Ballestri *et al. Metab Target Organ Damage* 2023;3:13
**DOI:** 10.20517/mtod.2023.21

**Metabolism and Target Organ Damage**

**Commentary**

**Open Access**

Check for updates

# Ultrasonographic fatty liver indicator (US-FLI): a reliable biomarker for non-invasive NAFLD stratification

**Stefano Ballestri[1], Amedeo Lonardo[2]**

[1]Internal and Rehabilitation Medicine, Department of Internal Medicine, Hospital of Pavullo, Modena 41026, Italy.
[2]Metabolic and Neural Sciences, Azienda Ospedaliero Universitaria di Modena - Ospedale di Baggiovara, Modena 41026, Italy.

**Correspondence to:** Dr. Stefano Ballestri, Internal and Rehabilitation Medicine, Department of Internal Medicine, Hospital of Pavullo, Azienda USL of Modena, Via Suore di S. Giuseppe Benedetto Cottolengo 5, Pavullo nel Frignano, Modena 41026, Italy. E-mail: stefanoballestri78@gmail.com

**How to cite this article:** Ballestri S, Lonardo A. Ultrasonographic fatty liver indicator (US-FLI): a reliable biomarker for non-invasive NAFLD stratification. *Metab Target Organ Damage* 2023;3:13. https://dx.doi.org/10.20517/mtod.2023.21

**Received:** 27 Jun 2023 **First Decision:** 9 Aug 2023 **Revised:** 16 Aug 2023 **Accepted:** 21 Aug 2023 **Published:** 29 Aug 2023

**Academic Editor:** Sonia Najjar **Copy Editor:** Yanbing Bai **Production Editor:** Yanbing Bai

## Abstract

Here, we comment on a recent article supporting the use of the ultrasonographic fatty liver indicator (US-FLI) as a point-of-care biomarker to be used in the community to rule out nonalcoholic steatohepatitis (NASH). To this end, we discuss definitions and characteristics of US-FLI, and we critically summarize the principal studies published from 2012 to 2023. We conclude that US-FLI exhibits high reproducibility. It finds utility across both the pediatric population and the point-of-care settings. Furthermore, it demonstrates a robust correlation with metabolic derangements, and also serves as a predictive tool for varying grades of hepatic steatosis and important liver histology endpoints. Notably, it excels in its capacity to differentiate between bland steatosis and true NASH. However, US-FLI reportedly exhibits limited accuracy among patient populations with obesity. Finally, we propose a detailed agenda to advance research on US-FLI.

**Keywords:** Liver biopsy, NAFLD, NASH, ultrasonography

Defined as liver steatosis occurring in the absence of competing etiologies of steatogenic chronic liver disease (CLD), nonalcoholic fatty liver disease (NAFLD) embraces bland steatosis through nonalcoholic

steatohepatitis (NASH), which also features inflammation and hepatocellular ballooning[1]. Of concern, global NAFLD burdens have increased since 1990[2], accounting for direct and indirect financial costs.

Alarmingly, compared to individuals with NAFLD, NASH patients exhibit increased odds of developing cirrhosis and mortality owing to liver-related causes[3], which supports the importance of differentiating NASH from steatosis. This differential diagnosis requires liver biopsy, although this procedure is invasive and not invariably safe, and thus cannot be used in epidemiological studies[4]. Imaging techniques, such as nuclear magnetic resonance (NMR) proton density fat fraction, are deemed to be the surrogate reference standard for the evaluation of NAFLD[5]. However, drawbacks of NMR imaging comprise high costs, limited sampling of hepatic regions, susceptibility to motion artifacts from the heart, time consumption, and uneven availability across various institutions and countries[6]. Taken collectively, issues with liver biopsy and inconvenience with NMR make ultrasonographic techniques an excellent non-invasive imaging modality for use in the NAFLD arena[7]. Point-of-Care ultrasound (POCUS) refers to the use of echography to diagnose problems wherever a patient is being treated, conducted by a non-radiologist physician who is directly involved in the patient's care[8]. However, the application of POCUS in the field of NAFLD has been limited so far.

