POLISH ARCHIVES OF
Internal Medicine
POLSKIE ARCHIWUM MEDYCYNY WEWNĘTRZNEJ

ONLINE FIRST

This is a provisional PDF only. Copyedited and fully formated version will be made available soon.

# Ultrasound and biomarker based assessment of hepatic steatosis in patients with severe obesity

**Authors:** Michał Byra, Cezary Szmigielski, Piotr Kalinowski, Rafał Paluszkiewicz, Bogna Ziarkiewicz-Wróblewska, Krzysztof Zieniewicz, Grzegorz Styczynski

**Article type:** Original article

**Received:** May 4, 2022.

**Revision accepted:** September 13, 2022.

**Published online:** September 13, 2022.

**ISSN:** 1897-9483

Pol Arch Intern Med.

doi:10.20452/pamw.16343

# Ultrasound and biomarker based assessment of hepatic steatosis in patients with severe obesity

Michał Byra[1], Cezary Szmigielski[2], Piotr Kalinowski[3], Rafał Paluszkiewicz[3], Bogna Ziarkiewicz-Wróblewska[4], Krzysztof Zieniewicz[3], Grzegorz Styczynski[2]

1 Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

2 Department of Internal Medicine, Hypertension and Vascular Diseases, Medical University of Warsaw, Warsaw, Poland

3 Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Warsaw, Poland

4 Department of Pathology, Center for Biostructure Research, Medical University of Warsaw, Warsaw, Poland

**Correspondence to:** Michal Byra, PhD, Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland, ul. Pawinskiego 5B, Warsaw, Poland, phone: (+48) 22 826 89 11, email: mbyra@ippt.pan.pl

**What's new?**

Non-alcoholic fatty liver disease (NAFLD) is among the most common liver abnormalities. Various methods based on ultrasound imaging and clinical biomarkers have been proposed for the non-invasive diagnosis of the fatty liver disease in the general population. However, the usefulness of these methods for the patients with severe obesity has not been adequately investigated yet. In this work, we compare the fatty liver disease classification performance of

the ultrasound hepatorenal index technique, the hepatic steatosis index and the NAFLD logit score technique in patients with severe obesity. Results demonstrate that the investigated methods require adjustments in order to work well in patients with severe obesity. Our study also indicates that the ultrasound hepatorenal index method outperforms biomarker-based techniques.


## Abstract

**Introduction:** Nonalcoholic fatty liver disease (NAFLD) is a common liver abnormality, but its non-invasive diagnosis in patients with severe obesity remains difficult.

**Objectives:** To investigate the usefulness of the ultrasound (US) based hepatorenal index (HRI) technique, and two biomarker-based methods, including the hepatic steatosis index (HSI) and NAFLD logit score for the diagnosis of NAFLD in subjects referred for the bariatric surgery.

**Patients and methods:** 162 subjects, 106 with NAFLD, admitted for the bariatric surgery participated in the study. Fat fraction level and the presence of NAFLD were determined using surgical liver biopsy. Each patient underwent liver US examination and blood tests to determine the HRI, HSI and NAFLD logit score.

**Results:** For the NAFLD diagnosis, the HRI, HSI and NAFLD logit score techniques achieved areas under the receiver operating characteristic curves of 0.879, 0.577 and 0.825, respectively. The Spearman's correlation coefficients between the liver fat fraction values and the HRI, HSI and NAFLD logit score were equal to 0.695, 0.215 and 0.595, respectively. The optimal cut-off values for the NAFLD diagnosis for the HRI, HSI and NAFLD logit score were equal to 1.12, 56.1 and 0.59, and significantly differed from the cut-off values reported for the general population in the literature.

**Conclusions:** Our study confirms the usefulness of only two out of three techniques, the HRI and the NAFLD logit score for the diagnosis of NAFLD in patients with severe obesity. Methods designed for the general population require different cut-off values to achieve accurate performance in severe obesity.

**Key words**

biomarkers, fatty liver disease, hepatorenal index, obesity, ultrasound

## Introduction

The increasing prevalence of obesity has become a major public health concern [1,2]. Obesity is defined as a body mass index (BMI) greater than or equal to 30 kg/m$^2$ and it is regarded as a major preventable risk factors for morbidity and mortality [3,4]. Complications resulting from obesity may account for 5 to 15% of all deaths [5].

Non-alcoholic fatty liver disease (NAFLD) is a common liver disease present in a large percent of people with obesity [6–8]. NAFLD is related to various health complications, from simple hepatic steatosis and non-alcoholic steatohepatitis (NASH), cirrhosis, hepatocellular carcinoma and even death [8–11]. Patients with severe obesity referred for the bariatric surgery belong to a high risk group for NAFLD [12]. Severe obesity is recognized in patients with BMI equal or greater than 40 kg/m$^2$ or in patients with BMI greater than 35 and at least one serious obesity-related health condition [13]. Obesity status should be considered when designing effective treatments for the prevention of the hepatic steatosis[14]. Nowadays, the continuous expansion of the treatment options for patients with liver diseases requires accurate initial characterization of the liver structure and function, especially in the presence of NAFLD [15]. This is especially important in patients with severe obesity. A definitive diagnosis of NAFLD is based on histopathological analysis. However, in clinical practice, liver biopsy and histological assessment of the liver tissue is rarely performed due to

