ISSN 2150-5330 (online)

World Journal of *Gastrointestinal Pathophysiology*

Bimonthly Volume 15 Number 1 April 22, 2024

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Pathophysiology

Contents

Bimonthly Volume 15 Number 1 April 22, 2024

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Study

Observational Study

SYSTEMATIC REVIEWS

World Journal of Gastrointestinal Pathophysiology

Bimonthly Volume 15 Number 1 April 22, 2024

Contents

ABOUT COVER

AIMS AND SCOPE

The primary aim of the *World Journal of Gastrointestinal Pathophysiology* (*WJGP*, *World J Gastrointest Pathophysiol*) is to provide scholars and readers from various fields of gastrointestinal pathophysiology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJGP mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal pathophysiology and covering a wide range of topics including disorders of the esophagus, stomach and duodenum, small intestines, pancreas, biliary system, and liver.

INDEXING/ABSTRACTING

The *WJGP* is now abstracted and indexed in PubMed, PubMed Central, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL

World Journal of Gastrointestinal Pathophysiology

ISSN

ISSN 2150-5330 (online)

LAUNCH DATE

April 15, 2010

FREQUENCY

Bimonthly

EDITORS-IN-CHIEF

Kusum K Kharbanda, Tsutomu Nishida, Somchai Amornyotin

EDITORIAL BOARD MEMBERS

https://www.wjgnet.com/2150-5330/editorialboard.htm

PUBLICATION DATE

April 22, 2024

INSTRUCTIONS TO AUTHORS

https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS

https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH

https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS

https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT

https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE

https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS

https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION

https://www.f6publishing.com

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2024 April 22; 15(1): 92864

DOI: 10.4291/wjgp.v15.i1.92864

ISSN 2150-5330 (online)

MINIREVIEWS

Changes in the terminology and diagnostic criteria of non-alcoholic fatty liver disease: Implications and opportunities

Muhammed Mubarak

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade C
Creativity or Innovation: Grade C
Scientific Significance: Grade B

P-Reviewer: Dai DL, China

Received: February 8, 2024
Revised: April 4, 2024
Accepted: April 10, 2024
Published online: April 22, 2024

Muhammed Mubarak, Javed I. Kazi Department of Histopathology, Sindh Institute of Urology and Transplantation, Karachi 74200, Pakistan

Corresponding author: Muhammed Mubarak, MD, Full Professor, Javed I. Kazi Department of Histopathology, Sindh Institute of Urology and Transplantation, Chand Bibi Road, Karachi 74200, Pakistan. drmubaraksiut@yahoo.com

Abstract

Fatty liver disease (FLD) is a highly prevalent pathological liver disorder. It has many and varied etiologies and has heterogeneous clinical course and outcome. Its proper nomenclature and classification have been problematic since its initial recognition. Traditionally, it was divided into two main categories: Alcohol-associated liver disease and nonalcoholic FLD (NAFLD). Among these, the latter condition has been plagued with nomenclature and classification issues. The two main objections to its use have been the use of negative (non-alcoholic) and stigmatizing (fatty) terms in its nomenclature. Numerous attempts were made to address these issues but none achieved universal acceptance. Just recently, NAFLD has received a new nomenclature from an international collaborative effort based on a rigorous scientific methodology. FLD has been renamed steatotic liver disease (SLD), and NAFLD as metabolic dysfunction-associated SLD. Metabolic dysfunction-associated steatohepatitis was chosen as the replacement terminology for non-alcoholic steatohepatitis. This is a significant positive change in the nomenclature and categorization of FLD and will likely have a major impact on research, diagnosis, treatment, and prognosis of the disease in the future.

Key Words: Fatty liver disease; Metabolic syndrome; Non-alcoholic fatty liver disease; Steatotic liver disease; Steatohepatitis

Core Tip: Non-alcoholic fatty liver disease (NAFLD) is a very common illness in adult patients throughout the world and its prevalence has reached epidemic proportions in many parts of the world. Its nomenclature and classification have been controversial since its initial recognition compounded by rapid developments in understanding of its epidemiology and pathogenesis. In June 2023, its nomenclature was changed to steatotic liver disease (SLD) and NAFLD has been renamed metabolic dysfunction-associated SLD. This change in nomenclature and classification has not only implications for clinical practice but also provides opportunities to better understand the disease and its treatment.

Citation: Mubarak M. Changes in the terminology and diagnostic criteria of non-alcoholic fatty liver disease: Implications and opportunities. *World J Gastrointest Pathophysiol* 2024; 15(1): 92864
URL: https://www.wjgnet.com/2150-5330/full/v15/i1/92864.htm
DOI: https://dx.doi.org/10.4291/wjgp.v15.i1.92864

INTRODUCTION

Fatty liver disease (FLD) is not new, but its formal recognition and categorization is a relatively recent development (Figure 1). The association of hepatic steatosis with alcohol abuse is a well-known pathological entity with a well-recognized and non-controversial terminology of alcoholic liver disease (ALD). However, nonalcoholic FLD (NAFLD), a historical overarching term used for the spectrum of FLDs not linked to significant alcohol abuse or other known etiologic agents, was formally recognized only in the 1980s[1,2]. Since then, it has been plagued with nomenclature and diagnostic controversies. Its predecessor term, non-alcoholic steatohepatitis (NASH), was first named by Ludwig *et al*[3] in 1980. In the seminal report of 20 patients whose liver biopsy specimens exhibited striking fatty and necro-inflammatory changes, Mallory bodies, fibrosis, and cirrhosis, Ludwig *et al*[3] used the name "NASH" to distinguish it from the well-characterized pathological condition of ALD. The study subjects exhibited a high prevalence of obesity, female gender, type 2 diabetes (T2D), gallstones, and thyroid disease[3]. The broader term NAFLD was first used in a review article by Schaffner and Thaler[4] in 1986. Since these earlier descriptions of FLD, considerable progress has been made in understanding the disease's epidemiology, etiology, pathogenesis, pathology, treatment, and prognosis[5-10]. During the ensuing years, the prevalence of the disease has risen steeply throughout the world and it has attained epidemic proportions globally affecting more than 30% of the world's adult population. It has also attained the status of the dominant source of chronic liver disease and the dominant indication of liver transplantation globally[11-16]. The rise in prevalence has, in particular, paralleled the rise in the incidence of obesity, T2D, and metabolic syndrome (MS)[17-21]. Several studies also reported an association of the disease with cardiovascular disease, thus further complicating the disease's etiology and pathogenesis[22-25]. Although there is also no specific treatment for the disease to date, considerable progress has been made in understanding the pathogenesis and molecular mechanisms of the disease. These advancements have made it possible to develop targeted therapies, paving the way for personalized medicine[26-30]. This huge expansion of knowledge and advancements in understanding the disease were not reflected until very recently either in the nomenclature of the disease or its diagnostic criteria. All the above developments necessitated a revisiting of the terminology and classification of the disease[31,32]. This mini-review aims to summarize the history of nomenclature changes of this disease. This change is not merely a semantic process but has considerable implications not only for hepatologists but for several other stakeholders.