In 2012, Ballestri *et al.* conceived a novel ultrasound-based scoring system that they named ultrasonographic fatty liver indicator (US-FLI)[9]. US-FLI was based on the ultrasound semeiotics as follows: (severity of) liver-kidney contrast, posterior attenuation (of ultrasound beam), (difficult) visualization of the diaphragm, vessel blurring, (difficult) visualization of gallbladder wall, and focal sparing[9]. US-FLI could range from 2 to 8, and NAFLD was identified by a score ≥ 2. [Figures 1 and 2 reproduced, with permission from Ballestri *et al.*[9]]. Interestingly, US-FLI was correlated with metabolic variables [homeostasis model (HOMA), insulin, serum uric acid, ferritin, alanine transaminase (ALT), and bilirubin. Moreover, US-FLI was also associated with some histological features [such as steatosis extent and nonalcoholic steatohepatitis (NASH), except for fibrosis]. At multivariate analysis, US-FLI independently predicted NASH (OR 2.236; $P = 0.007$) and a US-FLI < 4 excluded severe NASH at histology with a negative predictive value of 94%.

Sourianarayanane and McCullough have recently validated US-FLI as a marker of NASH in the setting of POCUS delivered by primary care providers[10]. In their study, these authors found that US-FLI can reliably stratify NASH patients. This important study is only the last adjunct to a series of studies summarized in Table 1.

Taken collectively, the studies illustrated above[7,9-13] offer a complete outlook of the points of strengths and weaknesses of US-FLI. The former include high reproducibility[9,13], applicability to the pediatric population[12] and to the point-of-care setting[10], its strong association with metabolic derangements[9,11,12], different grades of hepatic steatosis assessed with transient elastography[6], and important liver histology endpoints, notably including its capacity to differentiate bland steatosis from true NASH[7,9,10,11]. As a result, US-FLI outperforms the fatty liver index (FLI)[13], a validated non-invasive biomarker proposed by Bedogni *et al.*, to help in picking out those subjects to submit to hepatic ultrasonography scanning to confirm steatosis (and whose scope has now been expanded to many other areas)[14] and represents an invaluable tool for triaging those patients in whom hepatic biopsy for suspected NASH is indicated. Coming to the limitations, US-FLI reportedly has limited accuracy among patient populations with obesity, given that these individuals often exhibit scores ≥ 5[7]. Moreover, US-FLI was devised at a time when the dichotomy bland steatosis vs. NASH was deemed the key research and clinical question to address. However, over time, assessment of fibrosis has become comparatively more important given that fibrosis dictates the hepatic and extra-hepatic outcomes of NAFLD, and several biomarkers and algorithms are

