Annals of Clinical and Analytical Medicine
Original Research

The relationship between biopsy findings of liver fibrosis and non-alcoholic steatohepatitis and hematological scales

NASH and LF biopsy results and hematological scales

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Abstract

Aim: Liver biopsy is the gold standard in the diagnosis of Liver Fibrosis (LF) and Nonalcoholic Steatohepatitis (NASH). Liver biopsy is the gold standard in the diagnosis of Liver Fibrosis (LF) and Nonalcoholic Steatohepatitis (NASH). Its invasiveness limits its use. However, the reliability of non-invasive markers is still controversial. In this study, according to the results of liver biopsy in patients with LF and NASH; Fibrosis-4 Index (FIB-4), Aspartate Aminotransferase Platelet Ratio Index (APRI), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), mean platelet volume (MPV), and Mean Platelet Volume/Platelet Ratio (MPR) we aimed to reveal the relationship.

Material and Methods: 1350 liver biopsies were reviewed retrospectively. 197 patients (88 men and 109 women) who met the criteria were included in the study. All pathology results were re-evaluated with Fibrosis score (FS) and Nonalcoholic Fatty Liver Disease Activity Score (NAS). Efforts were made to exclude possible disturbing factors that may affect hematological parameters. NLR, PLR, MPV, MPR, APRI and FIB-4 values were calculated and the relationship between NAS score and FS was examined.

Results: APRI was significant according to the results with and without NASH (p=0.002). In patients with LF; MPR, MPV, APRI, FIB-4 values were statistically significant and correlated compared to those without fibrosis. (p<0.001, p=0.029, p=0.001, p<0.001, respectively). Surprisingly, there was no statistically significant relationship between PLR and NLR, neither NASH nor LF (p<0.18, p=0.60 respectively).

Discussion: This study is one of the few studies with biopsy results. For the reliability of these parameters, extensive studies are needed in this area.

Keywords

Non-Alcoholic Steatohepatitis, Liver Fibrosis, Liver Biopsy, Mean Platelet Volume To Platelet Ratio, Hematological Scales

DOI: 10.4328/ACAM.21966 Received: 2023-09-11 Accepted: 2024-03-12 Published Online: 2024-08-26 Printed: 2024-10-01 Ann Clin Anal Med 2024;15(10):672-676
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This study was approved by the Ethics Committee of Karadeniz Technical University (Date: 2021-04-15, No: 2021/98)

Introduction

NASH is an inflammatory and progressive liver disease characterized by the accumulation of fat in the liver, usually observed in individuals who consume little or no alcohol. NASH progresses to the stages of advanced fibrosis (F3) or cirrhosis (F4) in patients, depending on time and etiology [1, 2]. LF is an independent risk factor for death due to liver disease, leading to hepatocellular carcinoma and mortality [3].

Liver biopsy is considered the gold standard for diagnosis of LF and NASH. This method is both risky and costly. This has made the search for less invasive and more cost-effective alternatives in hepatology popular [4].

The hematological scales Fibrosis-4 Index (FIB-4) and Aspartate Aminotransferase Platelet Ratio Index (APRI) are used to assess the degree of LF [5]. In addition, blood parameters such as neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), mean platelet volume (MPV) have been proposed as indirect indicators of inflammation and fibrosis processes [6]. Mean Platelet Volume/Platelet Ratio (MPR) has been studied less in liver diseases compared to other parameters, but it has been suggested that it may be related to LF [7, 8].

In LF and NASH, many studies have been conducted on PLR, NLR, MPV, MPR FIB-4 score ANDAPRI. However, it is still a matter of debate whether these markers can be a reliable indicator. Some studies advocate for their consideration as markers, while others suggest that the evidence is insufficient [9].

In this study, we aimed to Decipher the relationship between NLR, PLR, MPV, FIB-4 score, APRI and MPR in patients with and without NASH and LF according to the biopsy results.

Material and Methods

This study was conducted using liver biopsy samples collected from two different pathology centers. The pathology results were retrospectively reviewed. Biopsies of patients suspected of having NASH and LF were examined. 1350 patients who underwent liver biopsy between 2010 and 2020 were screened. Among them, 197 patients meeting the criteria were included in the study. In this study, accompanying hepatitis and clinical findings were not considered. All samples were re-evaluated using the NAS. The NAS score was classified as 0-2: hidden, 3-4: suspicious, and 5-8: steatohepatitis.