its invasive nature, potential complications and cost. Other methods for screening for fatty liver disease, like computed tomography and magnetic resonance imaging, have limited availability for large populations [10]. Accordingly, an approach incorporating a feasible, safe and widely available screening technique for NAFLD diagnosis would be important. Ultrasound (US) imaging is usually preferred for screening, because of its safety, availability, and relatively low cost. However, when using traditional imaging criteria, the accuracy of US based diagnosis of NAFLD in patients with severe obesity is limited, mostly because of the difficulties in acquiring high quality images [16]. In recent years, to improve the US quantitative assessment of the liver fat, the hepatorenal index (HRI) approach has been developed and applied for the patients assessed for liver steatosis [17–24]. The goal of the HRI approach is to compare the echogenicity of the liver to the echogenicity of the kidney cortex. Normal liver and renal tissue show similar echogenicity in healthy subjects. However, brightness of the liver is higher compared to the kidney in the presence of the liver steatosis. Therefore, HRI proportionally increases with the liver fat accumulation.

Recently, several laboratory biomarkers have been proposed to improve NAFLD diagnosis in the general population [25], including hepatic steatosis index (HSI) [26] and NAFLD logit score [27]. However, these methods have been developed and validated using data collected from general population, and their usefulness for accurate NAFLD diagnosis in patients with severe obesity needs to be investigated.

In this work, we compare the HRI, HSI and NAFLD logit score methods for the diagnosis of hepatic steatosis in patients with severe obesity and NAFLD confirmed by liver biopsy during bariatric surgery.

**Patients and methods**

Data were collected retrospectively from 162 patients admitted for bariatric surgery (laparoscopic sleeve gastrectomy) between year 2016 and 2019, in a tertiary care, university

hospital. The study was approved by the local institutional review board. No secondary causes of the hepatic steatosis other than obesity (such as alcohol abuse, viral infections or hepatotoxic drugs) were present in our patients according to medical history and clinical evaluation. Patients investigated in our study had no reported history of kidney diseases. All patients provided an informed consent for the examinations. No specific diet was required as a preparation for the surgery. Each patient underwent a wedge liver biopsy during the bariatric surgery, implemented as a routine procedure in our surgical clinic. During biopsy, tissue samples were extracted from the subcapsular part of the left lobe of the liver. The liver fat fraction was defined based on the percentage of hepatocytes with fatty infiltration, determined by an experienced pathologist. The fatty liver was defined if more than 5% of hepatocytes had steatosis. Fatty liver disease was diagnosed in 106 patients (Fig. 1). Clinical characteristics of the patients were analyzed, including age, sex, weight, body surface area (BSA), BMI, and diagnosis of hypertension (HT), as well as diabetes mellitus (DM).

All blood tests and US examinations were performed within 24-48 hours before the bariatric surgery. The following 15 biomarkers were determined: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), glucose, c-peptide, total cholesterol (THC), low-density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), triglyceride (TG), white blood cell count (WBC), hemoglobin A1c ($HbA_{1c}$), insulin and homeostatic model assessment for insulin resistance (HOMA-IR), serum creatinine and glomerular filtration rate (eGFR) calculated with the Salazar-Corcoran formula dedicated for the obese patients [28].

The HSI parameter was calculated with the following formula [26]:

$$HSI = 8 \times ALT/AST\ ratio + BMI\ (+2, \text{if DM}; +2, \text{if female}).$$

The NAFLD logit score, ranging from 0 to 1, was estimated with the following equation [27]:

$$NAFLD\ logit\ score = \exp(-7.338+0.046 \times ALT-1.277 \times HDL+0.486 \times TG+0.911 \times HbA_{1c}+$$

$$0.207\times WBC+0.589\times HT) \,/[1 + \exp(-7.338+0.046\times ALT-$$

$$1.277\times HDL+0.486\times TG+0.911\times HbA_{1c}+0.207\times WBC+0.589\times HT)].$$

The US images, displaying liver/kidney views, were acquired during preoperative cardiac echocardiographic evaluation up to two days before the bariatric surgery with an ultrasound scanner (GE Vivid E9, GE Healthcare, Horten, Norway), equipped with a 2.5 MHz sector probe. Figure 2 shows examples of the US images of the livers with various steatosis levels. A single physician determined the HRI values for each US image, without knowledge of the biopsy results. Two regions of interest (ROIs) corresponding to the uniform parts of the liver and the kidney cortex were identified and used to calculate the HRI parameter, defined as the ratio of the average liver and kidney US image brightness levels (Fig. 3).

Statistical analysis

Continuous variables were described using median and with the first and third quartiles. Comparison of the continuous variables, between subjects with and without NAFLD, was performed with the Wilcoxon rank sum test at the significance level of 0.05. Nominal variables were presented as the number of cases in each category (percentage) and compared using Fisher exact test. Additionally, the Spearman's correlation coefficients (SCCs) were calculated between each continuous variable and the liver fat fraction level. For diagnostic performance assessment, first, we determined the regular performance of these techniques for the diagnosis of cases with the fat fraction higher than 5%. Second, we additionally assessed the performance of the techniques in patients with the fat fraction higher than 30%, representing moderate and severe steatosis that may be more clinically significant than mild steatosis [29–31].