History of nomenclature changes

The term NASH was coined almost four decades ago by Ludwig and his colleagues in a seminal paper published in Mayo Proceedings journal[3]. Soon, it became the subject of intense research on multiple aspects of the disease to define its epidemiology, pathogenetic mechanisms, diagnosis, and treatment. Its overarching term, NAFLD, was first used by Schaffner and Thaler[4] in 1986 in a review article on FLDs (Figure 2). With the expansion of knowledge about different aspects of the disease and its widespread prevalence, it was soon realized that the original name does not reflect the continuously expanding knowledge and understanding of the disease's etiology and pathogenesis. Many acquired and genetic risk factors were identified and the heterogeneity and complexity of disease pathogenesis became obvious[33-35]. In a Single Topic Conference on NAFLD held in 2002 and sponsored by the American Association for the Study of Liver Diseases (AASLD), it was hotly debated to change the name of the disease to better reflect the etiopathogenesis of the disease and many alternative names were considered but no one name was approved[31].

The two main objections to the use of the term NAFLD were that it was based on negative or exclusionary diagnostic criteria and there were some stigmatizing terms in the name, *i.e.*, alcohol and fatty[36-40]. There was no link to the underlying etiology or risk factors of the disease in the name. In addition, the previous terminology excluded individuals harboring risk factors for NAFLD, for instance, T2D, who consumed greater quantities of alcohol than the non-alcoholic thresholds envisaged in the criteria.

In 2020, metabolic dysfunction-associated FLD (MAFLD) was proposed as a replacement for the acronym NAFLD by a small number of international liver experts, to emphasize the importance of systemic metabolic dysfunction in the etiopathogenesis of this disease[41,42]. They suggested MAFLD as the single overarching term for the entire spectrum of NAFLD. They also proposed positive diagnostic criteria for an affirmative diagnosis of MAFLD (Figure 2). The change of the terminology of "NAFLD" to "MAFLD" was done to highlight the dominant role of metabolic factors in the disease

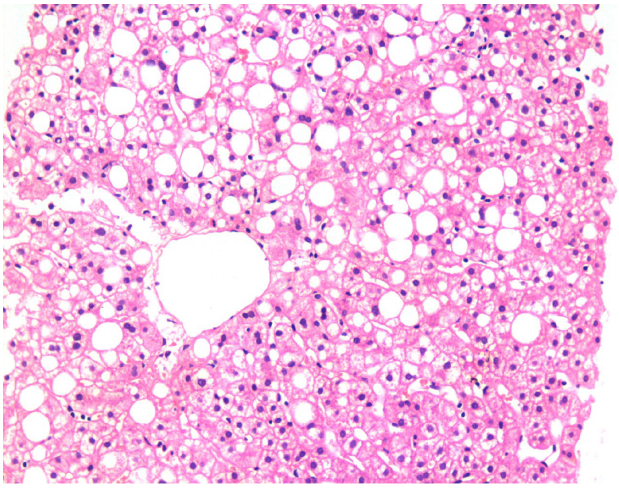

Figure 1 Liver biopsy showing fatty changes. There is a predominant macro-vesicular fatty change in the centrilobular area. There is no significant hepatocellular damage, inflammation, or fibrosis. This represents one end of the spectrum of fatty diseases of the liver (HE, × 200).

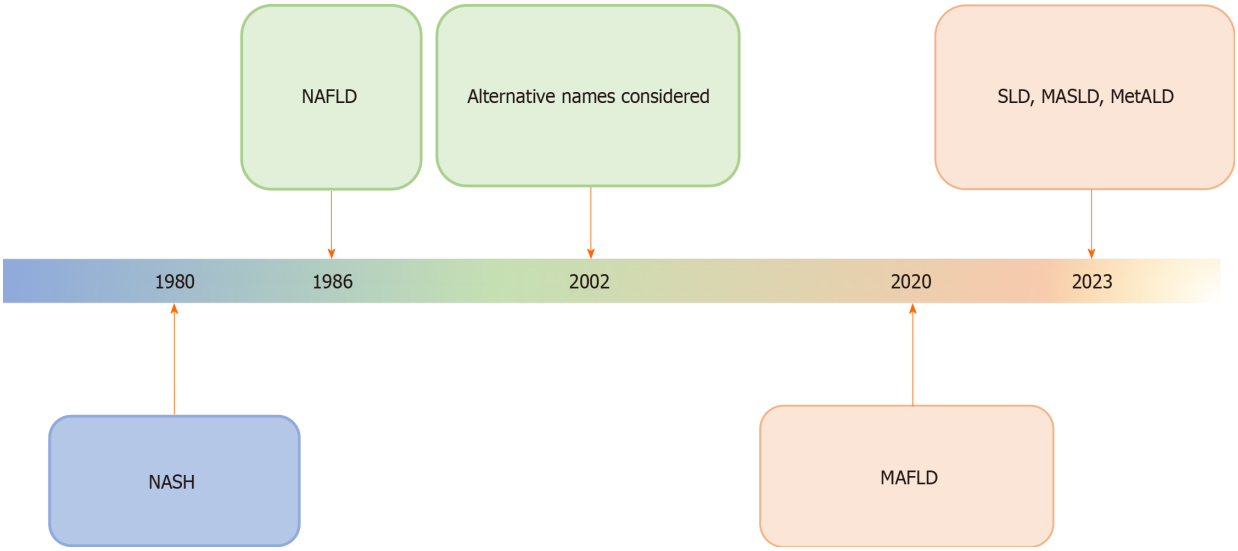

Figure 2 Schematic diagram showing the history of evolution of nomenclature changes of fatty liver diseases, particularly, non-alcoholic fatty liver disease. The most recent name suggested is the overarching term of steatotic liver disease. The rationalization of classification approaches is highlighted by changes in color. NASH: Non-alcoholic steatohepatitis; MAFLD: Metabolic dysfunction-associated fatty liver disease; MASLD: Metabolic dysfunction-associated SLD; MetALD: MASLD, and moderate alcohol intake; NAFLLD: Non-alcoholic fatty liver disease; SLD: Steatotic liver disease.

etiology and pathogenesis, thus improving patient understanding of the disease, facilitating patient-physician communication, and emphasizing the importance of public health interventions in its prevention and management[43-45]. However, the new term still included stigmatizing terms and did not cover the whole spectrum of steatotic liver disease (SLD). Some specific examples of discrepancies include, for example, not all cases of NAFLD are seen in obese or fatty individuals. These can be seen in lean individuals. Fat may not be demonstrated in the liver tissue in advanced stages of SLD resulting in cirrhosis. The new name was accepted by many societies dedicated to the study of liver diseases. Nevertheless, a broader international agreement was not accorded and some of the key pan-national and national societies did not fully endorse the terminology as many other stakeholders. Many liver experts described it as a premature attempt[46,47]. Concerns were also raised on the robustness and transparency of the methodology used for changing the name. They also removed the term steatohepatitis, a key lesion in progressive forms of FLD, from the nomenclature (Figure 3). The heterogeneity of NAFLD etiology and pathophysiology was ignored in MAFLD. In initial clinical trials, investigators concentrated on curtailing metabolic risk factors or insulin resistance, as NAFLD was predominantly thought of as a hepatic expression of the MS. However, most of these clinical trials with anti-obesity treatments, lipid-lowering agents, and insulin sensitizers, did not succeed in NAFLD treatment. The development of NAFLD is mediated by a variety of mechanisms and is much more complicated than formerly stipulated. Thus, a dominant focus on metabolic dysfunction could mask new treatment targets and delay the development of targeted therapeutics. Some important risk factors such as dysbiosis of the intestinal microbiota, genetic factors, and sarcopenia, were not given due consideration in the MAFLD transition. However, these factors are important contributory factors in the development of

