	0	21 (53.8%)	
F3	5 (12.8%)	0	5 (12.8%)	
F4	0 (0%)	0	0	

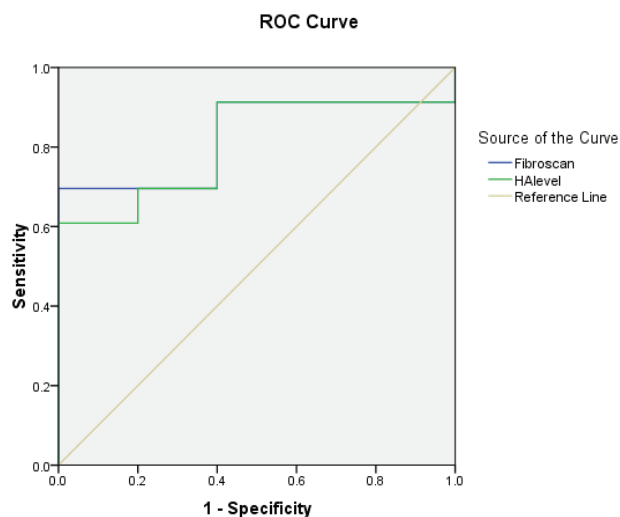


Figure 1. Receiver operating characteristics curves (ROCs) of serum hyaluronic acid and transient elastography in predicting severe liver fibrosis (F2-4).

Area Under the Curve

Test Result Variable(s)	Area
Fibroscan(tm)	0.83
HA level	0.81

cut-off value of 6.5 kPa had the maximum AUC with sensitivity of 87%, specificity of 50%, PPV and NPV of 87% and 50%, respectively as shown in Figure 1.

DISCUSSION

With regard to NAFLD, the determination of the severity of liver fibrosis is important in the evaluation of the prognosis of patients. Nowadays, the liver biopsy is the gold standard method to assess the liver fibrosis among the patient, however, it is quite invasive, expensive and occasionally presents with a sampling error resulting from the heterogenous distribution of liver fibrosis. Based on our study, transient elastography and serum hyaluronic acid are the very promising non-invasive tools with high positive predictive value for detection of severe liver fibrosis in Thai NAFLD patients comparable to the liver histology, especially when combined with BARD scoring system or using both of them altogether. We can omit the liver biopsy in 50% of our patients by using the combination of BARD score and serum hyaluronic acid instead and 60% in case of using the combination of

Table 7. Diagnostic performance of transient elastography for the diagnosis of severe liver fibrosis in NAFLD.

Stage	AUROC	Cutoff (kPa)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥F2	0.83 (0.68-0.99)	6.5	87.0	50.0	87.0	50.0
≥F3	0.72 (0.52-0.92)	8.2	70	80	66.7	35.7
		9.1	100	58.3	22.7	94.7
		9.4	80.0	62.5	22.7	94.7

Table 8. Diagnostic performance of serum hyaluronic acid for the diagnosis of severe liver fibrosis in NAFLD.

Stage	AUROC	Cutoff (ng/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
≥F2	0.82 (0.65-0.98)	76	91.3	60.0	84.0	66.7
≥F3	0.73 (0.41-1.04)	110	59.3	78.6	84.2	50
		200	66.7	80.0	36.4	93.3
		245	50	85.7	37.5	90.9

Table 9. Sensitivity, specificity, positive predictive value and negative predictive value of combination of BARD scoring system and serum hyaluronic acid or transient elastography for predicting severe liver fibrosis (F2-4) in NAFLD.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BARD score ≥2 and serum HA ≥76 ng/mL	96.2%	38.5%	84.6%	83.3%
BARD score ≥2 and Fibroscan(tm) ≥6.5kPa	92%	60%	83.3%	60%
Serum HA ≥76ng/mL and Fibroscan(tm) ≥6.5kPa	100%	20%	93.3%	ND
BARD score ≥2 and serum HA ≥76ng/mL and Fibroscan(tm) ≥6.5kPa	100%	ND	100%	ND

ND= no data

BARD score and transient elastography. Although there are some previous studies about the diagnostic performance of TE or serum HA in NAFLD patient⁽²⁰⁻²⁴⁾, our study is the first prospective one conducting in Thai NAFLD patients who may have some differences due to the ethnic variations of the disease. Moreover, our study use the ELISA kit, developed in our own country to measure the serum hyaluronic acid level make it more available and accessible to use in the real clinical practice. Compared to the previous study⁽²⁵⁾, the higher NAS score and older age are the only significant predictors for severe liver fibrosis in NAFLD with-

out the effect of BMI. This may result from the significant younger age in the group of morbid obese patient, and may be explained by the slow progression of liver fibrosis in NAFLD.

However, there are some limitations in our study. Firstly, there is too small number of cases with advanced liver fibrosis (F3-4) to conclude the accurate diagnostic performance of TE and serum HA in this group, so we need the more number of patients to validate the result. Second, at the time of the study we have only the standard (M probe) and usual software of Fibroscan(tm) in our institution, these results in the

inability to measure TE in some patients with high BMI or morbid obesity. Third, we found the discordance of the result of the non-invasive test and the liver histology in 5-6% of our patients. Although the liver biopsy is the gold standard method to detect the liver fibrosis in NAFLD, there is still a sampling error from a tiny tissue which is at most of 1/50,000 of the total mass of the liver, comparable to TE that measures the volume at least 100 times bigger than a biopsy sample⁽¹⁷⁾. However, not only the fibrosis but also the necroinflammatory activity of the liver that influence the liver stiffness measurement⁽²⁶⁾ make the falsely high value of TE in case of the elevation of transaminase enzyme.

In conclusion, the combination of BARD scoring system and serum hyaluronic acid or transient elastography is a promising tool with very high positive predictive value (84.6%) for predicting severe liver fibrosis (F2-4) in NAFLD patients. However, the further study in the patient with advanced liver fibrosis and the group who have discordant result of the non-invasive tools and the liver histology is required.

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