**Table 1. Principal published studies on US-FLI from 2012 to 2023**

| Author | Method | Findings | Conclusion |
|---|---|---|---|
| Ballestri *et al.*[9] | 53 individuals were submitted to both US and LB | US-FLI was correlated with metabolic parameters, associated with hepatic histology. US-FLI, independently predicted NASH (OR 2.236; *P* = 0.007) and US-FLI < 4 ruled out severe NASH (NPV = 94%) | This study was the first to propose US-FLI and validate it *vs.* liver histology in NAFLD/NASH arena |
| Ballestri *et al.*[11] | 352 patients were submitted to both US and LB: 173 had HCV, 23 HBV, 123 NAFLD (70.7% of whom had NASH), and 33 other liver diseases | In patients with NAFLD: (A) US-FLI ≥ 2 detected steatosis ≥ 10% (sensitivity 85.7%. specificity 87.5%) (AUC 0.893) and ≥ 5%-10% (sensitivity 95.9%-97.5%; specificity 66.7%) (AUC 0.956). (B) US-FLI was correlated with WC, BMI, HOMA and the number of traits of the MetS. (C) US-FLI was strongly correlated with steatosis extent (rho = 0.883; *P* < 0.001 in the whole sample). Moreover, among NAFLD patients, it was moderately correlated with the severity of lobular inflammation (rho = 0.490; *P* < 0.001); ballooning degeneration (rho = 0.485; *P* < 0.001); strongly correlated with Brunt's inflammatory grading (rho = 0.622; *P* < 0.001); and weakly correlated with portal fibrosis and stages of fibrosis | US-FLI detects mild-moderate hepatic steatosis (≥ 10% on histology) accurately and is correlated to histological and metabolic variables in CLD owing to different etiologies, notably including NAFLD |
| Liu *et al.*[12] | 117 children (10-18 years) were submitted to anthropometric and laboratory assessment. Hepatitis was defined by ALT > 40 units/L. LB was not performed in any patients | At multivariate analysis, US-FLI score was associated with WHR, WHtR, UA, adiponectin, and M30 levels (all *P* < 0.05) among children with obesity<br>US-FLI ≥ 6 was the best cut-off value for predicting hepatitis in children with NAFLD [PPV = 71.4%; AUC = 0.710 (95% CI: 0.572-0.847); *P* = 0.005] | Among children with obesity, US-FLI is correlated with anthropometric measures and laboratory tests<br>US-FLI ≥ 6 identifies raised ALT among children with NAFLD |
| Nelson *et al.*[7] | LB and US metrics were available in 208 adult obese individuals (mean age 47 years; age range 22 to 72; BMI 32.8 ± 5.1) with normal liver, bland steatosis, or NASH (*n* = 14; 89; and 105, respectively) | Poor gallbladder wall visualization was specific for NASH (89%), and vessel blurring was sensitive for NASH (93%)<br>US-FLI ≤ 4 ruled out NASH (NPP 88%; sensitivity 91%)<br>At LRA, vessel blurring predicted NASH (*P* ≤ .01). However, when the US-FLI score was ≥ 5, it performed poorly in differentiating steatosis from NASH (AUC = 0.649) | US-FLI accuracy may be low in differentiating steatosis from NASH among individuals with obesity and US-FLI ≥ 5 |
| Xavier *et al.*[13] | 31 patients were initially evaluated for assessing inter-observer reproducibility; 96 additional patients with NAFLD were submitted to the assessment of anthropometric, clinical, laboratory parameters, US and TE. Cut-off for steatosis > S1 was 268 dB/m and > S2 was 280 dB/m. LB was not performed | (A) Inter-observer agreement on the total US-FLI score was excellent [average Interclass Correlation Coefficient of 0.972(95% CI: 0.949-0.986)]<br>(B) US-FLI ≤ 3 had a NPV 100% for steatosis > S2 and US-FLI ≥ 6 points had a PPV of 94.0% for steatosis > S2<br>(C) AUC of FLI vs. US-FLI in discriminating the same CAP cut-offs was significantly different for both cut-offs (*P* < 0.001), indicating that FLI, compared to US-FLI, displayed a weaker capacity to differentiate both grades of steatosis | US-FLI is highly reproducible and accurately discriminates among different steatosis grades<br>US-FLI ≤ 3 rules out significant steatosis and scores ≥ 6 points have a PPV of 94,0% for steatosis > S2<br>US-FLI performs better than FLI in discriminating different steatosis grades |
| Sourianarayanane and McCullough[10] | 11 normal livers; 24 bland steatosis; and 78 NASH individuals were submitted to both US and LB | US-FLI ≥ 6 ruled in NASH and US-FLI ≤ 3 ruled out NASH (sensitivity 81% and 100%, respectively). In multivariate analysis, the difficult visualization of the gallbladder wall was the only independent predictor of NASH (LR 4.2, 95%CI: 1.07-8.7 *P* = 0.0226) | By confirming that it is a useful tool in excluding NASH, this study brings US-FLI to the community level and supports its use in epidemiological studies |

ALT: alanine transaminase; AUC: area under the curve; BMI: body mass index; CI: confidence interval; FLI: fatty liver index; HBV: hepatitis B virus; HCV: hepatitis C virus; HOMA: homeostasis model of insulin resistance; LB: liver biopsy; LR: likelihood ratio; LRA: logistic regression analysis; M30: caspase-cleaved cytokeratin fragment of cytokeratin 18; MetS: metabolic syndrome; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; NPV: negative predictive value; PPV: positive predictive power; rho: Spearman's coefficient; TE: transient elastography; UA: uric acid; US: ultrasonography; US-FLI: ultrasonographic fatty liver indicator; WC: waist circumference; WHR: waist-to-hip; WHtR: weight-to-height ratio.

available to this end[15,16].

