Original Article

Controlled Attenuation Parameter with Serum High Sensitivity-CRP Measurement for Differentiating Non-alcoholic Steatohepatitis from Simple Steatosis in Patients with Non-alcoholic Fatty Liver Disease

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a disorder which ranges over a spectrum extending from simple steatosis to non-alcoholic steatohepatitis (NASH). The latter could progress to liver cirrhosis. Liver biopsy is the gold standard to help differentiate between simple steatosis and NASH. Controlled attenuation parameter (CAP) is a recent non-invasive method for the detection and quantification of hepatic steatosis, while serum high sensitivity C-reactive protein (hs-CRP) is a reliable inflammatory marker.

Objective: To assess whether CAP and hs-CRP could differentiate between simple steatosis and NASH in patients with NAFLD.

Method: Patients with chronic hepatitis and suspected NAFLD were performed liver biopsy. All patients were measured CAP, serum hs-CRP, and basic laboratorys parameters. History and physical examination were obtained. Data were analyzed using SPSS statistic.

Results: Seventy-seven NAFLD patients were included for study, 28 with simple steatosis and 49 with NASH. Baseline characteristics and basic laboratory values were not different between the two groups. The optimal cut-off value of CAP and serum hs-CRP in differentiating simple steatosis from NASH, as assessed by ROC analysis, were 292 dB/m (sensitivity 87.8%, specificity 96.4%, and accuracy 90.9%) and 0.92 mg/L (sensitivity 100%, specificity 53.6%, and accuracy 83.1%), respectively. In multivariate analysis, CAP increased 1 dB/m (OR=1.05, 95% CI; 1.02-1.1 p=0.006) and serum hs-CRP increased 1 mg/L (OR=7.91, 95% CI; 1.62-38.6 p=0.011)

Conclusions: In NAFLD patients, CAP and serum hs-CRP appeared useful non-invasive tool for differentiating between simple steatosis and NASH.

Key words : NAFLD, NASH, CAP, hs-CRP .

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Introduction

Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of liver disorders encompassing benign steatosis, non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis[1]. NASH is recognized as an important cause of cryptogenic cirrhosis[2] and is associated with an increased risk of hepatocellular carcinoma, even in patients without cirrhosis[3]. The prevalence of NAFLD has increased rapidly over the years, paralleling the increase of the metabolic syndrome. NAFLD is now one of the most common causes of chronic liver disease worldwide[4].

NASH is defined by the presence lobular necroinflammatory activity, with or without the presence of perisinusoidal fibrosis in the liver biopsy. Differentiating between simple steatosis and NASH is important, because up to 28% of patients with NASH may progress to cirrhosis[5], with an inherent risk of ultimate liver failure and development of hepatocellular carcinoma. Simple steatosis, on the other hand, which often remains stable for a number of years probably does not progress in many patients[6,7].

Traditionally, the only reliable method for differentiating simple steatosis from NASH is liver biopsy, an invasive procedure with significant complications. Non-invasive biochemical assays as well as a recent novel technology called transient elastography have been shown to accurately distinguish between steatosis and NASH. Such novel tools would potentially reduce the need for liver biopsy while providing satisfactory prognostic information. Such non-invasive approaches for assessing the severity of fibrosis in NAFLD incorporate varying combinations of clinical features and routine laboratory investigations[8-11].

Recently, an elevated serum hs-CRP was reported as a strong predictor of future cardiovascular events and was also related to be metabolic syndrome and atherosclerosis[12-17]. An elevated serum hs-CRP was also reported as a diagnostic tool and a predictor of disease progression in patients with NAFLD.

A recent technology called transient elastography (TE) has been used to estimate liver stiffness. TE findings correlate well with liver biopsy histopathological fibrosis staging. This has opened up a non-invasive and accurate staging of fibrosis in NAFLD patients, which decreasing amplitude of the ultrasound wave as it propagates through the liver tissue using an ultrasound-based vibration-controlled transient elastography device[18]. Another novel technology called controlled attenuation parameter (CAP) has also been shown to correlate well with hepatic steatosis. Several publications have explored CAP for the estimation of hepatic steatosis in patients with chronic liver disease of various etiologies[19-25]. All but one study were conducted in limited numbers of NAFLD patients. Only one study included a homogeneous cohort of chronic hepatitis C patients[20]. Currently, there is no histological confirmation or grading to help distinguish between simple steatosis and steatohepatitis[26-30]. We therefore conducted a prospective study to evaluate the diagnostic performance of CAP in the estimation of hepatic steatosis specifically in NAFLD patients, and to study whether CAP together with serum hs-CRP and other metabolic parameters could differentiate and predict NASH in patients with NAFLD.