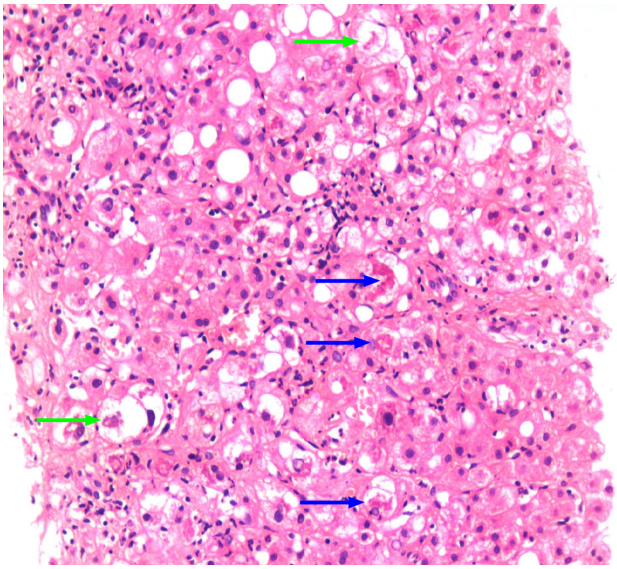

Figure 3 Liver biopsy showing morphological changes of non-alcoholic steatohepatitis, now named metabolic dysfunction-associated steatohepatitis. There is the macro-vesicular fatty change associated with ballooned hepatocytes (green arrow), inflammation, and fibrosis. A few Mallory bodies are seen in the ballooned hepatocytes (blue arrow). This represents the progressive form of fatty disease of the liver that may progress to cirrhosis if left untreated (HE, × 200).

NAFLD and may serve as targets for drug discovery. The new characterization of MAFLD also increased the spectrum of the study population in phase III clinical trials, since it also encompassed subjects with ALD or viral hepatitis. Moreover, the definition of the resolution of MAFLD as the key endpoint in clinical trials could result in controversial results. Presently, the resolution of NASH without augmentation of hepatic fibrosis is utilized as a tangible endpoint in clinical trials for NAFLD[43,44].

The new nomenclature

Acknowledging the above deficiencies, a wide-ranging and all-inclusive effort was started by the AASLD, the Asociación Latinoamericana para el Estudio del Hígado, and the European Association for the Study of the Liver to systematically and scientifically address this issue. This multi-stakeholder initiative not only involved hepatologists, but also included hepatopathologists, gastroenterologists, endocrinologists, and obesity and public health experts, along with representatives from regulatory agencies, industry, and patient advocacy groups. Their combined expertise and varied viewpoints helped achieve a new agreement on changing the diagnostic criteria and terminology for NAFLD[48].

The new nomenclature was developed from 2020 to early 2023 and was finalized in June 2023. The global consultation process used the structured, transparent, multistage survey-based Delphi technique along with hybrid meetings (Figure 4). During the process, a total of 236 panelists from 56 countries, and members of the NAFLD Nomenclature Consensus Group, contributed to four online surveys and two in-person meetings with a final response rate of > 75% for four rounds of data collection. In a preliminary survey, the terms 'non-alcoholic' and 'fatty' were considered to be stigmatizing by 61% and 66% of those who responded, respectively[48]. As the term ‘non-alcoholic’ was already replaced, the term ‘fatty’ was replaced by steatosis, a scientific and non-stigmatizing term. Thus, SLD was selected as an umbrella term to include all possible causes of steatosis including ALD (Figure 5). Five diagnostic sub-categories were created to encompass the entire spectrum of FLDs including ALD and combined forms of the disease. The term steatohepatitis was retained as it was considered to represent a crucial step in the progressive form of liver damage caused by fat accumulation and an integral part of the natural course of the disease. The term metabolic dysfunction-associated SLD (MASLD) was chosen in place of NAFLD. A consensus was also reached on changing the defining criteria. The presence of at least one among the five cardiometabolic risk factors was considered essential for the diagnosis. These are different for adult and pediatric patients. Metabolic dysfunction-associated steatohepatitis (MASH) was selected to represent the former term NASH. Cases in which no metabolic parameters and no known causes were obvious were categorized as cryptogenic SLD (Figure 5). Because of the frequent concurrence of the two pathologies, a new category, designated MetALD was chosen to represent those patients with MASLD who consume beyond threshold amounts of alcohol per week. The Delphi panel devised an algorithmic approach for categorizing the disease in individual patients (Figure 6), which is very helpful in clinical practice. It should be noted that the name change does not alter the natural history, biomarkers, or trials. The staging and grading of the disease will also not be affected by this change of terminology. The Delphi panel defined and separated a sub-category, MetALD, that has not been studied till now, which will benefit from being included in clinical trials and integrated into care pathways. According to proponents of the new nomenclature, there is a need for more work to be done to enhance disease familiarity, eliminate stigma, and speed up biomarker and targeted drug development to improve outcomes of patients with MASLD and MetALD[48].

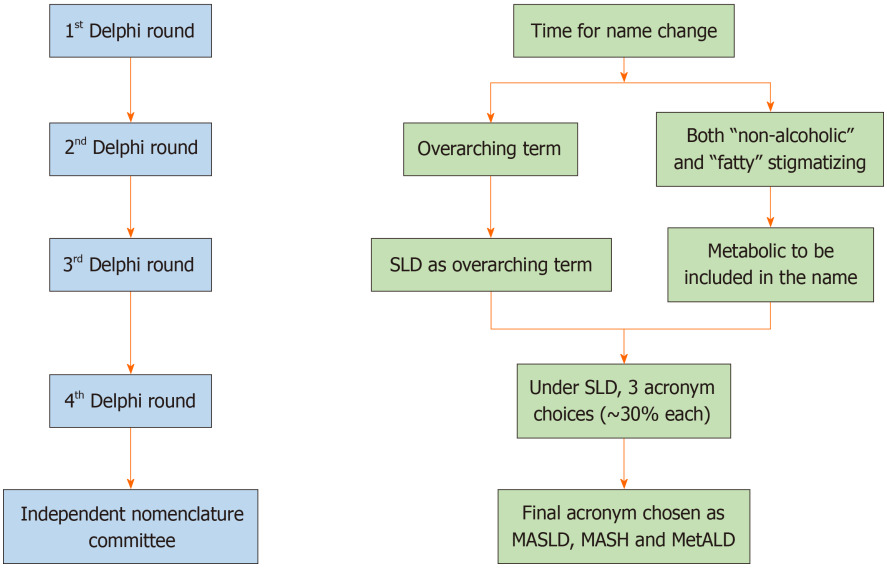

Figure 4 Overview of the main methodology used to change the name of fatty liver disease. The conclusions reached at each round of the Delphi method are shown on the right. Changes were also made in the diagnostic criteria (not shown here). An independent subcommittee comprising expert hepatologists, endocrinologists, pediatricians, and patients chose between the top three acronyms emerging from the fourth Delphi round. SLD: Steatotic liver disease; MASLD: Metabolic dysfunction-associated SLD; MASH: Metabolic dysfunction-associated steatohepatitis; MetALD: MASLD, and moderate alcohol intake.

