Research Article

Relationship between Serum Cytokeratin-18, Control Attenuation Parameter, NAFLD Fibrosis Score, and Liver Steatosis in Nonalcoholic Fatty Liver Disease

Sumitro Kosasih,
Wong Zhi Qin,
Rafiz Abdul Rani,
Nazefah Abd Hamid,
Ngiu Chai Soon,
Shamsul Azhar Shah,
Yazmin Yaakob,
and Raja Affendi Raja Ali

Gastroenterology and Hepatology Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, 56000, Malaysia
Gastroenterology Unit, Faculty of Medicine, Universiti Teknologi MARA, Shah Alam, Selangor, 40450, Malaysia
Department of Medical and Health Science, Universiti Sains Islam Malaysia, Nilai, Negeri Sembilan, 71800, Malaysia
Department of Public Health, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, 56000, Malaysia
Department of Radiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, 56000, Malaysia

Correspondence should be addressed to Raja Affendi Raja Ali; draffendi@ppukm.ukm.edu.my

Received 27 May 2018; Accepted 29 August 2018; Published 27 September 2018

Academic Editor: Heather Francis

Copyright © 2018 Sumitro Kosasih et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Backgrounds. The aim of this study was to appraise the relationship between serum fragmented cytokeratin-18(CK-18), controlled attenuation parameter (CAP), and liver steatosis assessed by ultrasound (US) in nonalcoholic fatty liver disease (NAFLD) patients.

Methods. Patients who underwent abdominal US were recruited, followed with measurement of CAP using Fibroscan and serum fragmented CK-18 using enzyme-linked immunosorbent assay. The degree of liver steatosis assessed by US was categorized into mild (S1), moderate (S2), and severe (S3).

Results. A total of 109 patients were included in our study. CAP and fragmented CK-18 level were significantly correlated with liver steatosis grade with $r_s = 0.56$ and $0.68$, $p = 0.001$, respectively. NAFLD Fibrosis Score was poorly correlated with liver steatosis grade ($r_s = -0.096$, $p = 0.318$). Using fragmented CK-18 level, area under receiver operating characteristic (AUROC) curves for $S \geq 2$ and $S \geq 3$ were excellent (0.82 and 0.84, respectively). Using CAP, AUROC curves for detection of $S \geq 2$ and $S \geq 3$ were good (0.76, 0.77, respectively). We also proposed cut-off value of CAP to detect $S \geq 2$ and $S \geq 3$ to be 263 and 319dB/m, respectively, and fragmented CK-18 level to detect $S \geq 2$ and $S \geq 3$ (194 and 294 U/L, respectively).

Conclusions. Both the fragmented CK-18 level and the CAP, but not NAFLD Fibrosis Score, were well correlated with hepatic steatosis grade as assessed by US.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is the liver pandemic in this 21st century, affecting 20-45% population around the world [1–5]. NAFLD has been proven to cause liver fibrosis, liver cirrhosis, and hepatocellular carcinoma [6–9]. Not only does it have an adverse outcome to the liver itself but NAFLD also has been associated with increased rate of metabolic syndrome [10], cardiovascular diseases, and chronic kidney disease [11, 12]. Although NAFLD is usually benign, it may be associated with inflammation and hepatocyte apoptosis resulting in nonalcoholic steatohepatitis (NASH) of 20–30% of subjects. One-fifth of these NASH subjects will progress to develop liver cirrhosis [13].

Liver biopsy is still the gold standard to stage liver fibrosis as it provides a multitude of information on the inflammation activity. However, considering its invasive nature, sampling variability, and cost, other noninvasive modalities of imaging and biomarkers have been developed. The NAFLD fibrosis score (NFS) developed by Angulo et al. utilizes six variables (age, body mass index (BMI), diabetes, aspartate transaminase (AST), alanine transaminase (ALT), and albumin) which are commonly available in patient's assessment. It has been shown to reduce the need for biopsy in most NAFLD patients [14]. In NASH, liver cell apoptosis and necroinflammation play a major role. Serum caspase-cleaved fragmented cytokeratin-18(CK-18) reflects the degree of apoptosis and has been shown as an independent predictor in diagnosis of
NASH [15–18]. One meta-analysis in 2014 [19] showed area under receiver operating characteristic (AUROC), sensitivity, and specificity of fragmented CK-18 to be 0.84, 0.83, and 0.71, respectively.

