Inflammatory-nutritional scores in the diagnosis of NASH and liver fibrosis

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ABSTRACT

BACKGROUND: The aim of the present study was to investigate the possible correlation between various inflammation-nutritional scores to histological determined nonalcoholic steatohepatitis (NASH) and other liver injury suggestive for non-alcoholic fatty liver disease (NAFLD) in a bariatric population.
METHODS: We consecutively and retrospectively evaluated all the patients referred to the Department of Bariatric Surgery in Trieste, Italy. Inflammation-nutritional scores were calculated starting from preoperative hematologic data. Liver biopsy was performed at the time of bariatric surgery (sleeve gastrectomy or gastric bypass) and pathological assessment was performed using Kleiner-Brunt staging system (NAS score).
RESULTS: Glasgow Prognostic Score/modified Glasgow Prognostic Score (GPS/ mGPS) and Prognostic Index (PI) were associated to the diagnosis of NASH (P=0.024 and P=0.03 respectively). The presence of perisinusoidal and/or periportal fibrosis was correlated to Prognostic Nutritional Index (PNI) and platelet-to-lymphocyte ratio (PLR) values (P=0.02 and P=0.009 respectively).
CONCLUSIONS: GPS/mGPS and PI are statistically associated to the histological diagnosis of NASH. Further studies on large series are needed to better understand the relationship between these serum markers and liver injury in obese patients.

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KEY WORDS: Liver; Obesity; Non-alcoholic fatty liver disease.

Non-alcoholic fatty liver disease (NAFLD) is the most important cause of chronic liver disease worldwide[1] and it is strictly associated with diabetes, obesity, and hyperlipidemia. NALFD could be considered the hepatic manifestation of the metabolic syndrome[2, 3] with a global overall prevalence of about 25%.[1] Due to the increasing incidence and prevalence of this disease, NAFLD has become the second leading cause of liver transplantation in the USA.[4] NAFLD is a spectrum of histological alterations, which accounts for different stages of pathological accumulation of triglycerides into hepatocytes, liver inflammation, and fibrosis.[5] It ranges from relatively benign conditions as non-alcoholic fatty liver (NAFL) to more severe and evolving diseases such as nonalcoholic steatohepatitis (NASH), cirrhosis, end-stage liver disease, and eventually, hepatocellular carcinoma (HCC). Among NAFLD patients, 41% experienced fibrosis progression, 25% cirrhosis, and 7% end-stage liver disease.[1] Fibrosis is the most important predictor

of NAFLD progression into advanced stages of liver dysfunction and NASH related mortality.[5] On this basis, the identification of early stages of liver fibrosis is crucial to select patients requiring close monitoring or specific targeted therapy. Liver biopsy is the gold standard for diagnosis of NAFLD, but costs and risks of potential complications associated with this invasive procedure makes it unsuitable for screening purposes.[6] In recent years, research is focusing on novel non-invasive diagnostic methods but none of them has still reached the accuracy comparable to the histological diagnosis.[7, 8] In the last decade, inflammatory-nutritional scores have been associated with several oncological diseases of the gastrointestinal tract,[9-12] hepatocellular carcinoma,[13, 14] pancreatic,[15] lung,[16] breast,[17] and female reproductive system tumors.[18] Our study aimed to investigate the possible association between inflammatory-nutritional scores and NAFLD. In particular, we studied the possible applications of these scores as noninvasive biomarkers for the diagnosis of NASH and liver fibrosis in a cohort of obese patients.