Fibrosis scores were classified as: Stage 1: Zone 3 fibrosis, perisinusoidal fibrosis, portal/periportal fibrosis, Stage 2: perisinusoidal and portal/periportal fibrosis, Stage 3: bridging fibrosis, Stage 4: cirrhosis [10].

Based on the dates of the liver biopsies, the patients' liver function test results, complete blood count values, used pharmacological agents, comorbidities, and demographic characteristics were retrospectively collected from the hospital data system.

Biochemical measurements were made enzymatically using original reagents on the Beckman Coulter AU5800 automatic analyzer. CBC parameters were analyzed on the Sysmex XN-9000 automatic analyzer. In this study, inflammation and platelet activation markers obtained from patients' routine blood tests were examined. NLR and PLR were calculated by dividing the absolute neutrophil count by the absolute lymphocyte count and the absolute platelet count by the absolute lymphocyte count, respectively. MPV, used to measure the average size of platelets in the blood, was obtained from routine blood tests. The MPR was calculated as MPV/platelet.

Indirect fibrosis markers accepted were APRI : [(AST/*ULN)/PLT × (109/L)] × 100; (*ULN- upper limit normal), FIB-4: [age × AST/PLT × (109/L)] × ALT1/2 were calculated.

Retrospectively efforts were made to exclude inflammatory events that could potentially affect blood parameters and those who met the following criteria were removed from the study.

- Active infection,
- Receiving treatment that alters neutrophil and leukocyte levels,
- Chronic Kidney Failure,
- Malignancies,
- Rheumatologic Diseases,
- Anti-Inflammatory and Steroid Drug Users,
- Using drugs that could affect the bone marrow,
- Inflammatory Bowel Disease,
- Immune Thrombocytopenic Purpura,
- Myeloproliferative Disease
- Bernard-Soulier Syndrome,
- Patients receiving treatment that could affect neutrophil and leukocyte levels

Statistical analysis

Data were analysed using SPSS for Windows version 23.0 (spss.ktu.edu.tr.). Continuous variables were expressed as median (25th-75th percentile) and categorical variables were expressed as frequencies (n) and percentages (%). The Normal distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Since the variables showed non-normal distribution, they were compared using the Kruskal-Wallis test followed by the post-hoc Bonferroni test. Predictive value of biomarkers in detecting steatohepatitis and fibrosis were analyzed using Receiver Operating Characteristics (ROC) curve analysis. In the presence of significant cutoff points, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of these cutoff points were calculated. A p value of <0.05 was considered significant.

Ethical Approval

This study was approved by the Ethics Committee of Karadeniz Technical University (Date: 2021-04-15, No: 2021/98).

Results

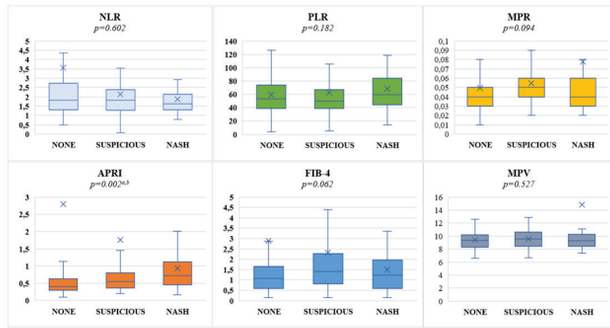
The 197 patients comprised 88 (44.7%) men and 109 (55.3%) women with a mean age of 45 (range, 31.0-57.0) years. Type 2 diabetes mellitus (T2DM) was present in 14.1% (n=27) and hypertension was present in 17.2% (n=33) (Table 1). Biopsy results indicated no fibrosis in 116 (58.9%) patients, while they showed perisinusoidal or periportal fibrosis in 33 (16.8%), perisinusoidal and portal/periportal fibrosis in 14 (7.1%), bridging fibrosis in 26 (13.2%), and cirrhosis in 8 (4.1%) patients. Additionally, the results showed no NASH in 84 (42.6%), suspicious NASH in 82 (41.6%), and NASH in 31 (15.7%) patients (Table 2). The APRI values were significantly higher in patients with NASH and suspicious compared to patients without NASH (p=0.002). (Figure 1) The MPR and MPV values were statistically significantly higher in patients with Stage 4 fibrosis compared