Patients and methods

Between December 2014 and December 2015, patients with chronic elevation of serum alanine aminotransferase (ALT) and/or aspatate aminotransferase (AST) more than 1.5 times the upper limit normal at least on two occasions in the preceding six months were recruited for study. All patients were negative for hepatitis B or C viral markers, with no evidence of other causes of chronic liver disease such as autoimmune liver disease, Wilson's disease and hemochromatosis. Additionally, there was neither history of herbal, hepatotoxic medication nor alcohol consumption of more than 20 g/week. Patients with clinical evidence of an active infection or inflammatory disorder at the time of serum analysis were excluded. All patients underwent abdominal ultrasonography and percutaneous liver biopsy. The study protocol was approved by the Hospital Ethical Committee. All participants gave an informed consent prior to the study.

Demographic data

The following data were recorded at the time of liver biopsy: age, sex, body mass index (BMI), history of diabetes mellitus, hypertension or hyperlipidemia. Diagnosis of the metabolic syndrome was assessed according to the modified Asia-Pacific guidelines of the NCEP III as follow:

(1) fasting plasma glucose ≥ 110 mg/dL,

(2) central obesity (waist circumference ≥ 90 cm. in men and ≥ 80 cm. in women),

(3) triglyceride level ≥ 150 mg/dL,

(4) blood pressure ≥ 130/85 mmHg or on treatment,

(5) HDL ≤ 40 mg/dL or ≤ 50 mg/dL in men and women, respectively.

Biochemical tests

After 10-hour overnight fasting, venous blood sample was drawn for liver biochemical test, glucose, total cholesterol, low-density cholesterol (LDL-C), high-density cholesterol (HDL-C), and triglyceride. Serum high sensitivity CRP (hs-CRP) was measured on the same day by nephelometry method using a standard curve and reported in unit of milligram per litre (mg/L).

Histological assessment

Liver biopsy specimens were fixed in 10% buffered formalin, and were examined by a pathologist who was unaware of the clinical and biochemical data. All specimens were stained with hematoxylin and eosin stain, and also Masson trichome stain. Histologic scoring followed the Brunt criteria[31]. Steatosis was graded as 0 to 3; ballooning degeneration was graded as mild, moderate and marked; and necroinflammation was graded as 0 to 3. Fibrosis was graded as 0 (absent) to 4 (cirrhosis), and significant fibrosis was defined as grade ≥ 2. Steatohepatitis was diagnosed from the presence of steatosis plus ballooning degeneration and/or necroinflammation.

Statistical analysis

The demographic and laboratory data were first compared between the simple steatosis and the NASH group (grade≥2), using Student's t-test for continuous variables and Fisher exact test for categorical variables. Continuous data were summarized as mean ± SD, and categorical variables as frequencies and percentages (%). Serum hs-CRP was compared between the two groups. In order to evaluate the discriminative ability of the independent variables with respect to diagnosis and to explore the appropriate cut-off, we performed a receiver operating characteristic (ROC) curve analysis. The cutoff value associated with the optimal combination of sensitivity and specificity was determined. Variables with p-value < 0.05 were selected to backward the likelihood ratio, and multivariate logistic re-

Table 1. Comparison of demographic and laboratory data between simple steatosis and NASH.

	Simple (n=28)	NASH (n=49)	*p*-value
Gender, Male	20 (71.4%)	22 (44.9%)	0.025*
Female	8 (28.6%)	27 (55.1%)	
Age, year	54.86±9.72	52.51±9.59	0.299
BMI, kg per m^2	29.12±4.52	29.85±5.65	0.556
WC, cm	103.29±10.45	106.55±12.07	0.235
Diabetes mellitus	12 (42.9%)	26 (53.1%)	0.389
Hypertension	14 (50%)	30 (61.2%)	0.388
Dyslipidemia	19 (67.9%)	34 (69.4%)	0.889
FBG, mmol/L	129±59.79	128.06±40.48	0.895
Hb_{A1c},%	6.86±1.72	6.86±1.37	0.988
Triglyceride, mmol/L	158.79±91.26	158.55±68.76	0.990
Cholesterol, mmol/L	184.14±35.78	185.61±38.75	0.870
HDL, mmol/L	50.18±15.78	48.82±11.49	0.664
LDL, mmol/L	122.11±32.47	127.45±37.53	0.531
ALP, IU/L	70.68±19.59	73.24±17.43	0.554
ALT, IU/L	50.96±33.63	62.39±26.71	0.105
AST, IU/L	39.32±18	48.02±20.16	0.062
hs-CRP, mg/L	1.01±0.77	4.26±1.75	<0.001*
CAP	244.32±45.24	335.82±40.92	<0.001*
Metabolic syndrome criteria ≥ 2	24 (85.71%)	49 (100%)	0.007*

Values presented as mean ± SD for continuous data and n (%) for categorical data. The p-value corresponds to t-test (continuous data) and Chi-Square test (categorical data).

gression analysis was used to determine factors associated with NASH. Data analysis was performed with STATA version 13.0. A p-value < 0.05 was taken as statistically significant.