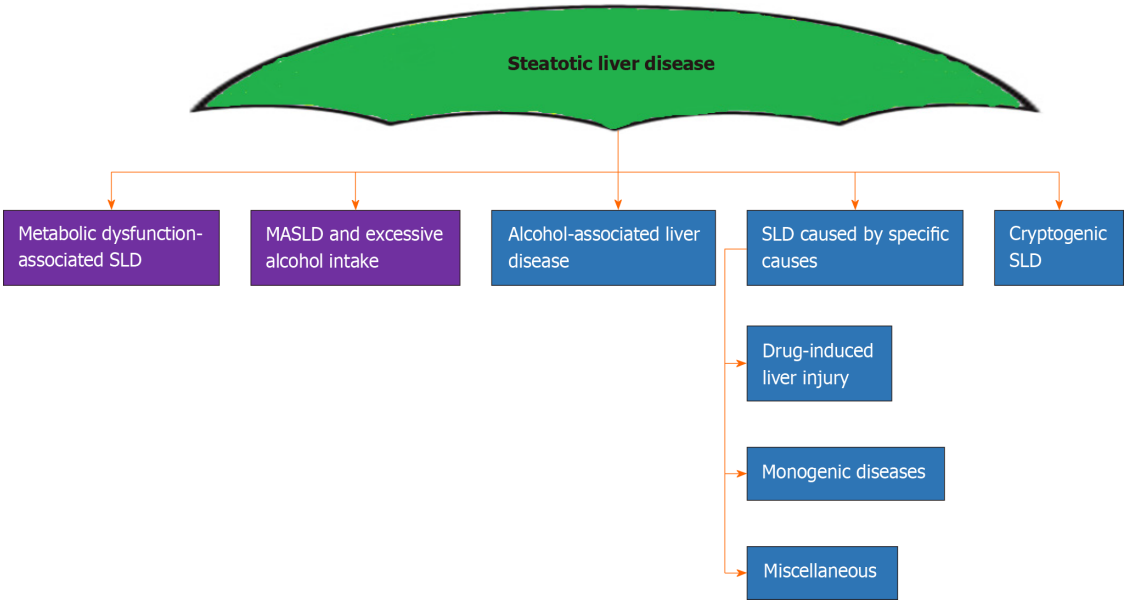

Figure 5 Steatotic Liver Disease and its sub-categorization. This figure shows the schema for steatotic liver disease (SLD) and its sub-categorization. SLD, diagnosed by imaging studies or histology, has many potential causes. Metabolic dysfunction-associated SLD, defined as hepatic steatosis together with one cardiometabolic risk factor and no other apparent cause, ALD, and an overlap of the two (MetALD), comprise the most common causes of SLD. Other specific causes of SLD need to be considered separately, as they exhibit distinct pathogenesis. Multiple etiologies of steatosis can coexist in one case. Those with no identifiable cause are currently placed under the cryptogenic SLD category. However, these may be reclassified in the future in response to an increase in our understanding of disease pathophysiology. SLD: Steatotic liver disease.

Implications and opportunities

The change of name from NAFLD to MASLD has many benefits and implications. It is an affirmative diagnosis with positive diagnostic criteria. It avoids the use of stigmatizing terms. It has been endorsed internationally and is widely accepted. It raises awareness of the disease process and its risk factors in primary care physicians and patients and elucidates treatment options more clearly, and encourages a comprehensive approach to managing patients with the disease. MASLD permits better management of patients with concurrent liver diseases other than MASLD. During the NAFLD era, chronic hepatitis C-infected patients, for example, were labeled as such regardless of the occurrence of liver steatosis. As a result, the significance of lifestyle changes in these patients was underestimated. Nevertheless, there is increasing evidence that concurrent liver steatosis aggravates the outcome in individuals with chronic viral hepatitis. Thus, the new initiative recognizes multiple causes of SLD and allows multidisciplinary management for such patients.

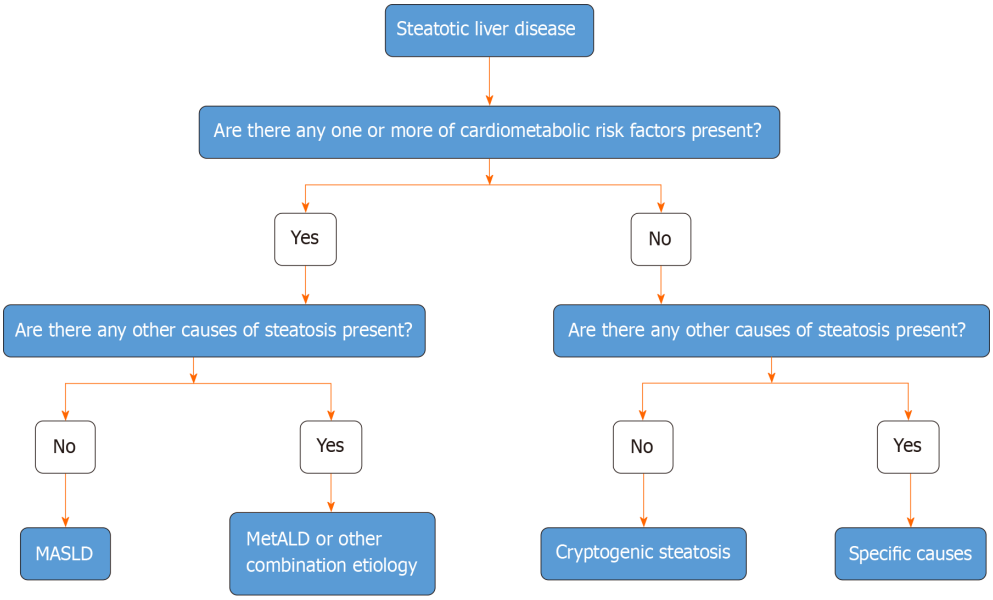

Figure 6 Algorithmic approach to the categorization of steatotic liver disease. In the presence of hepatic steatosis either on imaging or liver biopsy, the presence of any of a cardiometabolic risk factor (CMRF) will lead to a diagnosis of metabolic dysfunction-associated steatotic liver disease (SLD) in the absence of other causes of hepatic steatosis. If additional causes of steatosis are identified, then this will qualify for a combination etiology. In the case of alcohol, this is labeled MetALD. In the absence of overt CMRF, other causes must be excluded and if none is identified, this is labeled cryptogenic SLD. MASLD: Metabolic dysfunction-associated SLD; MetALD: MASLD, and moderate alcohol intake.

