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Clinical spectrum of non-alcoholic fatty liver disease in diabetic and non-diabetic patients☆

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ABSTRACT

Background: While non-alcoholic fatty liver disease (NAFLD) has been well characterised in patients with diabetes mellitus (DM), less is known about NAFLD in non-DM patients. We investigated the clinical characteristics of NAFLD patients with and without DM and accuracy of the NAFLD fibrosis score (NFS) in these two NAFLD groups.
Methods: Clinical, biochemical and histological variables were evaluated in this prospective cross-sectional study of 503 patients with biopsy proven NAFLD. Comparisons between patients with and without DM were analysed. NFS was correlated with liver histology to assess its robustness in patients with and without DM.
Results: There were 503 biopsy proven NAFLD patients with 48% of the cohort being diabetic. Relative to patients without DM, patients with DM were older (52 vs. 46 years, $p < 0.001$), with higher proportion of females (70% vs. 54%, $p < 0.001$), higher BMI (37 vs. 35, $p = 0.009$), higher prevalence of hypertension (73% vs. 44%, $p < 0.001$), higher prevalence of NASH (80.2% vs. 64.4%; $p < 0.001$) and advanced fibrosis (40.3% vs. 17.0%; $p < 0.001$). A considerable amount of patients without DM still had NASH (64%) and advanced fibrosis (17%). The clinical utility of the NFS differed between NAFLD patients with and without DM, with sensitivity to exclude advanced fibrosis being 90% of NAFLD patients with DM but only 58% of patients without DM.
Conclusion: Patients with DM have more severe NAFLD based on histology. However, NASH and advanced fibrosis also occur in a considerable proportion of NAFLD patients without DM. The lower utility of the NFS in NAFLD patients without DM emphasises the heterogeneous nature of the NAFLD phenotype.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome [1], represents a spectrum of histopathologic abnormalities ranging from simple steatosis to the more aggressive non-alcoholic steatohepatitis (NASH), characterised by steatosis, parenchymal inflammation, hepatocellular ballooning and other evidence of hepatic injury [2]. Patients with NASH are at risk of developing progressive fibrosis; reported in up to 50% of cases over 6 years [3]. There is increasing recognition that NAFLD is a heterogeneous disease with multiple pathways of pathogenesis and patients with different phenotypes of NAFLD can present with diverse disease manifestations [4]. Insulin resistance plays a dominant role in the pathogenesis of NAFLD [5]. Patients with type 2 diabetes mellitus (DM) have an increased risk of developing NAFLD, NASH and hepatic fibrosis/cirrhosis [6–9]. Furthermore, NAFLD patients with DM have three times the mortality compared to non-

Abbreviations: ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ACE-I, angiotensin-converting enzyme-inhibitor; ARB, angiotensin receptor blocker; apoB-100, apolipoprotein B-100; BMI, body mass index; CIs, confidence intervals; Chol, total cholesterol; DM, type 2 diabetes mellitus; ER, endoplasmic reticulum; FFAs, free-fatty acids; HDL, high density lipoprotein cholesterol; HOMA-IR, Homeostatic model assessment—insulin resistance; INR, international normalised ratio; LDL, low density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; NASH CRN, Non-alcoholic Steatohepatitis Clinical Research Network; NAS, NAFLD activity score; ORs, odd ratios; SDs, standard deviations; TGs, triglycerides; VLDL, very-low-density lipoproteins

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diabetic NAFLD patients [10]. The importance of DM in NAFLD is reflected by its inclusion in the majority of the non-invasive composite predictive scores for NASH and advanced fibrosis [11–14]. One such composite predictive score for predicting advanced fibrosis in NAFLD is the NAFLD fibrosis score (NFS), which has been validated and recommended for use in the American society guidelines [2,14]. Reiterating disease heterogeneity and that NAFLD may not conform to a "one size fits all approach", McPherson and colleagues had reported a difference in the reliability of NFS in the context of normal and abnormal ALT levels [15]. Other non-invasive fibrosis scores such as the BARD score and AST/ALT ratio have also been used to predict advanced fibrosis in NAFLD. We sought to characterise the clinical spectrum of NAFLD in patients with and without DM. In addition, we explored the utility of NFS and other established non-invasive fibrosis scores among these two groups.