RESULTS

During the study period, 77 patients with chronic elevation of serum aminotransferases and with negative results of other disease specific tests who were clinically suspected of having NAFLD were included. All underwent percutaneous liver biopsy. Of the 77 patients available for analysis, 28 had simple steatosis and 49 had NASH. The demographic and laboratory data of the two groups are shown in Table 1.

There were significant differences between the two groups regarding gender (p=0.025), metabolic syndrome ≥2 (p=0.007), CAP (p≤0.001) and serum hs-CPR (p≤0.001). The median of body mass index, waist circumference, fasting blood sugar, HbA1C, serum TG, HDL, LDL, AST and ALT were not significantly difference.

As for univariate analysis, four parameters were significantly associated with NASH, namely female sex, presence of metabolic syndrome criteria ≥2, CAP and serum hs-CRP. For multivariate logistic regression analysis, hs-CRP was independently associated with NASH with OR values of 1.05 (95% CI; 1.02-1.1, p=0.006) and 7.09 (95% CI; 1.62-38.61, p= 0.011), respectively (Table 2).

ROC analysis was applied using a model incorporating CAP ≥292 dB/m and serum hs-CRP ≥0.92 mg/L to differentiate simple steatosis and NASH. The values of AUC were 0.94 (95% CI; 0.885-0.995,

Table 2. Multivariate logistic regression analysis of predictive factors for NASH.

	OR	95%CI	*p*-value
Female	3.97	0.3-48.46	0.304
CAP	1.05	1.02-1.1	0.006*
hs-CRP	7.91	1.62-38.61	0.011*
Metabolic syndrome ≥2	1	0-1	0.999

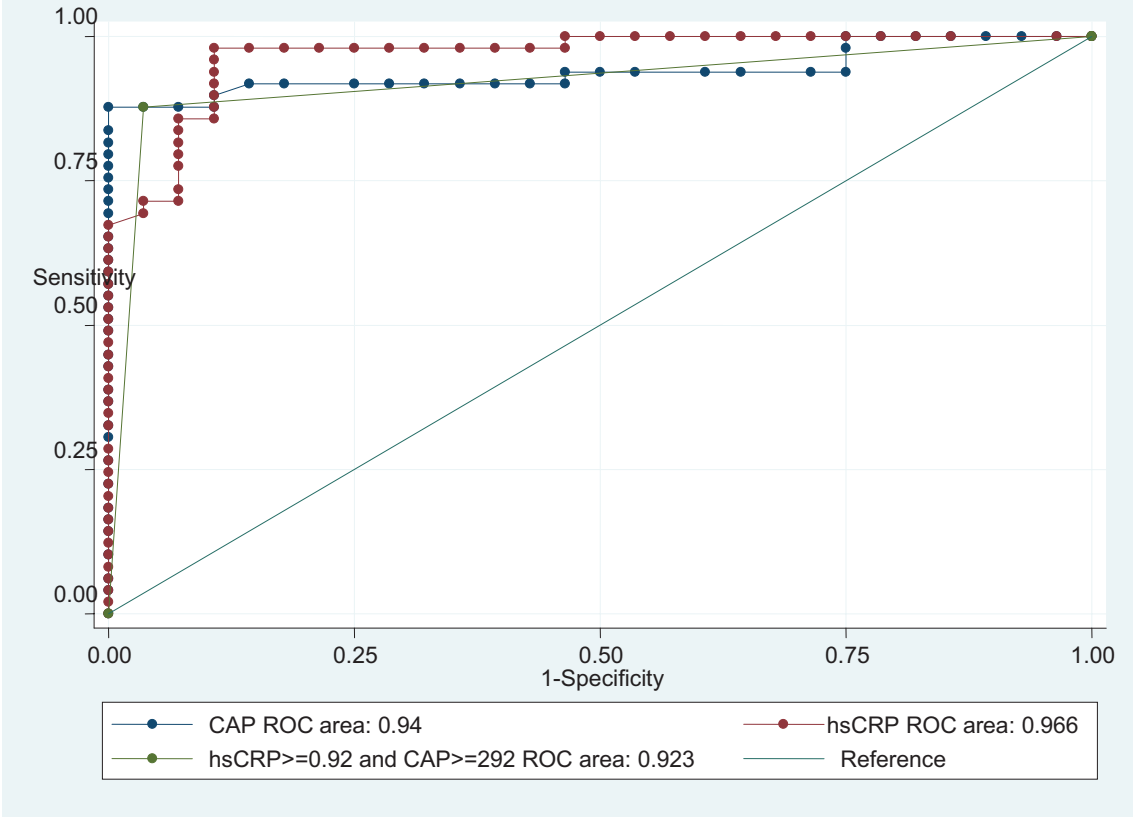

Figure 1. ROC analysis comparison between model that included CAP ≥292 and serum hs-CRP ≥0.92 with each or both parameters for differentiating NASH.