For each diagnostic method, we determined the receiver operating characteristic curve (ROC) and the area under the ROC curve (AUC). Accuracy, sensitivity and specificity were calculated for the cut-off value that was the closest to the upper left corner of the ROC curve

(optimal cut-off), as well as for the cut-off values corresponding to the sensitivity and specificity levels of 90% [32]. Standard errors of the metrics were calculated using bootstrapping. AUC values obtained for the selected techniques were compared with the DeLong test at the significance level of 0.05 [33]. All calculations were performed using Matlab's Statistics and Machine Learning Toolbox (MathWorks, USA).

**Results**

Characteristics of the patients and the results of the laboratory tests are presented in Table 1. Median values of 13 biomarker significantly differed between the NAFLD patients and the control group. Table 2 shows that the median values for the NAFLD patients were significantly higher for the HRI, HSI and NAFLD logit score techniques. Diagnostic performance of the HRI, HSI, and NAFLD logit score technique is summarized in Table 3. In the case of the NAFLD diagnosis (fat fraction>5%), the HRI technique and the NAFLD logit score achieved AUC values of 0.879 and 0.825, respectively. Compared to these two methods, the HSI technique achieved significantly lower AUC value of 0.599 (DeLong test P-*values*<0.001). Additionally, Table 3 illustrates the diagnostic performance for the subjects with moderate and high fat fraction (above 30%). In this case, the HRI and NAFLD logit score techniques achieved similar AUC values of around 0.82. In comparison, the AUC value for the HSI method was significantly lower and equal to 0.577 (DeLong test P-*values*<0.001). Moreover, Table 3 presents classification metrics calculated for the various cut-off values.

Table 4 presents the HRI based liver steatosis classification performance reported in the previous studies for the general population. In these studies, the reported HRI cut-off values ranged between 1.24 and 2.20, while the AUC values ranged between 0.92 and 0.996. In comparison, in our study for the severely obese patients the optimal cut-off value for the HRI was equal to 1.12. Similarly, Table 5 presents the steatosis diagnosis performance of the HSI, and NAFLD logit score techniques reported in the previous studies for the general

population. For the HSI, the cut-off values corresponding to approximately 90% sensitivity and 90% specificity were equal to 30 and 36, respectively. However, for our group of patients these cut-offs were much higher and equal to 49.3 and 64.9, respectively. For the NAFLD logit score, the cut-off values corresponding to approximately 90% sensitivity and 90% specificity were originally equal to 0.19 and 0.45, while for our group of patients these cut-off values were equal to 0.68 and 0.93, respectively. Moreover, we found that the minimal values of the HSI and NAFLD logit score for our group were equal to 45.2 and 0.133, respectively.

**Discussion**

In our study, we investigated the usefulness of the US based HRI and the laboratory based HSI and NAFLD logit score methods for the diagnosis of NAFLD in patients with severe obesity. In contrast to the previous studies, which derived and validated these methods in the general population, our work was dedicated to patients with severe obesity referred for the bariatric surgery. Generally, we obtained lower NAFLD classification scores for the investigated methods, compared to the results reported in the literature for the general population. The AUC value for the HRI technique was equal to 0.879 and was lower than the AUC values, ranging from 0.92 to 0.996, reported for the general population [17–24]. The lower performance of the HRI parameter in our work may be due to several factors. Mottin et al. presented that US imaging can be used as a diagnostic tool in patients with severe obesity, but the overall usefulness of the US imaging can be limited due to the lack of objective criteria for the NAFLD diagnosis and by various technical problems associated with the US scanning [12]. It is usually more difficult to perform US examination and to acquire high quality US images in patients with severe obesity, compared to lean persons. The lower quality of the US images could result in worse estimation of the HRI parameter. Moreover, in comparison to the previous studies, dedicated to the general population, our dataset was from

the beginning targeted towards patients with high values of liver fat. Inclusion of patients with lower liver fat values could improve the classification performance of the HRI technique.

The laboratory biomarker based methods, the HSI and NAFLD logit score, similarly achieved worse NAFLD diagnosis performance in obese patients than in the papers targeting the general population [26,27]. The HSI method achieved AUC value of 0.599, which was much lower than the AUC value from the original work of Lee et al., reported as 0.812 [26]. This large difference was probably caused by the severe obesity of our patients. In the work of Lee et al., the average BMI values for the controls and patients with fatty liver disease were significantly different and corresponded to a low range of BMI values of 22.9 and 25.3, respectively [26]. In contrast, in our study the median BMI values for the control and NAFLD cases were similar and high, equal to 43.30 and 43.78, respectively. This issue probably influenced the performance of the HSI score [26]. For the NAFLD logit score, we obtained the AUC value of 0.825, which was lower than the AUC value of 0.87 originally reported by Yip et al. for the general population [27]. In patients with the fat fraction above 30%, representing moderate and severe liver steatosis, we found that the HRI and NAFLD logit score techniques achieved similar performance, with AUC values equal to around 0.82. In contrast, the HSI method did not provide accurate results, with low AUC value of 0.577 in patients with moderate and severe steatosis.