2. Materials and methods

2.1. Study design and population

This is a prospective cross sectional study with patients enrolled from two hepatology outpatient clinics in Cleveland, Ohio (Cleveland Clinic and MetroHealth Medical Center). Study received approval from the institutional review board.

The study included patients 18 years of age and over, with histologically proven NAFLD, who had not received any prior therapies that may have been beneficial for NAFLD, such as Vitamin E, pentoxifylline, pioglitazone and prescribed diet & exercise weight loss programmes. Patients with excessive alcohol consumption (>21 drinks per week and >14 drinks per week for males and females respectively) were excluded. Similarly, patients with other contributory causes of liver disease including those with hepatotoxic drug history, viral hepatitis, hemochromatosis, autoimmune hepatitis, Wilson's disease or alpha 1 antitrypsin disease were excluded.

2.2. Ascertainment of clinical data

Demographic and clinical information was obtained by two of the authors (SD or AM) for all patients from an electronic medical record system that is common to both hospitals. The diagnosis of DM was diagnosed based on American Diabetes Association (ADA) criteria with or without the use of antidiabetic medications [16]. Hypertension was diagnosed by the Joint National Committee (JNC) 7 criteria [17]. All diagnoses were verified based on documentation in the electronic medical records by one of the investigators (SD or AM). Body mass index (BMI) were collated, as were liver function tests [serum albumin, bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT)], platelet count, international normalised ratio (INR), total cholesterol (Chol), triglycerides (TGs), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and HbA1C. To minimise inter-observer variability, liver biopsy specimens were read using standardised well defined histological criteria [18] established by the Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN). As recommended, the diagnosis of NASH was based on the overall impression of histopathologists and not the NAFLD activity score [NAS] [19]. Fibrosis was classified into 4 stages with advanced fibrosis defined as stage 3–4 fibrosis (bridging fibrosis–cirrhosis). Only clinical variables obtained within 6 months of the liver biopsy were included in the analysis. The use of statins and angiotensin-converting enzyme-inhibitor (ACE-I)/angiotensin receptor blocker (ARB) within 6 months prior to the liver biopsy was also examined. Insulin resistance was assessed with Homeostatic model assessment—insulin resistance (HOMA-IR) based on the formula: [fasting glucose (mg/dL) × insulin (μU/mL)] divided by 405 [20]. NFS was calculated according to the published formula; NFS: $-1.675 + 0.037 \times$ age (years) $+ 0.094 \times$ body mass index (BMI; kg/m^2) $+ 1.13 \times$ impaired fasting glycaemia or DM (yes = 1, no = 0) $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelet ($\times 10^9$/L) $- 0.66 \times$ albumin (g/dL) [14]. The cut-off points of the NFS used to categorise fibrosis were: <−1.455, −1.455 to 0.676, and >0.676 for low indeterminate and high probability for advanced fibrosis, respectively [14]. Calculation of the BARD score was also performed; the BARD score was a 4 point score derived from the weighted sum of three variables (BMI > 28 = one point, AST/ALT ratio > 0.8 = two points, diabetes = one point) where a score of two or more suggestive of advanced fibrosis [13]. Similarly, AST/ALT ratio more than 0.8 itself has been suggested to be useful in predicting advanced fibrosis [21].

2.3. Statistical analysis

Descriptive statistics was computed for all variables and reported as means and standard deviations (SDs) for continuous variables or frequencies and percentages for categorical variables. Baseline characteristics and differences in demographic, clinical, histological and laboratory indices between patients with and without DM were ascertained, using Student's T tests and Pearson's chi-square testing for continuous and categorical variables, respectively. Mann–Whitney U test was also performed when applicable. The utility of NFS was correlated with histological staging of fibrosis in both DM and non-DM patients. With regard to advanced fibrosis, Spearman's correlation analysis for each individual component of the NFS was performed in both patients with and without DM. In addition, the utility of using fibrosis scores such as the BARD score and AST/ALT ratio was also assessed in both patients with and without DM. All analyses were performed using SPSS version 21 statistical software (Chicago, Illinois, USA). Two sided p values were used. p values < 0.05 were considered statistically significant.

3. Results

Of the 503 NAFLD patients in the data set, 62% were female, 58% had concomitant hypertension, 48% had concomitant DM and the mean BMI was 36.1 kg/m^2. NASH and advanced fibrosis were present in 71.8% and 28.1% of the cohort respectively.