Materials and methods

This retrospective cohort study based on a prospective database was approved by the Institutional Review Board (IRB) of the Local Ethical Committee (Protocol N. 22979 FVG, SSN). Patients eligible for bariatric surgery were enrolled after obtaining written informed consent. Patients with other forms of chronic liver disease, including suspected/confirmed hepatocellular carcinoma, alcoholic liver disease (>25 g/day alcohol consumption), or known HBV, HCV, and HIV positivity were excluded. Clinical and anthropometric data were recorded on average 1 week before surgery. The day before surgery, serum samples were collected and laboratory tests including C-reactive protein mg/L (CPR), albumin g/L, white blood cell count 10^9/L (WBC), total lymphocyte count 10^9/L, neutrophil count 10^9/L, platelet count 10^9/L, and monocyte count 10^9/L were recorded. The following systemic inflammatory and nutritional scores were calculated: Glasgow Prognostic Score/modified Glasgow Prognostic Score (GPS/mGPS), Prognostic Index (PI), Prognostic Nutritional Index (PNI), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), Systemic Immune-Inflammation Index (SII). During the bariatric operation, a surgical liver biopsy on the left hepatic lobe was performed and analyzed by a single experienced pathologist who was blinded to all clinical and laboratory parameters. The histological diagnosis of NASH and fibrosis was made following Kleiner-Brunt classification.[18] The histopathological findings analyzed were: steatosis grade and localization, presence of microvesicular steatosis, fibrosis stage, lobular inflammation, microgranulomas, lipogranulomas, portal inflammation, hepatocyte ballooning, apoptotic bodies, presence of pigmented macrophages (Kupffer cells), mega-mitochondria, Mallory bodies, and glicogenated nuclei. NASH was defined as a total NAFLD activity score ≥5. In addition, regarding the fibrosis stage, it was scored as follows: 0 (none); 1A (mild, zone 3, perisinusoidal fibrosis); 1B (moderate, zone 3, perisinusoidal fibrosis); 1C (portal/periportal fibrosis); 2 (zone 3, perisinusoidal and portal/periportal fibrosis); 3 (bridging fibrosis); 4 (cirrhosis). In our sample, patients were categorized into two groups regarding the score of fibrosis: <2 (minimal fibrosis) and ≥2 (moderate/severe fibrosis).

Statistical analysis

Continuous variables were summarized as mean. χ^2 tests or Fisher's Exact Test, when appropriate, were used to compare univariate associations of histological features and the systemic inflammatory/nutritional scores (GPS, mGPS, PI, PNI, NLR, PLR). The area under the ROC curve (AUROC) was used to assess the accuracy of the most significant candidate Diagnostic Index detecting fibrosis. Youden indices were used to determine the optimal threshold among the candidate scores. The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated for both the literature validated threshold and the cut-off found in our cohort. The risk of detecting early fibrosis stages using systemic inflammatory/nutritional scores was estimated through odds ratio

(OR) with a 95% confidence interval (95% CI). A significative level (P) of 0.05 was used for all analyses.

Results

Two-hundred thirty-seven patients who underwent laparoscopic gastric bypass or sleeve gastrectomy were retrospectively evaluated. Two patients were excluded because no liver biopsy was performed at the time of surgery thus, 235 patients were included in the present study. Patient characteristics are shown in Table I. Mean age was 45 (19; 63) years and 71.1% were female. Mean BMI was 43.8 (30.2; 70.4). One hundred thirty-six patients underwent laparoscopic gastric by-pass while 99 laparoscopic sleeve gastrectomy. Among the entire cohort, 144 patients had previous medical comorbidity (61.3%) and, in particular, 41 patients showed unexplained altered liver enzymes. Liver biopsy characteristics are presented in Table II. At pathological evaluation, 142 (60.4%) patients were affected by active steatohepatitis (NASH) with a total Kleiner-Brunt score ≥5. Considering liver fibrosis stage, 194 (82.6%) patients had from none to mild liver fibrosis (score <2) while 41 patients presented with at least moderate fibrosis (score ≥2); among them, only 8 patients presented with advanced stages of fibrosis, in particular, 6 (2.6%) biopsies demonstrated the presence of bridging fibrosis while only 2 patients were cirrhotic. Nutritional/Inflammation scores and their correlation with histopathological characteristics of NAFLD are presented in Supplementary Digital Material

TABLE I.—*Patient characteristics.*

Parameters	Values
Number of patients	235
Female	167
Male	68
Age, years	45 (19; 63)
Body Mass Index	43.8 (30.2; 70.4)
Type of surgery	
Sleeve gastrectomy	99
Gastric bypass	136
Comorbidity	144
Diabetes type 2	57
Hypertension	71
Dyslipidemia	75
Unexplained abnormal liver enzyme	41