Our results agree with the findings reported by Parente et al., who investigated the usefulness of the HSI method in a study based on a small group of 32 patients qualified for bariatric surgery [34]. Similarly, Coccia et al. investigated the accuracy of the HSI method in a group of 90 morbidly obese patients undergoing bariatric surgery [35]. Our study involved 162 patients and indicated that higher cut-off values were required, both for the HSI and the NAFLD logit score technique, to diagnose NAFLD in patients with severe obesity. Lee et al. originally reported the better performing HSI cut-off for the NAFLD classification to be equal

to 36 for the general population [26]. Parente et al. reported the optimal HSI cut-off value of 53 (AUC value of 0.777) for the classification of NAFLD patients qualified for the bariatric surgery [34]. Coccia et al. reported the optimal HSI cut-off value of 52 (AUC value of 0.76) for the morbidly obese subjects [35]. In our study the optimal cut-off was equal to 56.9. Similarly, diagnosis based on the NAFLD logit score technique required higher cut-off values as well. Originally, in the work of Yip et al. the cut-off values of 0.19 and 0.45 corresponded to the sensitivity and specificity of 90% for the general population. However, in our study the NAFLD logit score cut-off values of 0.68 and 0.93 corresponded to the sensitivity and specificity of 90%, respectively. In the case of the HRI technique, the optimal cut-off value determined for the NAFLD diagnosis was equal to 1.12 and was much lower than the previously reported cut-off values for the general population, ranging from 1.24 to 2.20 [17–24]. This discrepancy could result from several factors. First, previous studies have reported different cut-off values for the HRI technique. Calculations of the HRI might be affected by the settings of the US scanner employed for the data acquisition. However, additional studies are required to address this problem. Second, the severe obesity of our patients could influence the acquisition of the US images. For example, the thick layer of tissues, including fat, between the imaging US probe and the liver/kidney regions impacts the imaging US pulse propagation. Third, fatty liver disease, commonly associated with hypertension and diabetes, is regarded as a risk factor for chronic kidney disease, which may increase renal cortex echogenicity, potentially influencing the HRI [36,37]. However, the patients in our study had no reported history of the chronic kidney disease and they had normal eGFR values (above 60 ml/min), suggesting low probability of significant kidney disease.

Our work has several limitations. We did not implement and evaluate the fatty liver index technique, which is one of the biomarker based methods designed for the fatty liver disease assessment in the general population [38]. Fatty liver index requires to determine

waist circumference, which we found to be impractical in the case of the patients with severe obesity, for whom it is difficult to accurately indicate the waist in a repeatable manner. Similarly, we did not implement the lipid accumulation product index for the same reasons related to the requirement for the waist circumference parameter [39]. Moreover, in our study we did not consider some advanced modalities, including magnetic resonance imaging, transient elastography, or electrical bioimpedance [40,41]. In the future, it would be interesting to compare these techniques with the biomarker and ultrasound-based methods in the fatty liver disease assessment in severe obesity.

We evaluated the usefulness of several non-invasive techniques for the NAFLD diagnosis in patients with severe obesity referred for the bariatric surgery. Ultrasound based HRI and laboratory based NAFLD logit score demonstrated good accuracy in severely obese patients, but the laboratory based HSI score achieved low performance. We also demonstrated that the laboratory based diagnostic techniques designed for the general population might require higher cut-off values to achieve accurate performance in severe obesity.

**Contribution statement** MB, CZ, GS contributed to the data analysis methodology and result interpretation. MB, CS, GS contributed to the conceptualization and methodology of the study. MB CZ, PK, GS drafted the original manuscript. All authors contributed to the data collection and data curation.

**Conflict of interest statement** None declared.

## References

1. Hales CM, Fryar CD, Carroll MD, et al. Trends in obesity and severe obesity prevalence in US youth and adults by sex and age, 2007-2008 to 2015-2016. JAMA. 2018; 319: 1723–1735.

2. Placzkiewicz-Jankowska E, Czupryniak L, Gajos G, et al. Management of obesity in the times of climate change and COVID-19: an interdisciplinary expert consensus report. Pol Arch Intern Med. 2022; 132: 16216.
3. Aune D, Sen A, Norat T, et al. Body mass index, abdominal fatness, and heart failure incidence and mortality: a systematic review and dose-response meta-analysis of prospective studies. Circulation. 2016; 133: 639–649.
4. Kenchaiah S, Evans JC, Levy D, et al. Obesity and the risk of heart failure. N Engl J Med. 2002; 347: 305–313.
5. Flegal KM, Graubard BI, Williamson DF, Gail MH. Excess deaths associated with underweight, overweight, and obesity. JAMA. 2005; 293: 1861–1867.
6. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002; 346: 1221–1231.
7. McPherson S, Hardy T, Henderson et al. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol. 2015; 62: 1148–1155.
8. Pischon T, Boeing H, Hoffmann K, et al. General and abdominal adiposity and risk of death in Europe. N Engl J Med. 2008; 359: 2105–2120.
9. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat Rev Gastroenterol Hepatol. 2013; 10: 330–344.
10. Bedogni G, Miglioli L, Masutti F, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology. 2005; 42: 44–52.
11. Mari A, Sbeit W, Haddad H, et al. The impact of overweight on diverticular disease: a cross-sectional multicenter study. Pol Arch Intern Med. 2022; 132: 16177.
12. Mottin CC, Moretto M, Padoin A V, et al. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. Obes Surg. 2004; 14: 635–637.