The term MASLD underscores metabolic dysfunction as the fundamental mechanism of hepatic steatosis, both through its terminology and the required diagnostic criteria. This change in nomenclature also would facilitate an instinctive elucidation of causes and management options for patients. The metamorphosis of name from NAFLD to MASLD is more than a semantic process and will have implications for research, government policies, the pharmaceutical industry, and insurance companies. The change in name to "MASLD" will require substantial changes in the designs of ongoing clinical trials of NAFLD, their main endpoints, clinical outcomes of final approval, and treatment targets due to the new inclusion criteria[48,49]. The transition to a new nomenclature for the FLD will require a step-by-step methodology to ensure its successful implementation and universal acceptance across the globe. The true implications of the changes in nomenclature and diagnostic criteria will become more obvious as the nomenclature and diagnostic modifications take effect in real life.

CONCLUSION

In conclusion, the new terminologies and diagnostic criteria have garnered widespread support, are non-stigmatizing, and provide a new and all-inclusive platform from which the medical community can increase disease cognizance, eradicate stigma, and speed up biomarker and drug development for better outcomes for patients with MASLD, MASH, and MetALD.

FOOTNOTES

Author contributions: Mubarak M is the sole author of the manuscript, and he conceived and designed the study, performed the research, participated in primary and final drafting, and has read and approved the final manuscript.

Conflict-of-interest statement: There is no conflict of interest associated with any of the senior author or other coauthors contributed their efforts in this manuscript.

Country/Territory of origin: Pakistan

ORCID number: Muhammed Mubarak 0000-0001-6120-5884.

S-Editor: Qu XL
L-Editor: A
P-Editor: Zhao YQ

REFERENCES

1 **Targher G**. What's Past Is Prologue: History of Nonalcoholic Fatty Liver Disease. *Metabolites* 2020; **10** [PMID: 33049948 DOI: 10.3390/metabo10100397]
2 **Ng CH**, Huang DQ, Nguyen MH. Nonalcoholic fatty liver disease versus metabolic-associated fatty liver disease: Prevalence, outcomes and implications of a change in name. *Clin Mol Hepatol* 2022; **28**: 790-801 [PMID: 35545437 DOI: 10.3350/cmh.2022.0070]
3 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]
4 **Schaffner F**, Thaler H. Nonalcoholic fatty liver disease. *Prog Liver Dis* 1986; **8**: 283-298 [PMID: 3086934]
5 **Diehl AM**, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. *N Engl J Med* 2017; **377**: 2063-2072 [PMID: 29166236 DOI: 10.1056/NEJMra1503519]
6 **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; **65**: 1038-1048 [PMID: 26823198 DOI: 10.1016/j.metabol.2015.12.012]
7 **Li B**, Zhang C, Zhan YT. Nonalcoholic Fatty Liver Disease Cirrhosis: A Review of Its Epidemiology, Risk Factors, Clinical Presentation, Diagnosis, Management, and Prognosis. *Can J Gastroenterol Hepatol* 2018; **2018**: 2784537 [PMID: 30065915 DOI: 10.1155/2018/2784537]
8 **Byrne CD**, Targher G. NAFLD: a multisystem disease. *J Hepatol* 2015; **62**: S47-S64 [PMID: 25920090 DOI: 10.1016/j.jhep.2014.12.012]
9 **Pafili K**, Roden M. Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. *Mol Metab* 2021; **50**: 101122 [PMID: 33220492 DOI: 10.1016/j.molmet.2020.101122]
10 **Nassir F**. NAFLD: Mechanisms, Treatments, and Biomarkers. *Biomolecules* 2022; **12** [PMID: 35740949 DOI: 10.3390/biom12060824]
11 **Riazi K**, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, Swain MG, Congly SE, Kaplan GG, Shaheen AA. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2022; **7**: 851-861 [PMID: 35798021 DOI: 10.1016/S2468-1253(22)00165-0]
12 **Teng ML**, Ng CH, Huang DQ, Chan KE, Tan DJ, Lim WH, Yang JD, Tan E, Muthiah MD. Global incidence and prevalence of nonalcoholic fatty liver disease. *Clin Mol Hepatol* 2023; **29**: S32-S42 [PMID: 36517002 DOI: 10.3350/cmh.2022.0365]
13 **Le MH**, Yeo YH, Li X, Li J, Zou B, Wu Y, Ye Q, Huang DQ, Zhao C, Zhang J, Liu C, Chang N, Xing F, Yan S, Wan ZH, Tang NSY, Mayumi M, Liu X, Rui F, Yang H, Yang Y, Jin R, Le RHX, Xu Y, Le DM, Barnett S, Stave CD, Cheung R, Zhu Q, Nguyen MH. 2019 Global NAFLD Prevalence: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2022; **20**: 2809-2817.e28 [PMID: 34890795 DOI: 10.1016/j.cgh.2021.12.002]
14 **Younossi Z**, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 11-20 [PMID: 28930295 DOI: 10.1038/nrgastro.2017.109]
15 **Quek J**, Chan KE, Wong ZY, Tan C, Tan B, Lim WH, Tan DJH, Tang ASP, Tay P, Xiao J, Yong JN, Zeng RW, Chew NWS, Nah B, Kulkarni A, Siddiqui MS, Dan YY, Wong VW, Sanyal AJ, Noureddin M, Muthiah M, Ng CH. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2023; **8**: 20-30 [PMID: 36400097 DOI: 10.1016/S2468-1253(22)00317-X]
16 **Younossi Z**, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, Wai-Sun Wong V, Yilmaz Y, George J, Fan J, Vos MB. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. *Hepatology* 2019; **69**: 2672-2682 [PMID: 30179269 DOI: 10.1002/hep.30251]
17 **Lee CH**, Lui DT, Lam KS. Non-alcoholic fatty liver disease and type 2 diabetes: An update. *J Diabetes Investig* 2022; **13**: 930-940 [PMID: 35080136 DOI: 10.1111/jdi.13756]
18 **Muzurović E**, Mikhailidis DP, Mantzoros C. Non-alcoholic fatty liver disease, insulin resistance, metabolic syndrome and their association with vascular risk. *Metabolism* 2021; **119**: 154770 [PMID: 33864798 DOI: 10.1016/j.metabol.2021.154770]
19 **Caussy C**, Aubin A, Loomba R. The Relationship Between Type 2 Diabetes, NAFLD, and Cardiovascular Risk. *Curr Diab Rep* 2021; **21**: 15 [PMID: 33742318 DOI: 10.1007/s11892-021-01383-7]
20 **Yki-Järvinen H**. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *Lancet Diabetes Endocrinol* 2014; **2**: 901-910 [PMID: 24731669 DOI: 10.1016/S2213-8587(14)70032-4]
21 **Wainwright P**, Byrne CD. Bidirectional Relationships and Disconnects between NAFLD and Features of the Metabolic Syndrome. *Int J Mol Sci* 2016; **17**: 367 [PMID: 26978356 DOI: 10.3390/ijms17030367]
22 **Targher G**, Byrne CD, Tilg H. NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications. *Gut* 2020; **69**: 1691-1705 [PMID: 32321858 DOI: 10.1136/gutjnl-2020-320622]
23 **Kasper P**, Martin A, Lang S, Kütting F, Goeser T, Demir M, Steffen HM. NAFLD and cardiovascular diseases: a clinical review. *Clin Res Cardiol* 2021; **110**: 921-937 [PMID: 32696080 DOI: 10.1007/s00392-020-01709-7]
24 **Boccatonda A**, Andreetto L, D'Ardes D, Cocco G, Rossi I, Vicari S, Schiavone C, Cipollone F, Guagnano MT. From NAFLD to MAFLD: Definition, Pathophysiological Basis and Cardiovascular Implications. *Biomedicines* 2023; **11** [PMID: 36979861 DOI: 10.3390/biomedicines11030883]
25 **Targher G**, Byrne CD, Tilg H. MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. *Gut* 2024; **73**: 691-702 [PMID: 38228377 DOI: 10.1136/gutjnl-2023-330595]
26 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9]
27 **Cobbina E**, Akhlaghi F. Non-alcoholic fatty liver disease (NAFLD) - pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. *Drug Metab Rev* 2017; **49**: 197-211 [PMID: 28303724 DOI: 10.1080/03602532.2017.1293683]
28 **Schwärzler J**, Grabherr F, Grander C, Adolph TE, Tilg H. The pathophysiology of MASLD: an immunometabolic perspective. *Expert Rev*