to patients without fibrosis (respectively p<0.001; p=0,029). The APRI value was statistically significantly higher in patients with stage 3 and stage 4 fibrosis compared to patients without fibrosis (p=0.001). The FIB-4 value was statistically significantly higher in patients with stage 1 and stage 4 fibrosis compared to patients without fibrosis (p<0.001). No significant difference was found for the remaining biomarkers (Figure 2). In the ROC analysis conducted to evaluate the predictive value of biomarkers for the diagnosis of NASH, the area under the ROC curve (AUC) values for APRI and FIB-4 were 0.636 and 0.588, respectively. In the ROC analysis conducted to evaluate the predictive value of biomarkers for fibrosis, the AUC values for MPR, APRI, FIB-4, and MPV were 0.822, 0.773, 0.874 and 0.746, respectively (Figure 3).

Discussion

The increasing prevalence of LF and NASH has led to a significant area of research in hepatology for the search of reliable non-invasive markers. Despite many studies being conducted, it is still not clear whether or not to include these tests in the follow-up panel [7]. In this context, many studies have been conducted on the potential diagnostic role of NLR, PLR, MPV, MPR, APRI, and FIB-4 scores. The aim of this study is to examine the relationship between these parameters and biopsy results, which are considered the gold standard.

From this perspective, only APRI showed an increasing trend



[a]None vs. Steatohepatitis, [b]None vs. Suspicious

Figure 1. Comparison of biomarkers in terms of the stages of steatohepatitis

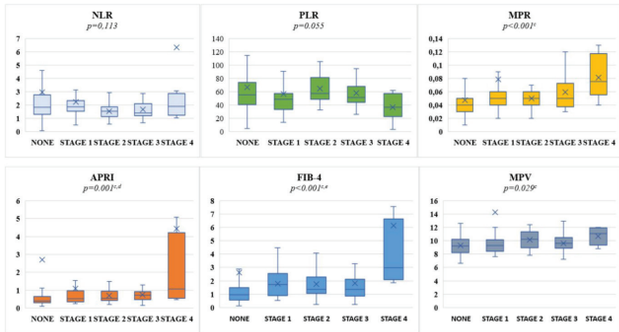


Figure 2. Comparison of biomarkers in terms of the stages of fibrosis

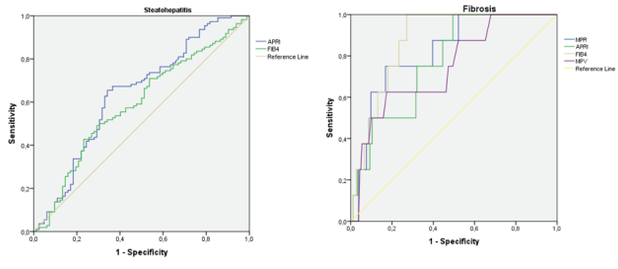


Figure 3. ROC analysis of biomarkers in the diagnosis of steatohepatitis and fibrosis

Table 1. Demographic and clinical characteristics

	Patients	
	n	%
Age (years)	45.0 (31.0-57.0)	
Gender (%)		
Female	109	55.3
Male	88	44.7
Comorbidities (%)		
T2DM	27	14.1
Wilson	2	1.0
Laboratory parameters (median, 25th-75th percentile)		
Glucose (mg/dL)	100.5 (89.0-121.5)	
ALT (U/L)	56.0 (33.0-103.0)	
AST (U/L)	42 (31.0-64.5)	
Triglyceride (mg/dL)	134 (91.8-189.5)	
HDL (mg/dL)	43 (35-55)	
LDL (mg/dL)	115 (88-139)	
GGT (U/L)	53 (30.0-102.3)	
Bilirubin (mg/dL)	0.7 (0.5-0.9)	
Creatinine (mg/dL)	0.72 (0.58-0.85)	
Albumin (g/L)	4.3 (4.0-4.6)	
ALP (U/L)	92 (75-145)	
LDH (U/L)	229.5 (195.8-311.3)	
TSH (mIU/L)	1.5 (1.0-2.3)	
CRP (mg/dL)	0.4 (0.2-0.9)	
Sedimentation (mm/h)	14.5 (6.0-37.5)	
HBG (g/dL)	13.8 (12.5-15.1)	
NLR	1.8 (1.3-2.4)	
PLR	51.8 (40.4-71.4)	
MPR	0.043 (0.035-0.060)	
APRI	0.486 (0.341-0.813)	
FIB-4	1.177 (0.660-2.045)	