Table 1 illustrates the characteristics of the whole cohort and also patients with and without DM. Compared to NAFLD patients without DM, patients with DM were older (52.5 vs. 46.0 years; $p < 0.001$), were more likely to be female (70.7% vs. 54.0%; $p < 0.001$) and have a higher BMI (37.2 vs. 35.2 kg/m^2; $p = 0.009$). Diabetic patients were also more likely to be hypertensive (73.2 vs. 43.9%; $p < 0.001$) with greater use of statins (42.0 vs. 15.2%; $p < 0.001$) and ACE-I/ARBs (51.9 vs. 28.8%; $p < 0.001$). However, there was no difference in the prevalence of advanced fibrosis among the DM patients taking or not taking statins ($p = 0.182$) and also among the DM patients taking or not taking ACE-I/ARB ($p = 0.357$). There were differences in ALT, albumin, platelet count, TG, LDL and HbA1C between the two groups. There was no difference in aggregated length of liver tissue examined between patients with and without DM (19.9 mm vs. 18.2 mm; $p = 0.225$). As shown in Table 2, patients with diabetes had more lobular inflammation ($p = 0.017$), ballooning ($p < 0.001$) and NASH ($p < 0.001$). The median NAS was higher in DM patients compared to non-DM patients ($p = 0.022$). More of the DM patients had grade 2 ballooning (41.7% vs. 24.0%; $p < 0.001$) and higher prevalence of NASH (80.2% vs. 64.4%; $p < 0.001$) compared to non-DM patients, while there was no differences in steatosis between patients with and without DM. DM patients also had a higher prevalence of advanced fibrosis (40.3% vs. 17.0%; $p < 0.001$) [Table 1]. When cirrhosis was considered specifically, significantly more of the DM patients had cirrhosis relative to the non-DM patients (20.6% vs. 5.7%) [Table 2]. Among the DM patients, the duration of DM did not differ between those with and without advanced fibrosis (5.79 vs. 5.42 years, $p = 0.791$). Similarly, HbA1C levels were comparable between diabetic patients with and without advanced fibrosis (7.5 vs. 7.3, $p = 0.414$). Among the patients without DM, there was no

Table 1
Characteristics of patients with NAFLD by the presence of DM status.

Characteristics	Total (n = 503)	Presence of DM		p value*
		Yes (n = 239)	No (n = 264)	
Age (years)	49 ± 12	52 ± 10	46 ± 12	<0.001
Proportion female (%)	311 (62.0)	169 (70.7)	142 (54.0)	<0.001
BMI (kg/m^2)	36.13 ± 8.43	37.16 ± 7.84	35.20 ± 8.84	0.009
Presence of HTN (%)	291 (57.9)	175 (73.2)	116 (43.9)	<0.001
Bilirubin (mg/dL)	0.67 ± 0.39	0.68 ± 0.42	0.66 ± 0.37	0.648
AST (U/L)	56.15 ± 63.65	53.52 ± 36.03	58.52 ± 80.85	0.388
ALT (U/L)	71.70 ± 55.60	64.18 ± 49.21	78.49 ± 60.09	0.004
AST/ALT ratio	0.85 ± 0.37	0.92 ± 0.35	0.79 ± 0.37	<0.001
Albumin (g/dL)	4.25 ± 0.49	4.19 ± 0.53	4.30 ± 0.45	0.017
INR	1.03 ± 0.17	1.04 ± 0.18	1.02 ± 0.16	0.262
Platelet count (k/uL)	238.38 ± 77.68	230.85 ± 78.72	245.11 ± 76.27	0.045
Chol (mg/dL)	197.74 ± 50.87	192.99 ± 53.57	201.88 ± 48.13	0.068
TG (mg/dL)	206.7 ± 181.20	231.8 ± 223.50	184.7 ± 130.20	0.007
HDL (mg/dL)	42.86 ± 10.85	41.94 ± 10.72	43.69 ± 10.93	0.096
LDL (mg/dL)	122.11 ± 42.86	114.6 ± 44.77	128.75 ± 40.04	0.001
HbA1C (%)	6.58 ± 1.47	7.40 ± 1.51	5.62 ± 0.56	<0.001
Ferritin (ng/mL)	231.3 ± 230.90	209.1 ± 233.90	250.9 ± 227.00	0.076
Presence of NASH (%)	361 (71.8)	190 (80.2)	168 (64.4)	<0.001
Presence of advanced fibrosis (%)	141 (28.1)	96 (40.3)	45 (17.0)	<0.001
Length of specimen (mm)	19.0 ± 10.8	19.9 ± 13.3	18.2 ± 8.0	0.225
Use of statin (%)	140 (27.9)	100 (42.0)	40 (15.2)	<0.001
Use of ACE-I/ARBs (%)	200 (39.8)	124 (51.9)	76 (28.8)	<0.001