For imaging, ultrasound (US) is the first method to be utilized as it is inexpensive, widely available and has a good sensitivity (70-94%) and specificity (70-97%) for liver steatosis [20, 21]. To enhance its sensitivity and specificity, hepatorenal index contrast has been used, resulting in 91% sensitivity and 84% specificity for liver steatosis.

Transient elastography (TE) has been used in several studies to predict steatosis grades in NAFLD patients by using control attenuated parameter (CAP), while stage of fibrosis is measured by liver stiffness measurement (LSM) [22–24]. There are different CAP cut-off values presented by different studies for distinct grades of liver steatosis defined by biopsy (ranging from S0, which indicates no steatosis, to S3, which indicates the highest level of steatosis); for S≥1(≥10% of hepatocytes with fat), the CAP cut-off values range from 214 to 289dB/m, with a 64%-91% sensitivity range and a 64%-94% specificity range; for S≥2(≥33% hepatocytes with fat), the CAP cut-off values range from 255 to 31dB/m, with a 57%-96% sensitivity range and a 62%-94% specificity range; finally, for S≥6 (66% hepatocytes with fat), the CAP cut-off values range from 281 to 310dB/m, with a 64%-100% sensitivity range and a 53%-92% specificity range [23]. A meta-analysis in 2014 [25] showed good pooled sensitivity and specificity for TE in diagnosing fibrosis (F) stage ≥3 (85% sensitivity, 85% specificity) and F4 (92% sensitivity, 92% specificity) and moderate accuracy to predict F≥2 in NAFLD.

The aims of this study are to evaluate the relationship between CAP, LSM, fragmented CK-18, and liver steatosis grade as assessed by US. We also would like to assess the diagnostic performance of CAP and fragmented CK-18 in liver steatosis. Lastly, we aim to compare the level and degree of association of various clinical and laboratory parameters in different liver steatosis grades. This is the first such study in South East Asia.

2. Materials and Methods

2.1. Patient Characteristics. The study was approved by Universiti Kebangsaan Malaysia (UKM) Ethics Committee and all patients gave written consent prior to participation. We recruited patients, aged more than 18 years old, who underwent ultrasound abdomen between June 2016 and September 2016. Patients with chronic liver disease, pregnancy, malignancy, and excessive alcohol use were excluded.

All recruited patients underwent US abdomen, clinical, laboratory examination, and Fibroscan® assessment.

2.2. Clinical Assessment. Comorbid illness (hypertension, diabetes, and dyslipidemia) and alcohol intake, together with anthropometric, laboratory, and past medical history, were obtained from all patients on the same day of ultrasound. Body mass index (BMI) was calculated as body weight in kilograms divided by body height in square meters (kg/m²). Waist circumference was measured in a standing position at a level of the umbilicus.

The diagnosis of metabolic syndrome [26–28] was made according to the joint statement of the International Diabetes Federation and World Heart Federation. Excessive alcohol use was defined by an average daily consumption of alcohol of <20g/day for men and <10g/day for women [29].

2.3. Ultrasonography. Ultrasound (US) of the abdomen was performed by single experienced consultant radiologist to omit interobserver bias. The degree of liver steatosis on ultrasound was categorized as mild (S1: increased liver echogenicity), moderate (S2: blurring of portal vein branches), or severe (S3: blurring of the diaphragmatic outline) [21, 30–32].

2.4. Laboratory Examination. Blood samples were obtained to measure AST, ALT, total cholesterol (TC), triglyceride(TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol(LDL-C), fasting blood sugar(FBS), and C-reactive protein(CRP). For measurement of fragmented CK-18, blood as initially processed to plasma and then stored frozen at -80 C. Plasma caspase-3 generated CK-18 fragments were quantitatively measured using the M30 Apoptosense ELISA kit (PEVIVA: Alexis. Grunwald, Germany).