TABLE II.—*Liver biopsy characteristics (Kleiner-Brunt staging system).*

Parameters	Values
NAFLD activity score	
<3 (not steatohepatitis)	49
≥3 and <5 (possible/borderline)	44
≥5 (definite steatohepatitis)	142
Steatosis (grade)	
0 (None)	52
1 (Mild)	87
2 (Moderate)	53
3 (Severe)	43
Location	
0 (Zone 3)	142
1 (Zone 1)	5
2 (Azonal)	55
3 (Panacinar)	33
Microvesicular	
0 (Absent)	181
1 (Present)	54
Fibrosis	
Stage 0 (None)	54
1 (Mild)	140
2 (Moderate)	33
3 (Bridging)	6
4 (Cirrhosis)	2
Inflammation	
Lobular inflammation	
0 (none)	84
1 (<2)#	123
2 (2-4)#	28
3 (>4)#	0
Microgranulomas	
0 (Absent)	212
1 (Present)	23
Lipogranulomas	
0 (Absent)	205
1 (Present)	30
Portal Inflammation	
0 (None to minimal)	174
1 (Greater than minimal)	61
Liver cell injury	
Hepatocyte ballooning	
0 (Absent)	132
1 (Few)	61
2 (Prominent)	42
Acidophil bodies	
0 (None to rare)	221
1 (Many)	14
Pigmented macrophages	
0 (None to rare)	174
1 (Many)	61
Megamitochondria	
0 (None to rare)	235
1 (Many)	0
Other findings	
Mallory's hyaline	
0 (None to rare)	234
1 (Many)	1
Glycogenated nuclei	
0 (None to rare)	157
1 (Many)	78

#Number of foci under 20× magnification.

1: Supplementary Table I. We calculated PNI, NLR, PLR, LMR, and PII for all 235 patients included in the present study but we determined nutritional/inflammation scores based on CRP value only in 176 patients. Our data showed that GPS, mGPS, and PI were significantly associated with NAFLD activity score ≥5 so with the diagnosis of steatohepatitis (P=0.013, P=0.012 and P=0.03 respectively). PNI and PLR values were significantly associated with fibrosis stage (P=0.02 and P=0.009 respectively). In particular, PLR score was significantly higher in patients with initial liver fibrosis (score <2) (IQR: 99.6; 159.0); Median PLR for patients with fibrosis ≥2 =108.7 (IQR: 91.3; 131.1). The accuracy of PLR in predicting liver fibrosis has been assessed by calculating the ROC curve that showed an Area Under the Curve (AUROC) of 0.63 (95% CI: 0.544; 0.716) (Figure 1). The reference cut-off value reported in almost all series considering PLR score in oncologic disease is 150 while in our study, the best threshold, calculated applying the Youden method, was 143,2. Data showed that the cut-off of 143,2 had higher specificity (SP=0.378 [95% CI: 0.310-0.451]), positive predictive value (PPV=0.236 [95% CI: 0.185-0.501] and negative predictive value (NPV=0.925 [95% CI: 0.834-0.975]) compared to the reference cut-off value reported in literature. Sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) calculated for both cut-offs are shown in Table III. From our analysis, patients with a PLR score <150 had a 3.5-fold higher risk to have liver fibrosis (OR=3.5; 95% CI: 1.31-9.35; P=0.012). On the other hand, using the 143.2-cut-off resulted from our analysis, the OR increased to 4.5 (OR=4.5; 95% CI: 1.69-12; P=0.0026). Data obtained from the correlation analysis between other nutritional/inflammation scores and available histopathological features were not statistically significant.

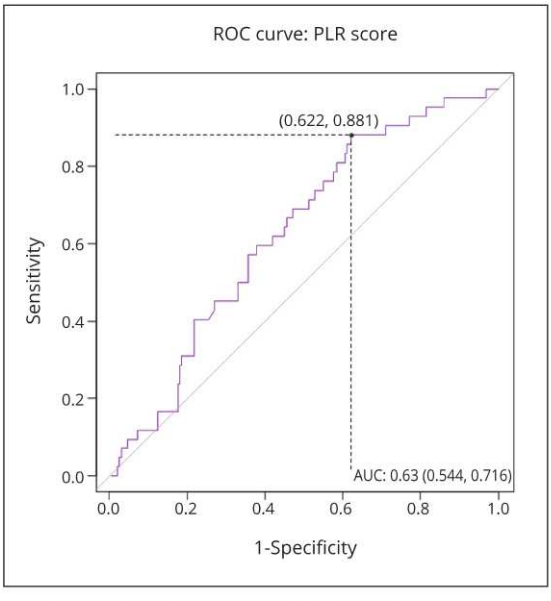

Figure 1. The accuracy of Platelet-to-Lymphocyte Ratio in predicting liver fibrosis.

Discussion

A characteristic of NAFLD is the inter-patient variation in disease progression therefore a histopathological definition of the hepatic damage is mandatory. Although liver biopsy is still the gold standard for diagnosis and staging of fibrosis, it is an invasive procedure does not bereft of potential complications[6] and its histopathological assessment depends on several variables such as the sampling technique and the observer skills. On this basis, its routine use for both screening and monitoring purposes is limited. Lately, an increasing number of noninvasive tests are being developed.[7, 8] These methods include both blood-based tests and radiological techniques. Blood-based tests could be categorized into "direct" markers, that measure deposition and turnover of molecules of the extracellular matrix, and "indirect" tests, which use patients' characteristics and hematological data.