Data are expressed as mean ± standard deviation or as number and percentage. BMI: body mass index, HTN: hypertension, AST: aspartate aminotransferase, ALT: alanine aminotransferase, INR: international normalised ratio, Chol: total cholesterol, TG: triglyceride, HDL: high density lipoprotein cholesterol, LDL: low density lipoprotein cholesterol, ACE-I: angiotensin-converting enzyme-inhibitor, ARB: angiotensin receptor blocker, DM: diabetes mellitus.
* p values were derived using Student's T tests and Pearson's chi-square tests on continuous and categorical variables respectively.

relationship between insulin resistance and presence of advanced fibrosis (p = 0.732) [Table 3]. Table 4 represents the correlation of NFS to the stage of fibrosis. In the whole cohort of patients, there were 334 patients without advanced fibrosis on histology, of which 26 patients (7.8%) were wrongly classified by the NFS. Hence, the NFS was able to correctly identify 92% of the patients as being without advanced fibrosis. Of the 129 patients with advanced fibrosis, 26 patients (20.2%) were wrongly classified by the NFS. Analysing the utility of the NFS in subgroups stratified by DM, 14.4% (19/132) of DM patients without advanced fibrosis as opposed to 3.5% (7/202) of non-DM patients without advanced fibrosis were wrongly predicted as the high risk NFS category. Of the DM patients with advanced fibrosis 10.1% (9/89) were inaccurately predicted as the low risk NFS category, while 42.5% (17/40) of the non-DM patients with advanced fibrosis were inaccurately predicted as the low risk NFS category. Spearman's correlation analysis of the individual components of NFS to advanced fibrosis in both the patients with and without DM was performed [Table 5]. This showed that in relation to advanced fibrosis, the correlation coefficients of the individual components used to calculate NFS were correspondingly lower in the non-DM patients compared to the DM patients. Other fibrosis scores developed for NAFLD, namely the BARD score and AST to ALT ratio were also tested in our cohort for their utility in patients with and without DM [Table 6]. Similar to our findings with the NFS, there were discrepancies in the utility of the BARD score and AST to ALT ratio between patients with and without DM.

Table 2
Comparison of liver histology between DM and non-DM patients.

Histology feature	Presence of DM		p value*
	Yes (n = 238)	No (n = 263)	
Grade of steatosis			0.500
0	5 (2.1%)	6 (2.3%)	
1	85 (36.0%)	81 (30.9%)	
2	79 (33.5%)	104 (39.7%)	
3	67 (28.4%)	71 (27.1%)	
Lobular inflammation			0.017
0	8 (3.4%)	28 (10.7%)	
1	104 (44.1%)	113 (43.1%)	
2	111 (47.0%)	109 (41.6%)	
3	13 (5.5%)	12 (4.6%)	
Ballooning			<0.001
0	39 (16.6%)	70 (26.7%)	
1	98 (41.7%)	129 (49.2%)	
2	98 (41.7%)	63 (24.0%)	
NAFLD activity score			0.200
NAS < 5	108 (46.0%)	136 (51.7%)	
NAS ≥ 5	127 (54.0%)	127 (48.3%)	
Median NAS (interquartile range)	5 (3)	4 (3)	0.022
Stage of fibrosis			<0.001
0	40 (16.8%)	98 (37.3%)	
1	55 (23.1%)	79 (30.0%)	
2	47 (19.7%)	41 (15.6%)	
3	47 (19.7%)	30 (11.4%)	
4	49 (20.6%)	15 (5.7%)	

DM: diabetes mellitus, NAS: NAFLD activity score, NASH: non-alcoholic steatohepatitis.
* p values were derived using Pearson's chi-square tests and Mann–Whitney U test.