The M30 Apoptosense® ELISA is a solid-phase sandwich enzyme immunoassay. Standards, controls, and samples react with a solid phase capture antibody M5 directed against K18 and the HRP-(horseradish peroxidase) conjugated M30 antibody directed against the K18Asp396 neoepitope. Unbound conjugate is removed by a washing step. TMB Substrate is added. The colour development is stopped and the absorbance is read. The resulting colour is directly proportional to the concentration of the analyte. By plotting a standard curve from known concentrations versus measured absorbance in the microplate reader, the amount of antigen in the sample can be calculated. The concentration of the antigen is expressed as Units per Litre (U/L).

2.5. NAFLD Fibrosis Score. This score was calculated based on the study by Angulo et al. [14] with formula of -1.675 + 0.037 x age(years) + 0.094 x BMI(kg/m²) +1.13 x diabetes/IFG(yes=1, no=0) + 0.99xAST/ALT ratio – 0.013 x platelet(x 10⁹/L) – 0.66 x albumin(g/dL). Score below -1.455 signified prediction of no advanced fibrosis while score more than 0.676 signified presence of advanced fibrosis, and the score in between is labeled as indeterminate.

2.6. Fibroscan. A Fibroscan 502, manufactured by Echosens (Paris, France), was used in the study. We considered results as reliable if interquartile range/median (IQR/M) is less than 30 percent and success rate is over 60 percent. Ten valid fibroscan readings were necessary for an examination to be deemed successful [33]. Controlled attenuation parameter (CAP) was measured to quantify liver steatosis, while the degree of liver fibrosis was displayed as liver stiffness measurement (LSM).

2.7. Statistical Analysis. Descriptive statistics were computed for all factors. These were presented in means (M) ± standard error of means (SEM) for normally distributed data,
3. Results

3.1. Patients’ Characteristic. We recruited 109 patients in this study, with 58 (53.2%) patients were male. Median age of patients in the study was 54, with range from 19 to 78 years of age.

Several comorbidities were found to be significantly higher in NAFLD patients compared to healthy subjects (Table 1), such as metabolic syndrome (n=56, 82% vs n=12, 29.2%, p<0.001), dyslipidemia (n=45, 79% vs n=23, 44.2%, and p<0.001), hypertension (n=35, 76% vs n=33, 52.4%, and p=0.009), and diabetes (n=40, 78% vs n=28, 48.2%, and p=0.001).

3.2. Relationship between Liver Biochemistry, CRP, Serum Fragmented CK-18, CAP LSM and Liver Steatosis Grade. All liver biochemistry (ALT and AST), as well as apoptotic markers (fragmented CK-18), was significantly higher in NAFLD group compared to healthy subjects (p<0.001) (Table 4). Fragmented CK-18 as shown in Figure 1 was significantly different across the healthy subjects, S1, S2, and S3 steatosis at 91(IQR 70-104), 189.5 (IQR 137-227.5), 277 (IQR 189.5-326), and 441(IQR 338-554.5) U/L, respectively.

CAP differed significantly between healthy subjects - 234 (IQR 95-266) dBm and NAFLD group (p<0.001) but was not significantly different in between the steatosis grades (S1-310(IQR 277-330), S2 – 331(IQR 279-364), and S3 - 334(IQR 323-374) dB/m).

3.3. NAFLD Fibrosis Score and Liver Steatosis. Only two (1.8%) of our patients were categorized as high risk for advanced fibrosis (F3-F4) according to NFS. The remaining were low risk (n=71, 65.1%) and indeterminate (n=36, 33%). We found that NFS was poorly correlated (rS=-0.096, p=0.318) with liver steatosis grade.

3.4. Diagnostic Performance of Fragmented CK-18 and CAP for Assessing Liver Steatosis. ROC analysis (Figure 2) was performed for all patients (n=109). By using a cut-off value of 194 U/L for diagnosis of liver steatosis S≥2, AUROC of fragmented CK-18 was 0.82 [95% Confidence Interval (CI), 0.74-0.91], while sensitivity and specificity were 70% and 82.6%, respectively. By using cut-off value of 294 U/L for liver steatosis S≥3, AUROC was 0.84 (95%CI, 0.69-0.99), while sensitivity and specificity were 75%, and 87.6%, respectively.