Clin Immunol 2024; 20: 375-386 [PMID: 38149354 DOI: 10.1080/1744666X.2023.2294046]

29 **Pierantonelli I**, Svegliati-Baroni G. Nonalcoholic Fatty Liver Disease: Basic Pathogenetic Mechanisms in the Progression From NAFLD to NASH. *Transplantation* 2019; **103**: e1-e13 [PMID: 30300287 DOI: 10.1097/TP.0000000000002480]

30 **Chan WK**, Chuah KH, Rajaram RB, Lim LL, Ratnasingam J, Vethakkan SR. Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD): A State-of-the-Art Review. *J Obes Metab Syndr* 2023; **32**: 197-213 [PMID: 37700494 DOI: 10.7570/jomes23052]

31 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219 [PMID: 12717402 DOI: 10.1053/jhep.2003.50193]

32 **Loria P**, Lonardo A, Carulli N. Should nonalcoholic fatty liver disease be renamed? *Dig Dis* 2005; **23**: 72-82 [PMID: 15920328 DOI: 10.1159/000084728]

33 **Huang TD**, Behary J, Zekry A. Non-alcoholic fatty liver disease: a review of epidemiology, risk factors, diagnosis and management. *Intern Med J* 2020; **50**: 1038-1047 [PMID: 31760676 DOI: 10.1111/imj.14709]

34 **Juanola O**, Martínez-López S, Francés R, Gómez-Hurtado I. Non-Alcoholic Fatty Liver Disease: Metabolic, Genetic, Epigenetic and Environmental Risk Factors. *Int J Environ Res Public Health* 2021; **18** [PMID: 34069012 DOI: 10.3390/ijerph18105227]

35 **Del Campo JA**, Gallego-Durán R, Gallego P, Grande L. Genetic and Epigenetic Regulation in Nonalcoholic Fatty Liver Disease (NAFLD). *Int J Mol Sci* 2018; **19** [PMID: 29562725 DOI: 10.3390/ijms19030911]

36 **Tang SY**, Tan JS, Pang XZ, Lee GH. Metabolic dysfunction associated fatty liver disease: The new nomenclature and its impact. *World J Gastroenterol* 2023; **29**: 549-560 [PMID: 36688021 DOI: 10.3748/wjg.v29.i3.549]

37 **Méndez-Sánchez N**, Díaz-Orozco LE. Editorial: International Consensus Recommendations to Replace the Terminology of Non-Alcoholic Fatty Liver Disease (NAFLD) with Metabolic-Associated Fatty Liver Disease (MAFLD). *Med Sci Monit* 2021; **27**: e933860 [PMID: 34248137 DOI: 10.12659/MSM.933860]

38 **Fouad Y**, Waked I, Bollipo S, Gomaa A, Ajlouni Y, Attia D. What's in a name? Renaming 'NAFLD' to 'MAFLD'. *Liver Int* 2020; **40**: 1254-1261 [PMID: 32301554 DOI: 10.1111/liv.14478]

39 **Valenzuela-Vallejo L**, Mantzoros CS. Time to transition from a negative nomenclature describing what NAFLD is not, to a novel, pathophysiology-based, umbrella classification of fatty liver disease (FLD). *Metabolism* 2022; **134**: 155246 [PMID: 35780909 DOI: 10.1016/j.metabol.2022.155246]

40 **Fouad Y**, Elwakil R, Elsahhar M, Said E, Bazeed S, Ali Gomaa A, Hashim A, Kamal E, Mehrez M, Attia D. The NAFLD-MAFLD debate: Eminence vs evidence. *Liver Int* 2021; **41**: 255-260 [PMID: 33220154 DOI: 10.1111/liv.14739]

41 **Eslam M**, Sanyal AJ, George J; International Consensus Panel. MAFLD: A Consensus-Driven Proposed Nomenclature for Metabolic Associated Fatty Liver Disease. *Gastroenterology* 2020; **158**: 1999-2014.e1 [PMID: 32044314 DOI: 10.1053/j.gastro.2019.11.312]

42 **Eslam M**, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Järvinen H, Fan JG, Grønbæk H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V, George J. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J Hepatol* 2020; **73**: 202-209 [PMID: 32278004 DOI: 10.1016/j.jhep.2020.03.039]

43 **Alomari M**, Rashid MU, Chadalavada P, Ragheb J, Zafar H, Suarez ZK, Khazaaleh S, Gonzalez AJ, Castro FJ. Comparison between metabolic-associated fatty liver disease and nonalcoholic fatty liver disease: From nomenclature to clinical outcomes. *World J Hepatol* 2023; **15**: 477-496 [PMID: 37206648 DOI: 10.4254/wjh.v15.i4.477]

44 **Kim D**, Konyn P, Sandhu KK, Dennis BB, Cheung AC, Ahmed A. Metabolic dysfunction-associated fatty liver disease is associated with increased all-cause mortality in the United States. *J Hepatol* 2021; **75**: 1284-1291 [PMID: 34380057 DOI: 10.1016/j.jhep.2021.07.035]

45 **Younossi ZM**, Paik JM, Al Shabeeb R, Golabi P, Younossi I, Henry L. Are there outcome differences between NAFLD and metabolic-associated fatty liver disease? *Hepatology* 2022; **76**: 1423-1437 [PMID: 35363908 DOI: 10.1002/hep.32499]

46 **Younossi ZM**, Rinella ME, Sanyal AJ, Harrison SA, Brunt EM, Goodman Z, Cohen DE, Loomba R. From NAFLD to MAFLD: Implications of a Premature Change in Terminology. *Hepatology* 2021; **73**: 1194-1198 [PMID: 32544255 DOI: 10.1002/hep.31420]

47 **Méndez-Sánchez N**, Zheng MH, Kawaguchi T, Sarin SK. Editorial: The Metabolic (Dysfunction) Associated Fatty Liver Disease (MAFLD)-Non-Alcoholic Fatty Liver Disease (NAFLD) Debate: A Forced Consensus and The Risk of a World Divide. *Med Sci Monit* 2022; **28**: e938080 [PMID: 36017581 DOI: 10.12659/MSM.938080]