T2DM: Type 2 diabetes mellitus, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, GGT: Gamma-glutamyl transpeptidase; ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase, CRP: C-reactive protein, WBC: White blood cell count, HBG: Hemoglobin, NLR: Neutrophil-to-
lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, MPR: mean platelet volume-to-platelet-ratio, APRI: AST to platelet ratio

Table 2. Biopsy results

	n	%
Steatohepatitis		
None	84	42.6
Suspicious	82	41.6
Steatohepatitis	31	15.7
Fibrosis		
None	116	58.9
Stage 1	33	16.8
Stage 2	14	7.1
Stage 3	26	13.2
Stage 4	8	4.1

Table 3. Predictive values of biomarkers in the diagnosis of steatohepatitis and fibrosis

	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV
Steatohepatitis						
APRI	0.636	0.363	69.1	52.4	66.1	55.8
FIB-4	0.588	0.780	70.0	46.3	63.6	53.3
Fibrosis						
MPR	0.822	0.037	80.2	45.0	51.6	75.8
APRI	0.773	0.405	77.8	53.2	54.8	76.6
FIB-4	0.874	0.915	80.2	49.5	53.7	77.5
MPV	0.746	8.250	88.9	26.1	46.8	76.3

AUC: Area under the ROC curve

in stages 3 and 4 of NASH. In the ROC analysis, we found FIB-4 and APRI to be sensitive to NASH. Contrary to studies that have found NLR, PLR, MPV, and MPR to be associated with NASH, these parameters were not statistically significant for NASH according to our study design [8, 11, 12, 13]. The pathophysiology of NASH is associated with a series of inflammatory processes and factors such as smoking, obesity, hyperlipidemia, and metabolic syndrome are influential in the development of the disease [14, 15]. However, the influence of these factors has not been fully controlled in most NASH studies. It is difficult to determine the specific effect of NLR, considered an inflammation marker on NASH. We thought this could be due to a very rigorous screening phase for inflammatory disruptors and the non-evaluation of clinical hepatitis diagnosis.

We found that MPR was associated with advanced LF (Stage-4), but its discriminative power in the early stages of fibrosis was weak. In a study where Michalak et al. compared LF with the MELD score, they found a strong association with MPR in LF [8]. MPR is a parameter that has just begun to be investigated. We believe that more research is needed to determine its diagnostic benefits in LF.

In a study conducted by Lee et al., they found that APRI was associated with LF [16]. In our study, APRI was significantly higher in Stage 3 and Stage 4 fibrosis compared to those without fibrosis. This finding suggested that APRI could potentially be valuable in determining LF before progressing to advanced fibrosis.

FIB-4 score has been found to be correlated with advanced LF in two distinct studies [16, 17]. In our study, a statistically significant association was observed between LF(stage 4) and patients without fibrosis.

Contrary to previous studies on LF, our study found that the PLR was statistically insignificant in relation to LF [8, 18]. Surprisingly, contrary to numerous previous studies, our study found that the NLR was not statistically associated with either NASH or LF [8, 13]. Similarly, in a study conducted by Coskun et al., comparing NLR with pathological data, they found that NLR was not associated with fibrosis.[19] These findings suggest that the lack of association between NLR and LF or NASH may have been influenced by confounding factors that affect NLR in the study's outcome.

Limitation

The most significant limitation of this study is its retrospective design, which may have resulted in an inability to access the complete demographic data of the participants.

Conclusion

Numerous factors play a role in the etiopathogenesis of LF and NASH. The potential for the parameters studied to be affected by inflammatory processes is the most significant reliability issue. There is a need for large-scale studies in different populations in this area.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

Funding: *None*

Conflict of Interest

The authors declare that there is no conflict of interest.

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How to cite this article:
Sukriye Tascı, Ismail Sayın, Irem Dilaver, Birgul Tok, Nilay Turan. *The relationship between biopsy findings of liver fibrosis and non-alcoholic steatohepatitis and hematological scales. Ann Clin Anal Med 2024;15(10):672-676*

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