CAP has demonstrated satisfactory diagnostic performance in detecting liver steatosis. AUROC for liver steatosisS≥2 by using cut-off value of 263 dB/m was 0.76(95%CI, 0.65-0.88), with sensitivity of 86.7% and specificity of 47.5%, while AUROC for liver steatosisS≥3 (with a cut-off value of 319 dB/m) was 0.77(95%CI, 0.65-0.88), with sensitivity of 90.9% and specificity of 59.3%.

3.5. Factors Associated with CAP. Using univariate linear regression analysis, BMI (β=5.5, p=0.001), FBS (β=13.07, p=0.02), HDL-C (β=-76.35, p=0.004), waist circumference (β=-5.8, p=0.001), fragmented CK-18 (β=0.17, p=0.001), LSM(β=10.1, p=0.001), and steatosis grade (β=36.2, p=0.001) were associated with CAP in all our patients. Among all these factors, only LSM, TG, and steatosis grade were shown to be independent factors related to CAP in multivariate linear regression analysis (Table 5). With every increment of LSM of 1kPa and 1 mmol/L of TG, CAP score would increase by 79dB/m and 21.2dB/m, respectively, while CAP scores would
Normal Grade I fatty liver Grade II fatty liver Grade III fatty liver

US Result

Fragment CK-18

Figure 1: The distribution of CAP, LSM, and CK-18 according to steatosis grade assessed by US. Figure 1 showed significant difference in Fragmented CK-18, LSM, and CAP between nonliver steatosis patients and grades I, II, and III liver steatosis patients.

increase by 31.9 dB/m with every increment in liver steatosis grade.

4. Discussion

Liver biopsy is considered the gold standard for assessing the degree of hepatic steatosis and fibrosis; however, biopsy is rarely done due to its risk and limitation. Liver biopsy has several limitations such as small area of examination (only representing 1/50000 of whole liver), sampling variabilities and error, inter- and intraobserver variability [34–36]. Therefore, steatosis and fibrosis are now being more commonly assessed by using noninvasive modalities like imaging and biomarkers. The short examination time and noninvasiveness make abdominal ultrasonography the best initial screening method for NAFLD. CAP and LSM in fibroscan is a recent, novel way to diagnose NAFLD as well as quantifying hepatic steatosis and fibrosis accurately and in a convenient manner. Fragmented CK-18 is a biomarker that currently under investigation to diagnose NASH and assess the degree of fibrosis [15].

In our study, we found that patient with hypertension, dyslipidemia, diabetes, and metabolic syndrome had higher proportion of suffering from NAFLD, regardless of age and sex. We also found that higher BMI, waist circumference (Table 2), and liver biochemistry (ALT, AST ≥ 35 U/L) (Table 3) are associated with increased severity of liver steatosis, as assessed by US. These findings are consistent with previous studies [37, 38]. Chia et al. showed that there was a significant difference in ALT and AST values between mild and significant fatty liver, although they used broader definition of fatty liver population, which included non-NAFLD patients as well.

In a study by Angulo et al. [14], NFS was shown to have a high positive predictive value (90%) of diagnosing advanced fibrosis. However, in our study, we concluded that ultrasonography is not a good tool to differentiate degree of fibrosis, since many of our patients were categorized into low and indeterminate groups, although US showed steatosis grade ≥ 2. This is consistent with previous studies that suggested it is difficult to differentiate steatosis and fibrosis, as
Figure 2: ROC Curve using CAP and Fragmented CK-18, to predict liver steatosis in US. Figure 2 showed CAP value with cutoff value of 263 dB/m and 319 dB/m had good sensitivity and specificity. FragCK-18 with cut-off value of 194 U/L and 345 U/L had a good sensitivity to predict moderate-severe steatosis.