4. Discussion

The present study shows that the clinical spectrum of NAFLD is different among patients with and without DM. More aggressive NAFLD as suggested by the higher prevalence of NASH and advanced fibrosis was seen in DM patients compared to non-DM patients. Other factors contributing to more severe NAFLD in the DM patients include older age, higher BMI and increased prevalence of hypertension. This is compatible with previous reports observing that patients with DM have higher risk of NAFLD, higher rates of NASH and advanced fibrosis relative to patients without DM [10,22,23]. In addition, DM is associated with accelerated progression of hepatic fibrosis [24], which is consistent with the higher prevalence of cirrhosis among the DM patients relative to the non-DM patients in our study. NAFLD patients with DM have twice the mortality risk compared to the general population with DM and no NAFLD [25]. Similarly, NAFLD patients with DM have three times the overall mortality risk and twenty two times the liver related mortality risk compared to NAFLD patients without DM [10]. In part, this mortality risk relationship is contributed by DM patients having a higher prevalence of NASH and advanced fibrosis. Studies have observed a higher overall mortality in patients with NASH relative to the general population, in particular attributed to cardiovascular and liver related mortality [26,27]. Comparing patients with and without NASH, NASH was associated with higher rates of cirrhosis and liver-related mortality [28]. This reiterates the importance of DM in the context of NAFLD.

Liver biopsy remains the gold standard in diagnosing and predicting the severity of NAFLD, with several histological features that can predict clinically relevant outcomes in NAFLD [29]. With regard to liver histology, our study found that inflammation and ballooning, integral features required for the diagnosis of NASH, were more frequently seen among NAFLD patients with DM, relative to patients without DM. Hepatocyte injury in NAFLD is often noted in the form of hepatocellular ballooning, and is often considered the most important histological feature for distinguishing NASH [30,31]. Ballooning is associated with more

Table 3
Insulin resistance in relation to advanced fibrosis in patients without DM.

HOMA-IR	Advanced fibrosis		
	Yes	No	
Tertile 1	7 (36.8%)	28 (43.8%)	p = 0.732
Tertile 2	8 (42.1%)	27 (42.2%)	
Tertile 3	4 (21.1%)	9 (14.1%)	

HOMA-IR: Homeostatic model assessment—insulin resistance.

Table 4
Correlation of NFS and histology in patients with DM and without DM.

NFS	DM				Non-DM			
	Low risk	Indeterminate	High risk	Total	Low risk	Indeterminate	High risk	Total
Stages 0–2	25	88	19	132	141	54	7	202
Stages 3–4	9	36	44	89	17	17	6	40
Total	34	124	63		158	71	13	
Sensitivity	89.9%		49.4%		57.5%		15%	
Specificity	18.9%		85.6%		69.8%		96.5%	
PPV	42.8%		69.8%		27.4%		46.2%	
NPV	73.5%		71.5%		89.2%		85.1%	

NFS: NAFLD fibrosis score, DM: diabetes mellitus, PPV: positive predictive value, NPV: negative predictive value.

aggressive NAFLD and higher rates of cirrhosis [28]. Similarly, necroinflammation at index biopsy was reported to be the only predictor of fibrosis progression in an analysis of ten histo-pathological series with follow-up liver biopsies [32].

The lower serum albumin and platelet count in combination with a higher AST to ALT ratio seen with the DM patients reflect the higher prevalence of advanced fibrosis as they are recognised markers of hepatic synthetic function, portal hypertension and surrogates of advanced fibrosis respectively [21,33].

Although DM has been proven to be an established risk factor for NASH and advanced fibrosis, the potential for severe disease in patients without DM should not be overlooked. Several epidemiological studies have reported prevalence rates of DM in NAFLD cohorts between 7 and 41% [1,8,14,34]. Consequently, although prevalence rates of NASH and advanced fibrosis may be lower among non-diabetics, taking into account the larger number of patients in the non-diabetic group, the absolute number of patients with significantly severe NAFLD may not be significantly less than in diabetics. Indeed, in our study, a significant proportion of NAFLD patients without DM has NASH (64%) and advanced fibrosis (17%). This reiterates the need for cautious management of NAFLD in the non-DM patient without trivialising the potential for disease severity in this subgroup of patients. Our study did not find a significant association between severity of insulin resistance and advanced fibrosis among the patients without DM, which may suggest that factors other than insulin resistance play a more prominent role in fibrogenesis among non-diabetics. This is consistent with the evolving recognition that multiple factors (not just insulin resistance) are involved in the pathogenesis of NAFLD [35,36].