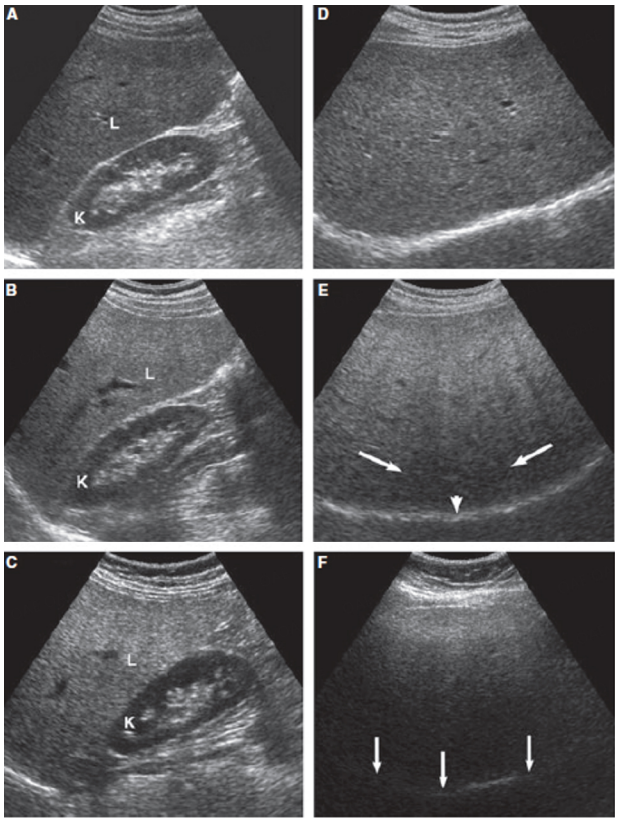


**Figure 1.** Illustration of US-FLI criteria and metrics: liver-kidney contrast, posterior attenuation of ultrasound beam and visualization of diaphragm. Absent -to – minimal liver-kidney contrast, which is normal, would receive a score = 0 (panel A). Conversely, the liver-kidney contrast of mild-moderate severity would be assigned a US-FLI score = 2 (panel B) and severe liver-kidney contrast would receive a US-FLI score = 3 (panel C). Absent posterior attenuation of ultrasound beam (PAUB) is normal and receives a US-FLI score = 0 (panel D). Conversely, PAUB present (highlighted with arrows) receives a US-FLI score = 1, although diaphragm remains visible, which is assigned a US-FLI score = 0 (panel E). Finally, impaired visualization of diaphragm (highlighted with arrows) owing to pronounced PAUB (panel F). These semiotics shown in panel F receive a US-FLI score = 1 each. Reproduced with permission from Ballestri *et al.*[9]. US-FLI: Ultrasonographic fatty liver indicator.

With this eminently diagnostic backset, it is somewhat unexpected that US-FLI may also contain some clues to better understand NASH pathomechanics. For example, the finding that a difficult visualization of gallbladder wall is specific for NASH[7] raises the possibility that accumulation of fat specifically either in the peri-cholecystic area or in the gallbladder wall may identify more aggressive liver histology. The vascular, histogenic or biochemical pathways underlying this phenomenon remain totally unexplored. However, it is possible that the hepato-cholecystic system behaves like a functional unit that is the target of severe metabolic derangements, as demonstrated, for example, by the association of NAFLD with gallstones. A recent umbrella review of 22 meta-analyses established that NAFLD carries an excess risk of gallstones[17] and, conversely, gallstones are an independent risk factor for NAFLD[18]. However, whether gallstones are also associated with more severe NAFLD remains controversial, with some studies supporting[19] and others disconfirming this contention[20]. Bringing this line of research further, authors have coined the definition of "fatty gallbladder disease" to identify increased gallbladder wall thickness and dysfunction of gallbladder motility, which are associated with NAFLD irrespective of gallstones[21].

Based on the study by Sourianarayanane and McCullough[10] as well as the others discussed here[7,9,11-13], it may be concluded that research on US-FLI needs to be further developed. Several lines of advancements in research can be envisaged. One possibility is to combine US-FLI with simple anthropometric indices or