Table 2: Relationship between anthropometric data with liver steatosis as assessed by US. BMI: Body Mass Index; M: means; SEM: standard error or means; \(r_s\): Spearman coefficient correlation; *: significant.

|                        | Non Liver steatosis (M±SEM) | Liver Steatosis Grade | \(p\) value | \(r_s\) |
|------------------------|-----------------------------|-----------------------|-------------|---------|
| BMI, kg/m\(^2\)        | 24.65±0.88                  | 29.96±0.90            | 29.99±1.02  | <0.001  | 0.46*   |
| Waist Circumference, inch | 33.40±0.78                  | 37.86±0.86            | 37.95±0.72  | 0.001   | 0.40*   |
respectively, and these were comparable with previous studies conducted. We demonstrated that optimal cut-off values for detecting S ≥ 2 and S ≥ 3 steatosis by using Youden index were 263 dB/m and 319 dB/m, respectively, and these were comparable with previous studies done in Canada and France [40, 41], with cut-off value of 250 dB/m and 317 dB/m for S ≥ 2 and S ≥ 3 reported, respectively. However, our cut-off values differed from those in other studies [42–44]. These differences might be explained by different study populations as some studies include not only NAFLD but also chronic viral hepatitis patients.

In our study, predictive value of fragmented CK-18 to detect liver steatosis grade as assessed by US was comparable to studies conducted previously. However, majority of the studies conducted were to detect steatohepatitis (NASH) rather than simple NAFLD on liver biopsy [18, 45, 46].

Our study also showed that liver steatosis grade detected by ultrasound, LSM, and TG was independently associated with CAP. However, study by Ahn et al. showed that only US liver steatosis grade independently affected the CAP score [39]. This could be explained by their different study populations which included alcoholic liver disease patients. None of the necroinflammatory markers such as ALT, AST, CRP, and fragmented CK-18 were independently associated with CAP. All these findings were quite consistent with other previous studies. [39, 41].

The strength of this study was its ability to show the relationship of CAP (a convenient but uncommonly used tool), with US (the most commonly available tool) for assessing NAFLD patients. In addition, we compared this relationship with fragmented CK-18, a new biomarker, in the NAFLD patients.

However, our study had several limitations. First, steatosis grade assessed by US has a limitation due to its subjective assessment,
**Table 5: Factors Influencing CAP (controlled attenuation parameter) in fibroscan. Using Univariate and Multivariate Linear Regression Analysis.**

| Variable          | β     | Uni p value | Multi β | Multi p value |
|-------------------|-------|-------------|---------|---------------|
| Age               | 0.42  | 0.53        | -0.505  | 0.442         |
| BMI               | 5.5   | 0.001       | -0.811  | 0.834         |
| FBS               | 13.07 | 0.02        | 7.54    | 0.064         |
| HDL               | -76.35| 0.004       | -26.3   | 0.337         |
| TG                | 16.6  | 0.112       | 21.2    | 0.04*         |
| TC                | -7.3  | 0.339       | -9.4    | 0.215         |
| WaistCircum       | 5.8   | 0.001       | 1.9     | 0.612         |
| ALT               | 0.47  | 0.07        | -0.17   | 0.588         |
| AST               | 0.92  | 0.04        | -0.69   | 0.362         |
| Albumin           | -0.69 | 0.81        | -0.06   | 0.98          |
| CRP               | 24.9  | 0.228       | -13.4   | 0.533         |
| Platelet          | 0.09  | 0.58        | -0.044  | 0.791         |
| CK18              | 0.172 | 0.001       | -0.052  | 0.394         |
| LSM               | 10.1  | 0.001       | 7.9     | 0.002*        |
| USResult          | 36.2  | 0.001       | 31.9    | 0.003*        |

interpretation. Secondly, single operator US may cause a bias in the result. Thirdly, we did not have a comparison against liver biopsy, regarded as gold standard as a reference for our US findings.

**5. Conclusion**

In conclusion, our study showed fragmented CK-18 and CAP were relatively well correlated with steatosis grade as assessed by US. NAFLD fibrosis score, however, did not show any correlation with US. We proposed the cut-off values for fragmented CK-18 and CAP in moderate and severe liver steatosis; fragmented CK-18 for S ≥ 2 and S ≥ 3 were 194U/L and 345U/L, respectively; CAP for S ≥ 2 and S ≥ 3 were 263dB/m and 319dB/m, respectively. However, larger scale studies are needed to confirm the optimal cut-off values.