With regard to non-invasive tools used in NAFLD, the NFS has been widely used as a reliable tool to predict advanced fibrosis in NAFLD [14,37–39]. In our study, the NFS performed well in identifying patients with and without advanced fibrosis in the entire group. However subgroup analysis suggested that the utility of the NFS had divergent clinical reliability for NAFLD patients with and without DM, particularly in excluding advanced fibrosis in patients without DM although this must be tampered in the context of a relatively lower prevalence of advanced fibrosis among the non-DM patients. Larger studies are warranted to clarify the utility of the NFS in non-DM patients with NAFLD. Nevertheless, this may illustrate the heterogeneous nature of the NAFLD phenotype and that the NFS may have to be adjusted in different subsets of NAFLD patients. Similar discrepant findings using the BARD score and AST/ALT ratios for predicting advanced fibrosis between patients with and without DM support the individualised approach to this heterogeneous disease. A recent study observed differences in sensitivity and specificity of NFS between NAFLD patients with and without normal ALT levels; sensitivity of NFS (at lower cut-off) was good in patients with normal ALT at 82% but specificity was low at 51% [15]. Yoneda et al. had similar findings and suggested resetting the cut-off values for NFS to achieve higher sensitivity and specificities in NAFLD patients with normal ALT levels [40].

Table 5
Correlation of NFS components with advanced fibrosis in patients with and without DM.

	Advanced fibrosis	Age	BMI	Platelet	Albumin	AST/ALT
DM	Rho	0.21	−0.72	−0.33	−0.18	0.36
	p value	0.001	0.269	<0.001	0.007	<0.001
Non-DM	Rho	0.20	0.05	−0.19	−0.14	0.31
	p value	0.001	0.459	0.003	0.027	<0.001

NFS: NAFLD fibrosis score, DM: diabetes mellitus, rho: correlation coefficient, AST: aspartate aminotransferase, ALT: alanine aminotransferase.

4.1. Strength and limitations

To our knowledge, this is the first study to explore the clinical utility of the commonly used NFS in NAFLD patients with and without DM. The strength of this study include the large cohort of patients with histologically proven NAFLD in addition to the wide range of available demographic, anthropometric, clinical, histological and laboratory data. However, there are a number of potential limitations. The cross-sectional nature of our study allows the evaluation of associations only. Furthermore, the use of electronic medical records to define the presence or absence of DM and hypertension may have led to some patients being misclassified. With regard to the NFS, the relatively small number of patients with advanced fibrosis in the non-diabetic group may impact on the test performance of the NFS, in particular the positive predictive value. Nevertheless, this study highlights important clinical concepts which are relevant to the everyday practical management of NAFLD.

5. Conclusion

While NAFLD patients with DM have higher prevalence of NASH and advanced fibrosis, the potential risk of severe NAFLD cannot be overlooked in patients without DM since both NASH and advanced fibrosis occur in a considerable proportion of these patients. The utility of the NFS and other fibrosis scores may have disparate clinical reliability for NAFLD patients with and without DM, which emphasises the heterogeneous nature of the NAFLD phenotype and that the NFS may have to be adjusted in different subsets of NAFLD patients. This warrants further studies to explore this relationship in greater detail.

Table 6
Correlation of BARD score and AST/ALT ratio to histology in patients with and without DM.

DM				Non-DM			
BARD	<2	≥2	Total	BARD	<2	≥2	Total
Stages 0–2	5	132	137	Stages 0–2	145	65	210
Stages 3–4	0	92	92	Stages 3–4	16	26	42
Total	5	224	229	Total	161	91	252
Sensitivity: 100%, specificity: 3.6%				Sensitivity: 61.9%, specificity: 69.0%			
PPV: 41.1%, NPV: 100%				PPV: 28.6%, NPV: 90.0%			
AST:ALT	<0.8	≥0.8	Total	AST:ALT	<0.8	≥0.8	Total
Stages 0–2	78	59	137	Stages 0–2	146	65	211
Stages 3–4	21	71	92	Stages 3–4	16	26	42
Total	99	130	229	Total	162	91	253
Sensitivity: 77.2%, specificity: 56.9%				Sensitivity: 61.9%, specificity: 69.2%			
PPV: 54.6%, NPV: 78.8%				PPV: 28.6%, NPV: 90.1%			

Conflict of interest

All authors declare no conflicts of interest.

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