48 **Rinella ME**, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, Arrese M, Bataller R, Beuers U, Boursier J, Bugianesi E, Byrne CD, Castro Narro GE, Chowdhury A, Cortez-Pinto H, Cryer DR, Cusi K, El-Kassas M, Klein S, Eskridge W, Fan J, Gawrieh S, Guy CD, Harrison SA, Kim SU, Koot BG, Korenjak M, Kowdley KV, Lacaille F, Loomba R, Mitchell-Thain R, Morgan TR, Powell EE, Roden M, Romero-Gómez M, Silva M, Singh SP, Sookoian SC, Spearman CW, Tiniakos D, Valenti L, Vos MB, Wong VW, Xanthakos S, Yilmaz Y, Younossi Z, Hobbs A, Villota-Rivas M, Newsome PN; NAFLD Nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* 2023; **78**: 1966-1986 [PMID: 37363821 DOI: 10.1097/HEP.0000000000000520]

49 **Yoon EL**, Jun DW. Waiting for the changes after the adoption of steatotic liver disease. *Clin Mol Hepatol* 2023; **29**: 844-850 [PMID: 37670441 DOI: 10.3350/cmh.2023.0291]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2024 April 22; 15(1): 91237

DOI: 10.4291/wjgp.v15.i1.91237

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Retrospective Study

Prognostic significance of tumor budding, desmoplastic reaction, and lymphocytic infiltration in patients with gastric adenocarcinoma

Aysen Yavuz, Kubra Simsek, Anil Alpsoy, Busra Altunay, Elif Ocak Gedik, Betul Unal, Cumhur Ibrahim Bassorgun, Ali Murat Tatli, Gulsum Ozlem Elpek

Specialty type: Pathology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Wang TJ, China

Received: December 25, 2023
Peer-review started: December 25, 2023
First decision: February 24, 2024
Revised: March 8, 2024
Accepted: March 22, 2024
Article in press: March 22, 2024
Published online: April 22, 2024

Aysen Yavuz, Kubra Simsek, Anil Alpsoy, Busra Altunay, Elif Ocak Gedik, Betul Unal, Cumhur Ibrahim Bassorgun, Gulsum Ozlem Elpek, Department of Pathology, Akdeniz University Medical School, Antalya 07070, Türkiye

Ali Murat Tatli, Department of Medical Oncology, Akdeniz University Medical School, Antalya 07070, Türkiye

Corresponding author: Gulsum Ozlem Elpek, MD, Professor, Department of Pathology, Akdeniz University Medical School, Dumlupinar Bulvarı, Antalya 07070, Türkiye.
elpek@akdeniz.edu.tr

Abstract

BACKGROUND

Recent studies have shown that the tumor microenvironment significantly influences the behavior of solid tumors. In this context, Accumulated data suggests that pathological evaluation of tumor budding (TB), desmoplastic reaction (DR), and tumor-infiltrating lymphocytes (TILs) may be crucial in determining tumor behavior in the gastrointestinal tract. Regarding gastric adenocarcinoma (GAC), although some results suggest that TB and TILs may be effective in determining the course of the disease, the data do not agree. Moreover, very few studies have investigated the relationship between DR and survival. At present, the associations between tumor TB, DR and TILs in GAC patients have not been determined.

AIM

To establish the relationships between TB, DR, and TILs in patients with GAC and to assess their influence on prognosis.

METHODS

Our study group comprised 130 patients diagnosed with GAC. The definition of TB was established based on the International TB Consensus Conference. The DR was categorized into three groups according to the level of tumor stroma maturation. The assessment of TILs was conducted using a semiquantitative approach, employing a cutoff value of 5%. The statistical analysis of the whole group and 100 patients with an intestinal subtype of GAC was performed using SPSS version 27.

RESULTS

A significant correlation between peritumoral budding (PTB) and intratumoral budding (ITB) was noted (r = 0.943). Tumors with high PTBs and ITBs had a greater incidence of immature DRs and low TILs (P < 0.01). PTB and ITB were associated with histological subtype, lymph node metastasis (LNM), and stage (P < 0.01). ITB, PTB, LNM, DR, and stage were significant risk factors associated with poor prognosis. The multivariate Cox regression analysis identified ITB, PTB, and LNM as independent prognostic variables (P < 0.05). In intestinal-type adenocarcinomas, a positive correlation between PTB and ITB was noted (r = 0.972). While univariate analysis revealed that LNM, stage, PTB, ITB, and DR were strong parameters for predicting survival (P < 0.05), only PTB and ITB were found to be independent prognostic factors (P < 0.001).

CONCLUSION

TB may be a potential prognostic marker in GAC. However, further studies are needed to delineate its role in pathology reporting protocols and the predictive effects of DR and TILs.

Key Words: Gastric cancer; Tumor budding; Desmoplastic stroma; Tumor-infiltrating lymphocytes; Prognosis

Core Tip: This study investigated the relationships between tumor budding, desmoplastic reaction (DR), and tumor-infiltrating lymphocytes (TILs) in patients with gastric adenocarcinomas (GAC) and assessed their influence on prognosis. Our results demonstrated that TB is a promising prognostic factor in GAC. While it could also be valuable in determining survival in patients with unresectable tumors, further studies are needed to draw a conclusion. Although the DR and TILs were not observed as independent parameters, their close association with TB in patients with GAC suggests their value in predicting tumor behavior merits further research to clarify their roles better.

Citation: Yavuz A, Simsek K, Alpsoy A, Altunay B, Gedik EO, Unal B, Bassorgun CI, Tatli AM, Elpek GO. Prognostic significance of tumor budding, desmoplastic reaction, and lymphocytic infiltration in patients with gastric adenocarcinoma. *World J Gastrointest Pathophysiol* 2024; 15(1): 91237

URL: https://www.wjgnet.com/2150-5330/full/v15/i1/91237.htm

DOI: https://dx.doi.org/10.4291/wjgp.v15.i1.91237

INTRODUCTION

Gastric adenocarcinoma (GAC), the sixth most common tumor in the world, are among the most lethal types of cancer worldwide and exhibit significant rates of recurrence even after curative surgical procedures[1,2]. While the tumor-node-metastasis classification is often preferred for predicting high risk, heterogeneity in the survival of patients at the same stage has necessitated the search for new prognostic indicators to better determine tumor behavior[3-5]. In recent years, much evidence has shown that epithelial-mesenchymal transition (EMT) plays a vital role in the aggressiveness of many cancers[6-8]. In this context, tumor budding (TB), which reflects EMT in particular, has been used in routine reporting protocols as an independent prognostic parameter in colorectal cancer (CRC) patients[9-11]. In GAC, although there is evidence that TB is associated with tumor behavior[12-14], the data do not reach an agreement[15-17]. Besides, different studies use different methods to evaluate TB, which limits the determination of the importance of this parameter in these tumors.

Recently, studies have demonstrated that the tumor microenvironment (TME) plays a more active role in tumor progression, contrasting with previous opinions that consider the formation of excessive fibrous or connective tissue, or, in other words, desmoplasia (DR), around a tumor as a simple host-related factor[18-20]. Therefore, the DR has been noted to be a determinant of tumor behavior in solid cancers, including CRC[21-23]. However, studies evaluating this parameter in GAC are rare[24-26].