**Appendix**

See Tables 1–5 and Figures 1 and 2.

**Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

**Conflicts of Interest**

The authors reported no potential conflicts of interest to disclose.

**Authors’ Contributions**

S. Kosasih researched the database, collected samples, and wrote the manuscript. N. A. Hamid, Y. Yaakob and S. Azhar contributed to laboratory, radiology, and statistics work, respectively. R. Abdul Rani, Z. Q. Wong, C. S. Ngui, and R. A. Raja Ali contributed in reviewing and editing the manuscript.

**Acknowledgments**

The manuscript is supported byUniversiti Kebangsaan Malaysia, no. FF-2014-212.

**References**

[1] Z. Li, J. Xue, P. Chen, L. Chen, S. Yan, and L. Liu, “Prevalence of nonalcoholic fatty liver disease in mainland of China: a meta-analysis of published studies,” *Journal of Gastroenterology and Hepatology*, vol. 29, no. 1, pp. 42–51, 2014.

[2] E. Magoso, M. A. Ansari, Y. Gopalan et al., “Prevalence of non-alcoholic fatty liver in a hypercholesterolemic population of northwestern peninsular Malaysia,” *Southeast Asian Journal of Tropical Medicine and Public Health*, vol. 41, no. 4, pp. 936–942, 2010.

[3] W.-K. Chan, A. T.-B. Tan, S. R. Vethakkan, P.-C. Tah, A. Vijayananthan, and K.-L. Goh, “Non-alcoholic fatty liver disease in diabetics - prevalence and predictive factors in a multiracial hospital clinic population in Malaysia,” *Journal of Gastroenterology and Hepatology*, vol. 28, no. 8, pp. 1375–1383, 2013.

[4] C.-H. Chen, M.-H. Huang, J.-C. Yang et al., “Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of Taiwan: metabolic significance of nonalcoholic fatty liver
disease in nonobese adults,” Journal of Clinical Gastroenterology, vol. 40, no. 8, pp. 745–752, 2006.

[5] E. M. Koehler, J. N. L. Schouten, B. E. Hansen et al., “Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study,” Journal of Hepatology, vol. 57, no. 6, pp. 1305–1311, 2012.

[6] S. Petta and A. Craxì, “Hepatocellular carcinoma and non-alcoholic fatty liver disease: From a clinical to a molecular association,” Current Pharmaceutical Design, vol. 16, no. 6, pp. 741–752, 2010.

[7] G. Baffy, E. M. Brunt, and S. H. Caldwell, “Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace,” Journal of Hepatology, vol. 51, no. 2, pp. 371–379, 2009.

[8] N. Bhula, P. Angulo, D. van der Poorten et al., “The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study,” Hepatology, vol. 54, no. 4, pp. 1208–1216, 2011.

[9] C. K. Argo, P. G. Northup, A. M. S. Al-Oasimi, and S. H. Caldwell, “Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis,” Journal of Hepatology, vol. 51, no. 2, pp. 371–379, 2009.

[10] V. T. Samuel, Z.-X. Liu, X. Qu et al., “Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease,” The Journal of Biological Chemistry, vol. 279, no. 31, pp. 32345–32353, 2004.

[11] G. Musso, R. Gambino, and J. H. Tabibian, “Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis,” PLoS Medicine, vol. 11, no. 7, Article ID e0010680, 2014.

[12] M. R. Ajmal, M. Yaccha, M. A. Malik et al., “Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients of cardiovascular diseases and its association with hs-CRP and TNF-α,” Indian Heart Journal, vol. 66, no. 6, pp. 574–579, 2014.

[13] P. Angulo, M. V. Machado, and A. M. Diehl, “Fibrosis in nonalcoholic fatty liver disease: Mechanisms and clinical implications,” Seminars in Liver Disease, vol. 35, no. 2, pp. 132–145, 2015.

[14] P. Angulo, J. M. Hui, G. Marchesini et al., “The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD,” Hepatology, vol. 45, no. 4, pp. 846–854, 2007.