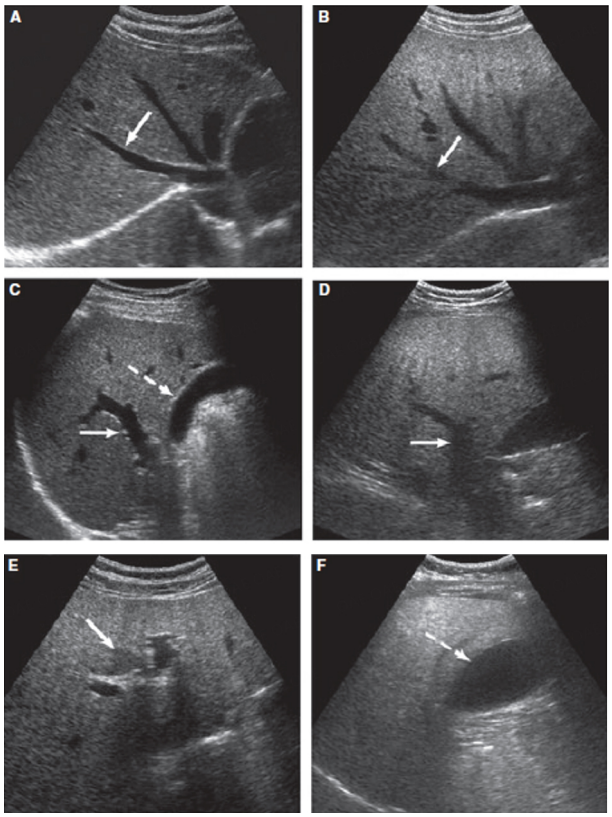


**Figure 2.** Illustration of US-FLI criteria and metrics: vessels blurring, focal sparing, and visualization of gallbladder wall. Normal appearance, with optimal visualization, of contours and lumen (highlighted with arrow) of hepatic veins (panel A). This semeiotics would receive a US-FLI score = 0. Conversely, impaired visualization of contours and lumen of hepatic veins would be assigned a US-FLI score = 1 (panel B). Panel C shows normal appearance of walls of portal branches (highlighted with an arrow) which would receive a US-FLI score = 0 and normal visualization of the gallbladder wall (highlighted with a dotted arrow) which is assigned a US-FLI score = 0. Impaired visibility of walls of portal branches (highlighted with an arrow) would receive a US-FLI score = 1 (panel D). An area of focal sparing (highlighted with an arrow) would be assigned a US-FLI score = 1 (panel E). Impaired visualization of wall of gallbladder (highlighted with a dotted arrow) is assigned a US-FLI score = 1 (panel F). Reproduced with permission from Ballestri *et al.*[9]. US-FLI: Ultrasonographic fatty liver indicator.

routine liver tests. Moreover, rather than attributing a similar score of 1 to each semeiotics, a more sophisticated analysis may eventually conduce to give a higher score to certain semeiotics such as vessel blurring and gallbladder wall visualization. Additionally, US-FLI must be validated among the cholecystectomized individuals. From a pathogenic perspective, it will be important to understand the histopathological grounds associating difficult visualization of the gallbladder wall with NASH. We highlight that NAFLD is associated with extra-hepatic complications, including a high risk of cardiovascular disease and cancer[15,16,22-24], although no data on the value of US-FLI in predicting such outcomes are available. US-FLI may also be combined with the measurement of spleen diameter and volume to detect the presence and severity of NAFLD[25]. Finally, it will be important to understand the accuracy of US-FLI among overweight patients and in the detection of the so-called "at-risk NASH", i.e., those patients with NAFLD activity score (NAS) ≥ 4, and fibrosis stage ≥ 2[26].

## DECLARATIONS

### Authors' contributions

Conception or design: Lonardo A

Acquisition, analysis, or interpretation of data: Lonardo A , Ballestri S
Creation of figures of the manuscript: Ballestri S
Drafting and revising the work: Lonardo A , Ballestri S
Final approval of the manuscript: Lonardo A , Ballestri S