13. Mechanick JI, Youdim A, Jones DB, et al. Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient—2013 update: Cosponsored by American association of clinical endocrinologists, The obesity society, and american society for metabolic & bariatric surgery. Obesity. 2013; 21: 1–27.
14. Huh JH, Kim KJ, Kim SU, et al. Obesity is an important determinant of severity in newly defined metabolic dysfunction-associated fatty liver disease. Hepatobiliary Pancreat Dis Int. 2022; 21: 241–247.
15. Kelley RK, Greten TF. Hepatocellular Carcinoma—Origins and Outcomes. N Engl J Med. 2021; 385: 280–282.
16. Hong CW, Marsh A, Wolfson T, et al. Reader agreement and accuracy of ultrasound features for hepatic steatosis. Abdom Radiol. 2019; 44: 54–64.
17. Webb M, Yeshua H, Zelber-Sagi S, et al. Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. AJR Am J Roentgenol. 2009; 192: 909–914.
18. Marshall RH, Eissa M, Bluth EI, et al. Hepatorenal index as an accurate, simple, and effective tool in screening for steatosis. AJR Am J Roentgenol. 2012; 199: 997–1002.
19. Zelber-Sagi S, Webb M, Assy N, et al. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. World J Gastroenterol. 2013; 19: 57–64.
20. Chauhan A, Sultan LR, Furth EE, et al. Diagnostic accuracy of hepatorenal index in the detection and grading of hepatic steatosis. J Clin Ultrasound. 2016; 44: 580–586.
21. Kozlowska-Petriczko K, Wunsch E, Petriczko J, et al. Diagnostic accuracy of non-imaging and ultrasound-based assessment of hepatic steatosis using controlled attenuation parameter (CAP) as reference. J Clin Med. 2021; 10: 1-14.

22. De Almeida e Borges VF, Diniz ALD, Cotrim HP, et al. Sonographic hepatorenal ratio: a noninvasive method to diagnose nonalcoholic steatosis. J Clin Ultrasound. 2013; 41: 18–25.

23. Mancini M, Prinster A, Annuzzi G, et al. Sonographic hepatic-renal ratio as indicator of hepatic steatosis: comparison with 1H magnetic resonance spectroscopy. Metabolism. 2009; 58: 1724–1730.

24. Martin-Rodriguez JL, Arrebola JP, Jimenez-Moleon JJ, et al. Sonographic quantification of a hepato-renal index for the assessment of hepatic steatosis in comparison with 3T proton magnetic resonance spectroscopy. Eur J Gastroenterol Hepatol. 2014; 26: 88–94.

25. Wong VWS, Adams LA, de Ledinghen V, et al. Noninvasive biomarkers in NAFLD and NASH—current progress and future promise. Nat Rev Gastroenterol Hepatol. 2018; 15: 461–478.

26. Lee JH, Kim D, Kim HJ, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis. 2010; 42: 503–508.

27. Yip TF, Ma AJ, Wong VS, et al. Laboratory parameter-based machine learning model for excluding non-alcoholic fatty liver disease (NAFLD) in the general population. Aliment Pharmacol Ther. 2017; 46: 447–456.

28. Salazar DE, Corcoran GB. Predicting creatinine clearance and renal drug clearance in obese patients from estimated fat-free body mass. Am J Med. 1988; 84: 1053–60.

29. Baccarani U, Adani GL, Isola M, et al. Steatosis of the graft is a risk factor for posttransplantation biliary complications. Transplant Proc. 2009; 4: 1313–1315.

30. Todo S, Demetris AJ, Makowka L, et al. Primary nonfunction of hepatic allografts with preexisting fatty infiltration. Transplantation. 1989; 47: 903–904.

31. Kang BK, Kim M, Shin SJ, Kim YJ. Correlation of clinical and histopathologic parameters with ultrasonographic grades in pediatric non-alcoholic fatty liver disease. J Korean Med Sci. 2019; 34: e298.

32. Fawcett T. An introduction to ROC analysis. Pattern Recognition Letters. 2006; 27: 861–874.

33. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988; 44: 837–845.

34. Parente DB, Perazzo H, Paiva FF, et al. Higher cut-off values of non-invasive methods might be needed to detect moderate-to-severe steatosis in morbid obese patients: a pilot study. Sci Rep. 2020; 10: 1–9.

35. Coccia F, Testa M, Guarisco G, et al. Noninvasive assessment of hepatic steatosis and fibrosis in patients with severe obesity. Endocrine. 2020; 67: 569–578.

36. Byrne CD, Targher G. NAFLD as a driver of chronic kidney disease. J Hepatol. 2020; 72: 785–801.

37. Faubel S, Patel NU, Lockhart ME, Cadnapaphornchai MA. Renal relevant radiology: use of ultrasonography in patients with AKI. Clin J Am Soc Nephrol. 2014; 9: 382–394.

38. Bedogni G, Bellentani S, Miglioli L, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006; 6: 1–7.

39. Bedogni G, Kahn HS, Bellentani S, Tiribelli C. A simple index of lipid overaccumulation is a good marker of liver steatosis. BMC Gastroenterol. 2010; 10: 1–8.