[15] N. Alkhouri, C. Carter-Kent, and A. E. Feldstein, “Apoptosis in nonalcoholic fatty liver disease: diagnostic and therapeutic implications,” Expert Review of Gastroenterology & Hepatology, vol. 5, no. 2, pp. 201–212, 2011.

[16] M. Tsutsumi, N. Tanaka, M. Kawakubo et al., “Serum fragmented cytokeratin 18 levels reflect the histologic activity score of non-alcoholic fatty liver disease more accurately than serum alanine aminotransferase levels,” Journal of Clinical Gastroenterology, vol. 44, no. 6, pp. 440–447, 2010.

[17] M. V. Machado and H. Cortez-Pinto, “Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal,” Journal of Hepatology, vol. 58, no. 5, pp. 1007–1019, 2013.

[18] A. E. Feldstein, A. Wieckowska, A. R. Lopez, Y.-C. Liu, N. N. Zein, and A. J. McCullough, “Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study,” Hepatology, vol. 50, no. 4, pp. 1072–1078, 2009.

[19] J. Chen, Y. Y. Zhu, Q. Zheng, and J. J. Jiang, “Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: a meta-analysis,” Hepatology Research, vol. 44, no. 8, pp. 854–862, 2014.

[20] M. Graif, M. Yanuka, M. Baraz et al., “Quantitative estimation of attenuation in ultrasound video images: Correlation with histology in diffuse liver disease,” Investigative Radiology, vol. 35, no. 5, pp. 319–324, 2000.

[21] S. H. Saverymuttu, A. E. A. Joseph, and J. D. Maxwell, “Ultrasound scanning in the detection of hepatic fibrosis and steatosis,” British Medical Journal, vol. 292, no. 6512, pp. 13–15, 1986.

[22] N. H. Afıdlal, “Fibroscan (transient elastography) for the measurement of liver fibrosis,” Journal of Gastroenterology and Hepatology, vol. 8, no. 9, pp. 605–607, 2012.

[23] I. Mikolasevic, L. Orlic, N. Franjic, G. Hauser, D. Stimac, and S. Milic, “‘Transit elastography (FibroScan(R)) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease - Where do we stand?’ World journal of gastroenterology : WJG, vol. 22, no. 32, pp. 7236–7251, 2016.

[24] V. W.-S. Wong, J. Vergniol, G. L.-H. Wong et al., “Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease,” Hepatology, vol. 51, no. 2, pp. 454–462, 2010.

[25] R. Kwok, Y.-K. Tse, G. L.-H. Wong et al., “Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease-the role of transient elastography and plasma cytokeratin-18 fragments,” Alimentary Pharmacology & Therapeutics, vol. 39, no. 3, pp. 254–269, 2014.

[26] B. Balkau, T. Drivsholm, K. Borch-Johnsen et al., “Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome,” Diabetes & Metabolism, vol. 28, no. 5, pp. 364–376, 2002.

[27] R. J. Molinario, “Metabolic syndrome: an up prevalence, criteria, and laboratory testing,” MLO: Medical Laboratory Observer, vol. 39, no. 11, pp. 24–27, 2007.

[28] C. M. Alexander, P. B. Landsman, S. M. Teutsch, and S. M. Haffner, “NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older,” Diabetes, vol. 52, no. 5, pp. 1210–1214, 2003.

[29] T. Gunji, N. Matsuhashi, H. Sato et al., “Alcohol consumption is inversely correlated with insulin resistance, independent of metabolic syndrome factors and fatty liver diseases,” Journal of Clinical Gastroenterology, vol. 45, no. 9, pp. 808–813, 2011.

[30] N. Khov, A. Sharma, and T. R. Riley, “Bedside ultrasound in the diagnosis of nonalcoholic fatty liver disease,” World Journal of Gastroenterology, vol. 20, no. 22, pp. 6821–6825, 2014.

[31] S. Carvalhalina, J. Leitão, A. C. Alves, M. Bourbon, and H. Cortez-Pinto, “How good is controlled attenuation parameter and fatty liver index for assessing liver steatosis in general population: Correlation with ultrasound,” Official Journal of the International Association for the Study of the Liver, vol. 34, no. 6, pp. e11–e17, 2014.