Table 3. Cut-off levels of CAP and serum hs-CRP for predicting NASH.

Variables	AUC	95%CI for AUC Lower-Upper	*p*-value
CAP	0.940	0.885-0.995	<0.001
hs-CRP	0.966	0.930-1.00	<0.001

Table 4. Diagnostic efficacy using CAP and serum hs-CRP to differentiate between simple steatosis and NASH.

Variable(s)	Cut off %	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %	LR+	LR-
CAP	292	87.8	96.4	97.7	81.8	90.9	24.57	0.13
hs-CRP	0.92	100	53.6	79.0	100	83.1	2.15	0.00

Table 5. Combined results of CAP and serum hs-CRP for differentiating NASH.

CAP ≥292 dB/m	hs-CRP ≥0.92 mg/L	Type of testing		NASH	Simple steatosis	Total
		Serial	Parallel			
CAP(+)	hs-CRP(-)	Neg	Pos	0	0	0
CAP(-)	hs-CRP(+)	Neg	Pos	6	12	18
CAP(+)	hs-CRP(+)	Pos	Pos	43	1	44
CAP(-)	hs-CRP(-)	Neg	Neg	0	15	15
Total (n)				**49**	**28**	**77**

Table 5.1 A 2x2 Table of result of serial testing for differentiate NASH.

	NASH n=49	Simple steatosis n=28
Positive test	43	1
Negative test	6	27

Sensitivity =43/49 = 87.76% Specificity =27/28 = 96.43%

p<0.001) and 0.966 (95% CI; 0.930-1.00, *p*<0.001). Comparing the efficacy using a model that included both parameters to differentiate NASH, it was found that AUC of the model that included CAP and serum hs-CRP was lower than compared with each parameter (AUC 0.923) (Figure 1 and Table 3).

Values presented as odds ratio (95% confident interval). *P*-value corresponds to logistic regression.

Using CAP and serum hs-CRP as a diagnostic tool for differentiating simple steatosis and NASH yielded the following results: CAP sensitivity 87.8%, specificity 96.4%, positive predictive value 97.7%, negative predictive value 81.8%, accuracy 90.9%; serum hs-CRP sensitivity 100%, specificity 53.6, positive predictive value 79.0%, negative predictive value 100% accuracy 83.1% (Table 4).

Lastly, using CAP≥292 dB/m combined with serum hs-CRP ≥0.92 mg/L by serial testing for diagnosing NASH yielded sensitivity 87.76% and specificity 96.43%. (Table 5 and Table 5.1).

DISCUSSION

Our study demonstrated that most NASH patients would be female, with the metabolic syndrome, greater CAP, and higher serum hs-CRP. The means of physical and laboratory characteristics were not significantly different between the two groups. The median of CAP and serum hs-CRP was statistically significantly higher in NASH compared with simple steatosis. ROC analysis was used to calculate the optimal cut-off value of

CAP and serum hs-CRP to differentiate NASH, CAP ≥292 dB/m and serum hs-CRP ≥0.92mg/L were statistical significantly with high sensitivity, specificity and accuracy (CAP ≥292; 87.8%, 96.4% and 90.9%, hs-CRP ≥0.92; 100%, 53.6% and 83.1%), respectively.

We then use each factor as a predictive model to differentiate between simple steatosis and NASH, ROC analysis of CAP and serum hs-CRP show that this model can be used as a good predictive tool which yields AUC of 0.94 and 0.966. Furthermore, when we use combine both factor are lower than use each factors AUC 0.923 but it high ROC area.

In the study, when we used CAP, serum hs-CRP or combined both, they are high efficacy to differentiate between simple steatosis and NASH. The histological of NASH was considered hepatic steatosis and inflammation, if we used combine both factors, it is good because CAP represented hepatic steatosis and serum hs-CRP represented systemic inflammation. Our study supports previous the studies that demonstrated relationship between CAP and grade of hepatic steatosis and serum hs-CRP and severity of liver histology in NAFLD patients.

We proposed an optimal cut-off level of CAP and serum hs-CRP for differentiating simple steatosis and NASH, easy to use for non-invasive method. Clinicians can use CAP or serum hs-CRP or combine both for screening and diagnosis; it's depended on available of each hospital.

In conclusion, our study showed that CAP and serum hs-CRP are non-invasive method for screening and diagnosis NASH in NAFLD patients. Suggested further studies with large population are needed to validation of this model.

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