40. Teixeira J, Marroni CA, Zubiaurre PR, et al. Phase angle and non-alcoholic fatty liver disease before and after bariatric surgery. World J Hepatol. 2020; 12: 1004–1019.

41. Di Vincenzo O, Marra M, Sacco AM, et al. Bioelectrical impedance (BIA)-derived phase angle in adults with obesity: a systematic review. Clin Nutr. 2021; 40: 5238–5248.

**Table 1.** Clinical characteristics of the patients and the results of the laboratory tests. The parameters were presented as medians (plus first and third quartiles) for the continuous variables and as the number of cases in each category (percentage) for the nominal variables. The Spearman's correlation coefficients were calculated between the continuous parameters and the liver fat fraction levels. Significantly different results were highlighted with a bold font (*P*-value <0.05).

| Parameter | Control n=56 | NAFLD n=106 | *P*-value, differentiation | Correlation coefficient | *P*-value, correlation |
|---|---|---|---|---|---|
| Age, years | 38 (33.5, 46.5) | 41.5 (37, 47) | 0.09 | 0.122 | 0.12 |
| Female sex, n | 47 (84%) | 65 (61%) | **0.003** | - | - |
| Weight, kg | 119 (112.5, 136) | 127 (114, 144) | 0.08 | 0.174 | **0.03** |
| BSA, $m^2$ | 2.36 (2.27, 2.53) | 2.42 (2.27, 2.67) | 0.052 | 0.185 | **0.02** |
| BMI, $kg/m^2$ | 43.30 (40.18, 46.15) | 43.78 (41.58, 47.53) | 0.25 | 0.104 | 0.19 |
| Hypertension, n | 25 (45%) | 74 (70%) | **0.002** | - | - |

| | | | | | |
|---|---|---|---|---|---|
| Diabetes type 2, n | 6 (11%) | 26 (25%) | **0.04** | - | - |
| AST, U/l | 23 (20, 27) | 30 (23, 42) | <**0.001** | 0.475 | <**0.001** |
| ALT, U/l | 26 (20.5, 37) | 39.5 (27, 64) | <**0.001** | 0.479 | <**0.001** |
| GGT, U/l | 24 (19, 31) | 38 (24, 65) | <**0.001** | 0.449 | <**0.001** |
| Glucose, mg/dL | 92 (87.5, 98) | 100 (90, 115) | <**0.001** | 0.328 | <**0.001** |
| C-peptide, ng/ml | 2.93 (2.33, 3.78) | 4.05 (3.14, 5.37) | <**0.001** | 0.383 | <**0.001** |
| TCH, mmol/L | 4.65 (4.05, 5.23) | 4.62 (3.90, 5.33) | 0.57 | -0.057 | 0.47 |
| LDL, mmol/L | 3.28 (2.89, 3.98) | 3.48 (2.89, 4.16) | 0.46 | 0.056 | 0.48 |
| HDL, mmol/L | 1.24 (1.09, 1.049) | 1.11 (0.93, 1.27) | <**0.001** | -0.350 | <**0.001** |
| TG, mmol/L | 1.32 (1.04, 1.70) | 1.73 (1.45, 2.62) | <**0.001** | 0.341 | <**0.001** |
| WBC, $10^9$ /L | 7.5 (6.5, 8.9) | 8.1 (6.6, 9.4) | 0.36 | -0.052 | 0.51 |
| $HbA_{1C}$, % | 5.4 (5.2, 5.6) | 5.8 (5.5, 6.2) | <**0.001** | 0.432 | <**0.001** |
| Insulin, IU/mL | 14.3 (10.9, 21.4) | 22.3 (14.2, 35.7) | <**0.001** | 0.405 | <**0.001** |
| HOMA-IR | 3.34 (2.35, 4.39) | 5.67 (3.71, 9.99) | <**0.001** | 0.474 | <**0.001** |

| | | | | | |
|---|---|---|---|---|---|
| Creatinine, umol/L | 67.18 (63.65, 75.14) | 68.07 (63.65, 78.67) | 0.99 | -0.033 | 0.68 |
| eGFR | 149 (122.1, 171.6) | 153 (128.5, 178.1) | 0.21 | 0.159 | **0.04** |

Abbreviations: BSA, body surface area, BMI, body mass index, AST, aspartate aminotransferase, ALT, alanine amonitransferase, GGT, gamma-glutamyl transpeptidase, TCH, total cholesterol, LDL, lipoprotein cholesterol, HDL, high density lipoprotein cholesterol, TG, triglyceride, WBC, white blood cell count, $HbA_{1C}$ , hemoglobin A1c, HOMA-IR, insulin and homeostatic model assessment for insulin resistance, eGFR, glomerular filtration rate

**Table 2.** Median values (plus first and third quartiles) for the three NAFLD diagnosis techniques and the correlation coefficients between the parameters and the liver fat fraction levels. Significantly different results were highlighted with a bold font (*P*-value <0.05).