[32] M. Lupşor-Platon, H. Stefănescu, D. Mureşcan et al., “Noninvasive assessment of liver steatosis using ultrasound methods,” Medical Ultrasonography, vol. 16, no. 3, pp. 236–245, 2014.

[33] European Association for Study of Liver and Asociacion Latinoamericana para el Estudio del Higado, “EASL-ALEH Clinical Practice Guidelines: non-invasive tests for evaluation of liver disease severity and prognosis,” Journal of Hepatology, vol. 63, no. 1, pp. 237–264, 2015.

[34] S. Gawrieh, D. M. Knoedler, K. Saelian, J. R. Wallace, and R. A. Komorowski, “Effects of interventions on intra- and
interobserver agreement on interpretation of nonalcoholic fatty liver disease histology,” *Annals of Diagnostic Pathology*, vol. 15, no. 1, pp. 19–24, 2011.

[35] Y. Sumida, A. Nakajima, and Y. Itoh, “Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis,” *World Journal of Gastroenterology*, vol. 20, no. 2, pp. 475–485, 2014.

[36] R. Vuppalanchi, A. Ünalp, M. L. Van Natta et al., “Effects of Liver Biopsy Sample Length and Number of Readings on Sampling Variability in Nonalcoholic Fatty Liver Disease,” *Clinical Gastroenterology and Hepatology*, vol. 7, no. 4, pp. 481–486, 2009.

[37] C. Wang, T. Tseng, T. Hsieh et al., “Severity of fatty liver on ultrasound correlates with metabolic and cardiovascular risk,” *Kaohsiung Journal of Medical Sciences*, vol. 28, no. 3, pp. 151–160, 2012.

[38] M. Hamaguchi, T. Kojima, Y. Itoh et al., “The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation,” *American Journal of Gastroenterology*, vol. 102, no. 12, pp. 2708–2715, 2007.

[39] J. M. Ahn, Y.-H. Paik, S. Y. Min et al., “Relationship between controlled attenuation parameter and hepatic steatosis as assessed by ultrasound in alcoholic or nonalcoholic fatty liver disease,” *Gut and Liver*, vol. 10, no. 2, pp. 295–302, 2016.

[40] R. P. Myers, A. Pollett, R. Kirsch et al., “Controlled Attenuation Parameter (CAP): A noninvasive method for the detection of hepatic steatosis based on transient elastography,” *Liver International*, vol. 32, no. 6, pp. 902–910, 2012.

[41] V. de L´edinghen, J. Vergniol, J. Foucher, W. Merrouche, and B. le Bail, “Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography,” *Liver International*, vol. 32, no. 6, pp. 911–918, 2012.

[42] J. K. Kim, K. S. Lee, J. R. Choi et al., “Usefulness of the controlled attenuation parameter for detecting liver steatosis in health checkup examinees,” *Gut and Liver*, vol. 9, no. 3, pp. 405–410, 2015.

[43] F. Shen, R. D. Zheng, and Y. Q. Mi, “Controlled attenuation parameter for non-invasive assessment of hepatic steatosis in Chinese patients,” *World Journal of Gastroenterology*, vol. 20, no. 16, pp. 4702–4711, 2014.

[44] K. S. Jung, B. K. Kim, S. U. Kim, Y. E. Chon, and K. H. Cheon, “Factors affecting the accuracy of controlled attenuation parameter (CAP) in assessing hepatic steatosis in patients with chronic liver disease,” *PLoS ONE*, vol. 9, no. 6, Article ID e98689, 2014.

[45] D. Joka, K. Wahl, S. Moeller et al., “Prospective biopsy-controlled evaluation of cell death biomarkers for prediction of liver fibrosis and nonalcoholic steatohepatitis,” *Hepatology*, vol. 55, no. 2, pp. 455–464, 2012.

[46] J. Shen, H. L. Chan, G. L. Wong, A. W. Chan, P. C. Choi, H. Y. Chan et al., “Assessment of non-alcoholic fatty liver disease using serum total cell death and apoptosis markers,” *Alimentary Pharmacology & Therapeutics*, vol. 36, no. 11-12, pp. 1057–1066, 2012.