### Availability of data and materials
Not applicable.

### Financial support and sponsorship
None.

### Conflicts of interest
All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate
Not applicable.

### Consent for publication
Not applicable.



## REFERENCES
1. Italian Association for the Study of the Liver (AISF). AISF position paper on nonalcoholic fatty liver disease (NAFLD): updates and future directions. *Dig Liver Dis* 2017;49:471-83. DOI PubMed
2. Wang D, Xu Y, Zhu Z, et al. Changes in the global, regional, and national burdens of NAFLD from 1990 to 2019: a systematic analysis of the global burden of disease study 2019. *Front Nutr* 2022;9:1047129. DOI PubMed PMC
3. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13:643-54.e1. DOI PubMed PMC
4. Lonardo A, Ballestri S. Perspectives of nonalcoholic fatty liver disease research: a personal point of view. Explor Med. 2020:1:85-107. 10.37349/emed.2020.00007. DOI
5. Yoshizawa E, Yamada A. MRI-derived proton density fat fraction. *J Med Ultrason (2001)* 2021;48:497-506. DOI PubMed
6. Ladd ME, Bachert P, Meyerspeer M, et al. Pros and cons of ultra-high-field MRI/MRS for human application. *Prog Nucl Magn Reson Spectrosc* 2018;109:1-50. DOI
7. Nelson SM, Hoskins JD, Lisanti C, Chaudhuri J. Ultrasound fatty liver indicator: a simple tool for differentiating steatosis from nonalcoholic steatohepatitis: validity in the average obese population. *J Ultrasound Med* 2020;39:749-59. DOI PubMed
8. Radonjić T, Popović M, Zdravković M, et al. Point-of-care abdominal ultrasonography (POCUS) on the way to the right and rapid diagnosis. *Diagnostics* 2022;12:2052. DOI PubMed PMC
9. Ballestri S, Lonardo A, Romagnoli D, et al. Ultrasonographic fatty liver indicator, a novel score which rules out NASH and is correlated with metabolic parameters in NAFLD. *Liver Int* 2012;32:1242-52. DOI
10. Sourianarayanane A, McCullough AJ. Accuracy of ultrasonographic fatty liver index using point-of-care ultrasound in stratifying non-alcoholic fatty liver disease patients. *Eur J Gastroenterol Hepatol* 2023;35:654-61. DOI PubMed
11. Ballestri S, Nascimbeni F, Baldelli E, et al. Ultrasonographic fatty liver indicator detects mild steatosis and correlates with metabolic/histological parameters in various liver diseases. *Metabolism* 2017;72:57-65. DOI
12. Liu HK, Yang MC, Su YT, et al. Novel ultrasonographic fatty liver indicator can predict hepatitis in children with non-alcoholic fatty liver disease. *Front Pediatr* 2018;6:416. DOI PubMed PMC
13. Xavier SA, Monteiro SO, Arieira CM, et al. US-FLI score - Is it possible to predict the steatosis grade with an ultrasonographic score? *Mol Genet Metab* 2021;132:204-9. DOI
14. Lonardo A, Ballestri S, Bedogni G, Bellentani S, Tiribelli C. The fatty liver index (FLI) 15 years later: a reappraisal. *Metab Target Organ Damage* 2021;1:10. DOI
15. Ballestri S, Mantovani A, Baldelli E, et al. Liver fibrosis biomarkers accurately exclude advanced fibrosis and are associated with higher cardiovascular risk scores in patients with NAFLD or viral chronic liver disease. *Diagnostics* 2021;11:98. DOI PubMed PMC

16. Ballestri S, Mantovani A, Girolamo MD, Baldelli E, Capitelli M, Lonardo A. Liver fibrosis in nonalcoholic fatty liver disease patients: noninvasive evaluation and correlation with cardiovascular disease and mortality. *Metab Target Organ Damage* 2023;3:1. DOI
17. Yi M, Peng W, Feng X, et al. Extrahepatic morbidities and mortality of NAFLD: an umbrella review of meta-analyses. *Aliment Pharmacol Ther* 2022;56:1119-30. DOI PubMed
18. Konyn P, Alshuwaykh O, Dennis BB, Cholankeril G, Ahmed A, Kim D. Gallstone disease and its association with nonalcoholic fatty liver disease, all-cause and cause-specific mortality. *Clin Gastroenterol Hepatol* 2023;21:940-948.e2. DOI PubMed
19. Kim YK, Kwon OS, Her KH. The grade of nonalcoholic fatty liver disease is an independent risk factor for gallstone disease: an observational study. *Medicine* 2019;98:e16018. DOI PubMed PMC
20. Yilmaz Y, Ayyildiz T, Akin H, et al. Gallstone disease does not predict liver histology in nonalcoholic fatty liver disease. *Gut Liver* 2014;8:313-7. DOI PubMed PMC
21. Colak Y, Bozbey G, Erim T, et al. Impaired gallbladder motility and increased gallbladder wall thickness in patients with nonalcoholic fatty liver disease. *J Neurogastroenterol Motil* 2016;22:470-6. DOI PubMed PMC
22. Tarantino G, Crocetto F, Di Vito C, et al. Association of NAFLD and Insulin resistance with non metastatic bladder cancer patients: a cross-sectional Retrospective study. *J Clin Med* 2021;10:346. DOI PubMed PMC
23. Mantovani A, Petracca G, Beatrice G, et al. Non-alcoholic fatty liver disease and increased risk of incident extrahepatic cancers: a meta-analysis of observational cohort studies. *Gut* 2022;71:778-88. DOI
24. Mantovani A, Csermely A, Tilg H, Byrne CD, Targher G. Comparative effects of non-alcoholic fatty liver disease and metabolic dysfunction-associated fatty liver disease on risk of incident cardiovascular events: a meta-analysis of about 13 million individuals. *Gut* 2023;72:1433-6. DOI PubMed
25. Tarantino G, Citro V, Balsano C. Liver-spleen axis in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2021;15:759-69. DOI PubMed
26. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. *Lancet Gastroenterol Hepatol* 2020;5:362-73. DOI PubMed PMC