| Method | Control n=56 | NAFLD n=106 | *P*-value | Spearman's correlation coefficient | *P*-value |
|---|---|---|---|---|---|
| HRI | 0.98 (0.88, 1.20) | 1.44 (1.20, 1.63) | **<0.001** | 0.695 | **<0.001** |
| HSI | 54.6 (51.1, 59.4) | 57.1 (53.1, 61.1) | **0.04** | 0.215 | **0.006** |
| NAFLD | 0.46 | 0.84 | **<0.001** | 0.595 | **<0.001** |

| | | | | | |
|---|---|---|---|---|---|
| logit score | (0.30, 0.65) | (0.65, 0.96) | | | |

Abbreviations: HRI, hepatorenal index, HSI, hepatic steatosis index, NAFLD, Nonalcoholic fatty liver disease

**Table 3.** Performance metrics (plus standard errors) of the HRI, HSI and NAFLD logit score methods calculated for various cut-off values (optimal, 90% sensitivity, 90% specificity) in the case of the NAFLD diagnosis (fat fraction>5%) and the diagnosis of patients with high fat fraction of 30%.

| Method | Cut-off | Accuracy | Sensitivity | Specificity | AUC |
|---|---|---|---|---|---|
| | Diagnosis of patients with fat fraction above 5%. | | | | |
| HRI | 1.12 | 0.820 (0.023) | 0.849 (0.026) | 0.767 (0.046) | 0.879 (0.019) |
| | 1.03 | 0.796 (0.026) | 0.896 (0.021) | 0.607 (0.054) | |
| | 1.23 | 0.771 (0.025) | 0.707 (0.033) | 0.892 (0.033) | |
| HSI | 56.1 | 0.605 (0.030) | 0.613 (0.037) | 0.589 (0.053) | 0.599 (0.036) |
| | 50.0 | 0.636 (0.029) | 0.896 (0.023) | 0.143 (0.036) | |
| | 62.7 | 0.425 (0.029) | 0.179 (0.028) | 0.893 (0.032) | |
| NAFLD logit score | 0.59 | 0.790 (0.023) | 0.821 (0.026) | 0.732 (0.046) | 0.825 (0.025) |
| | 0.42 | 0.753 (0.031) | 0.896 (0.024) | 0.482 (0.061) | |
| | 0.80 | 0.691 (0.028) | 0.585 (0.037) | 0.893 (0.033) | |
| | Diagnosis of patients with fat fraction above 30% | | | | |
| HRI | 1.34 | 0.783 (0.025) | 0.844 (0.044) | 0.760 (0.029) | 0.825 (0.026) |
| | 1.14 | 0.623 (0.028) | 0.911 (0.031) | 0.512 (0.035) | |
| | 1.60 | 0.771 (0.023) | 0.444 (0.054) | 0.897 (0.022) | |
| HSI | 56.9 | 0.580 (0.029) | 0.622 (0.053) | 0.564 (0.034) | 0.577 (0.038) |

| | | | | | |
|---|---|---|---|---|---|
| | 49.3 | 0.321 (0.023) | 0.911 (0.032) | 0.094 (0.020) | |
| | 64.9 | 0.673 (0.029) | 0.089 (0.034) | 0.897 (0.021) | |
| NAFLD logit score | 0.80 | 0.753 (0.031) | 0.800 (0.044) | 0.735 (0.031) | 0.825 (0.027) |
| | 0.68 | 0.685 (0.032) | 0.911 (0.033) | 0.598 (0.040) | |
| | 0.93 | 0.777 (0.026) | 0.467 (0.063) | 0.897 (0.022) | |

Abbreviations: HRI, hepatorenal index, HSI, hepatic steatosis index, NAFLD, Nonalcoholic fatty liver disease

**Table 4.** Performance of the HRI technique reported in the previous papers for the general population. The HRI cut-offs were selected to differentiate NAFLD cases (fat fraction>5%).

| Reference | Cut-off | Sensitivity | Specificity | AUC | Number of patients |
|---|---|---|---|---|---|
| Borges et al. 2012 [22] | 1.24 | 0.927 | 0.925 | 0.964 | 82 |
| Marshall et al. 2012 [18] | 1.27 | 1 | 0.54 | 0.92 | 101 |
| Martın-Rodrıguez et al. 2014 [23] | 1.28 | 0.947 | 0.957 | 0.991 | 121 |
| Kozłowska-Petriczko et al. 2021[21] | 1.41 | 0.916 | 0.862 | 0.94 | 167 |
| Webb et al. 2009 [17] | 1.49 | 0.91 | 0.91 | 0.992 | 111 |
| Chauhan et al. | 2.01 | 0.625 | 0.952 | 0.96 | 45 |

| | | | | | |
|---|---|---|---|---|---|
| 2016 [20] | | | | | |
| Mancini et al. 2009 [23] | 2.20 | 1 | 0.95 | 0.996 | 40 |

Abbreviations: HRI, hepatorenal index

**Table 5.** Performance of the HSI and NAFLD logit score techniques reported in the original papers for the general population. The cut-off values were selected to perform NAFLD diagnosis (fat fraction>5%).

| Method | Cut-off | Sensitivity | Specificity | AUC | Number of patients |
|---|---|---|---|---|---|
| HSI [26] | 30 | 0.913 | 0.90 | 0.812 | 10,724 |
| | 36 | 0.90 | 0.931 | | |
| NAFLD logit score [27] | 0.19 | 0.9041 | 0.6695 | 0.88 | 922 |
| | 0.45 | 0.5685 | 0.9011 | | |