TABLE III.—*Platelet-to-lymphocyte ratio and liver fibrosis.*

Parameters	Cut-off=150 (95% CI)	Cut-off=143.2 (95% CI)
Sensitivity	0.881 (0.744; 0.960)	0.881 (0.744; 0.960)
Specificity	0.321 (0.256; 0.392)	0.378 (0.310; 0.451)
Positive predictive value	0.220 (0.160; 0.291)	0.236 (0.185; 0.501)
Negative predictive value	0.925 (0.834; 0.975)	0.936 (0.851; 0.951)

Examples of "indirect" tests include NAFLD fibrosis score (NFS), BARD score, FibroTest, FIB4 Index, AST/platelet ratio (APRI) index, ELF score.[9] They showed an AUROC ranging from 0.7 to 0.86, a sensitivity ranging from 44 to 77%,[19] and a lower PPV compared to other "direct" indices of fibrosis. The "direct" indices include high sensitivity C-reactive protein, plasma pentraxin 3, interleukin-6, and cytokeratin[18, 19] as well as tests based on soluble markers such as microRNA (miRNA), and lipidomic panels, which remain highly experimental and require further validation. Several radiological techniques are used to estimate liver "stiffness," as a surrogate of fibrosis. The most accurate non-invasive methods to assess the stiffness of the liver and to dichotomize the patient affected by advanced *versus* non-advanced fibrosis include transient elastography (TE), magnetic resonance elastography (MRE), and emerging techniques such as shear wave elastography and acoustic radial force imaging. All of these methods have a sensitivity ranging from 65% to 88% and a positive predictive value from 65% to 68%.[19] Although MRI is reported to be the best test to assess advanced fibrosis (AUROC 0.92),[19] the high costs, the limited availability in hospitals, and the limitations related to the claustrophobia and patient's BMI do not make it the ideal tool for this purpose. Nutritional and inflammatory scores have been used in the oncological field to assess the progression and recurrence of several tumors as they are an independent indicator of a less favorable outcome and shorter survival.[8-17] Liver fibrosis, as a precursor of cirrhosis, is a crucial pathological process characterized by initial degeneration and necrosis of hepatocytes, evolution into the replacement of liver parenchyma by fibrotic tissues and regenerative nodules, and eventually, end-stage liver disease.[20] On this basis and considering that the etiology of NAFLD is the activation of intra-hepatic inflammation,[21] we supposed that inflammation-based scores could provide important prognostic information on fibrosis stage. Our study tested all nutritional and inflammatory scores available in literature and Platelet-to-Lymphocyte ratio (PLR score) showed the best correlation with the histopathological findings. Our data showed that PLR score was strongly inversely related to minimal fibrosis (stage <2); this result could have a great impact on the therapeutic point of view. Currently, specific therapy is still lacking but, it is well known that the initial stage of fibrosis, could be reversible.[22, 23] The awareness of initial liver disease could lead the patient toward a radical and lasting change in diet and lifestyle with a subsequent improvement of liver-related outcomes. PLR had a high sensitivity of 88%, which is comparable to the sensitivity of imaging-based tests.[19] Unfortunately, the AUROC was 0.63 and this unsatisfactory result, that does not allow us to reach the optimal decision threshold, could be probably due to the small sample size.

Limitations of the study

The main limitations of this study were its retrospective nature and the small sample size. We would like to highlight that we collected prospectively the clinical features of all consecutive patients treated in our institution even if the study has been carried out in a retrospective manner. Data were recorded in a standardized, password-protected and anonymized database. In our opinion, PLR score could be an ideal soluble marker to assess fibrosis stage, if tested on a larger number of patients. Alternatively, it could be included in a panel of existing "indirect" scores to increase their diagnostic accuracy. It is a cost-effective and easily available blood-based test and for these reasons suitable for both diagnosis and follow up of the disease. The strengths of this study were the biopsy-proven liver disease and the relatively high prevalence of initial fibrosis; in addition, to the best of our knowledge, no previous literature investigate the correlation between nutritional/inflammatory scores and NAFLD.

Conclusions

Our study aimed to propose an accurate, easily available, and non-invasive blood-tested marker of minimal and potentially reversible liver fibrosis. In our cohort, the best candidate among the nutritional and inflammatory scores considered was PLR, but larger prospective studies are needed to validate this marker.

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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