Abbreviations: HSI, hepatic steatosis index, NAFLD, Nonalcoholic fatty liver disease

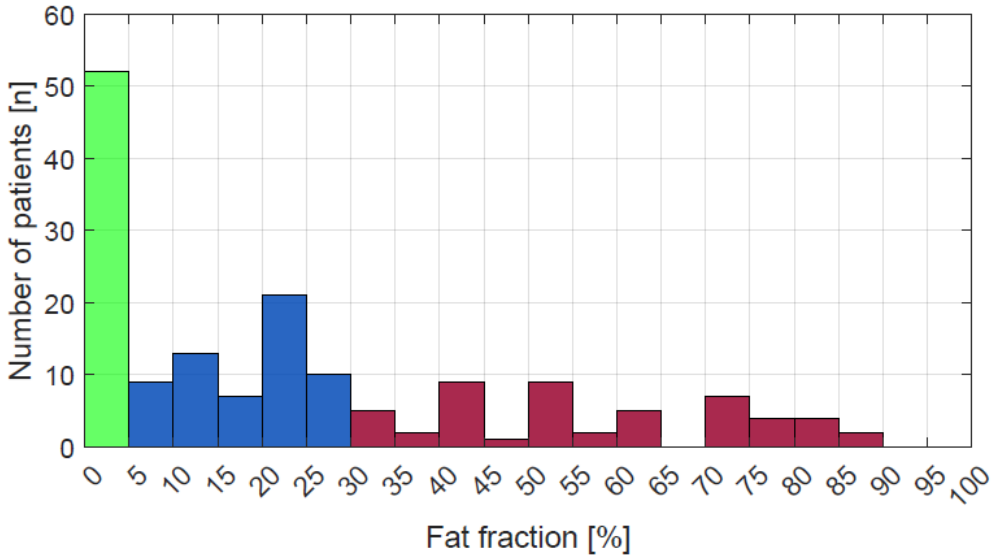


**Figure 1.** Distribution of the liver fat fraction values in the entire dataset of 162 cases.

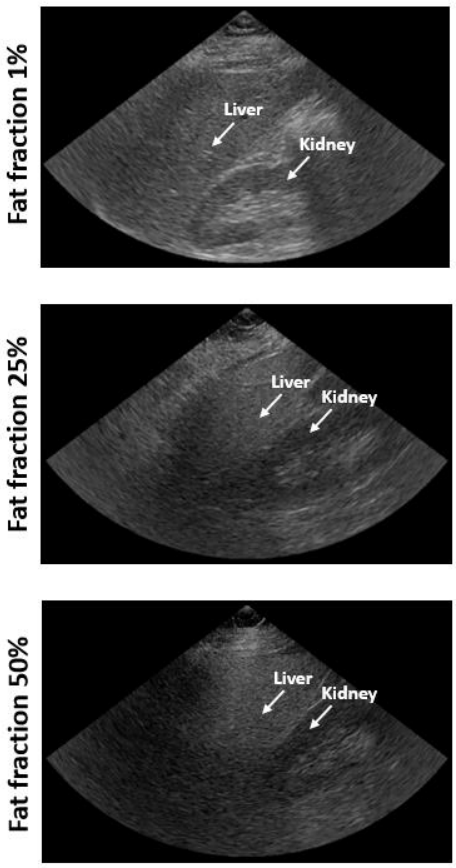


**Figure 2.** Liver/kidney view US images presenting cases with different levels of liver fat.

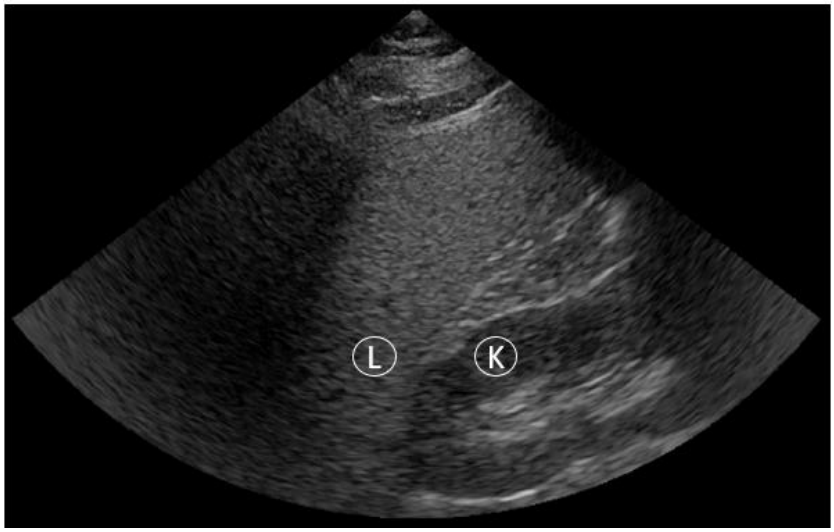


**Figure 3.** Example presenting the calculations of the hepatorenal index (HRI) parameter. Two regions of interest were used to outline uniform parts of the liver (letter L) and the kidney (letter K) to calculate average region pixel intensities and determine HRI.

**Short title:** Fatty liver disease assessment in patients with severe obesity