Moreover, immune cells that constitute a part of the TME, especially lymphocytes infiltrating the tumor, may play a role in determining tumor behavior in GAC, as noted in other organ tumors[27].

Recently, few studies in GAC have pointed to the association of high TB with immature stroma and tumor-infiltrating lymphocytes (TILs)[26,28]. Nonetheless, in patients with GAC, the interplay between these parameters and their efficiency in determining tumor behavior and survival have yet to be compared.

Therefore, this study aimed to investigate the relationships among DR, TB, TILs, clinicopathological parameters, and prognosis in GAC.

MATERIALS AND METHODS

Patient selection

This retrospective study included patients diagnosed at the Department of Pathology, Akdeniz University Medical School, Antalya, Türkiye, who underwent total or partial gastrectomy for GAC between 2004 and 2019. One hundred thirty patients were selected after excluding patients with other cancers, who underwent neoadjuvant therapy, or who had incomplete clinicopathological data. All patient-related data were collected and revised. Follow-up data were retrieved from patient records from the Department of Oncology of our institution. Tumor subtyping was performed according to the Lauren classification[29]. All patients were staged based on the eighth edition of the American Joint Committee on Cancer manual[30].

The study protocol was based on the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of Akdeniz University.

Histopathological evaluation of TB, DR, and TILs

Hematoxylin and eosin (H&E)-stained slides from tumor blocks were reevaluated using light microscopy, and slides with low maturation of the tumor stroma, high tumor bud density, and high lymphocytic infiltration were selected for further analysis.

The assessment of peritumoral budding (PTB) and intratumoral budding (ITB) in this study followed the International TB Consensus Conference (ITBCC) guidelines[31]. In brief, a single tumor cell or a cluster of up to four tumor cells at the invasive front and within the primary tumor body were considered PTB and ITB, respectively. The count was determined in a standardized field area of 0.785 mm^2 at 200 × total magnification, and both PTB and ITB were categorized into three grades: grade 1 (0-4 TB), grade 2 (5-9 TB), and grade 3 (> 10 TB) (Figure 1).

DRs were evaluated and classified into three groups based on the maturation of the tumor stroma, as described by Ueno *et al*[32]. Mature-type DR comprised fine collagen fibers in multiple layers (DR1). While intermediate-type DR contained keloid-like collagen (DR2), immature-type DR constituted from the myxoid stroma (DR3) and occupied more than a 40 × objective lens field on slides (Figure 1).

The evaluation of TILs was performed semiquantitatively based on a 5% cutoff value on H&E-stained slides at a magnification of 200 ×[33]. Lymphatic infiltrates outside the tumor borders were excluded from the evaluation (Figure 1).

Statistical analysis

The data were analyzed with SPSS 27.0. Spearman's correlation test was used to evaluate the relationship between PTB and the ITB. The categorical data were examined by the chi-square test. Univariate survival analysis was performed with the Kaplan-Meier method, and the log-rank test was used to compare survival rates. A multivariate Cox proportional hazards regression model was applied to predict parameters influencing patient prognosis[34]. A P value < 0.05 indicated statistical significance. Furthermore, similar tests were also performed in patients with intestinal-type GAC, which allowed the application of these analyses (100 patients).

RESULTS

Clinicopathological and prognostic findings in the whole cohort

The clinicopathological characteristics of the patients in the study group are presented in Table 1. In brief, the mean age was 62.14 years ± 12.00 years (range 28 years to 89 years), and 53 females and 77 males were included. Patients were categorized into two groups for further analysis based on their mean age and mean tumor diameter (1.86 cm ± 1.02 cm, range 1.0 cm to 6.8 cm); regarding the level of invasion, a great majority of patients were classified as having tumors limited to the subserosa (40.0%), followed by tumors limited to the muscularis propria (30.0%), and tumors with invasion beyond the serosa and adjacent organs (24.6%). Invasion of the mucosa and submucosa was observed in 5.4% of the patients. Lymph node metastasis (LNM) was observed in 39.6% of the patients. The median follow-up period was 39 months (2-120 months, mean 42.44 months).

The patients were divided into three groups according to their PTB status, which resulted in 26 patients (20.0%) being classified as PTB1, 42 patients (32.3%) as PTB2, and 62 patients (47.7%) as PTB3. The ITB groups were categorized as follows: 31 patients (23.8%) were classified as ITB1, 25 patients (19.2%) as ITB2, and 74 patients (56.9%) as ITB3.

According to the DR classification, a total of 58 (44.6%) patients were classified as DR1, 38 (29.2%) patients as DR2, and 34 (26.2%) patients as DR3. The number of patients with TILs less than the cutoff value (68 patients, 52.3%) outnumbered that with higher lymphocytic infiltration (62 patients, 47.7%).

The relationships between clinicopathological parameters and PTB, ITB, DR, and TILs are presented in Table 1. There was a positive correlation between PTB and invasion and distant metastasis (P < 0.05). Higher PTB and ITB were more frequently observed in patients with LNM (P < 0.001). Similarly, both parameters were associated with the disease stage (P < 0.001). Compared with those with intestinal carcinomas, patients with higher PTB and ITB were more likely to have diffuse and mixed subtypes (P < 0.01).

Spearman correlation analysis revealed a strong correlation between PTB and ITB (r = 0.943, Figure 2). In patients with either PTB or ITB, immature stroma (DR3) and low TILs were more frequent (P < 0.01) (Table 1).

In the total cohort, the median OS was 36.5 ± 14.26 (ranging from 2 to 120 months). According to the univariate analysis, histologic subtype, ITB, PTB, LNM, DR, and stage were identified as risk factors for poor prognosis (P < 0.01)

Table 1 Tumor budding, desmoplastic reaction and tumor-infiltrating lymphocytes in relation to clinicopathological parameters in the whole group

Parameters	*n*	PTB1 (%)	PTB2 (%)	PTB3 (%)	ITB1 (%)	ITB2 (%)	ITB3 (%)	DR1 (%)	DR2 (%)	DR3 (%)	TILs (%)	TILs (%)
Age, yr												
< 62.14 ± 12.00	64	11 (42.3)	25 (59.5)	28 (45.2)	15 (48.4)	14 (56.0)	35 (47.3)	29 (50.0)	20 (52.6)	15 (44.1)	34 (50.0)	30 (48.4)
≥ 62.14 ± 12.00	66	15 (57.7)	17 (40.5)	34 (54.8)	16 (51.6)	11 (44.0)	39 (52.7)	29 (50.0)	18 (47.4)	19 (55.9)	34 (50.0)	32 (51.6)
Gender												
Male	77	16 (61.5)	26 (61.9)	35 (56.5)	18 (58.1)	19 (76.0)	40 (54.1)	43 (74.1)	23 (60.5)	11 (32.4)[a]	36 (52.9)	41 (66.1)
Female	53	10 (38.5)	16 (38.1)	27 (43.5)	13 (41.9)	6 (24.0)	34 (45.9)	15 (25.9)	15 (39.5)	23 (67.6)	32 (47.1)	21 (33.9)
Diameter												
< 1.86 ± 1.02	84	16 (61.5)	29 (69.0)	39 (62.9)	16 (51.6)	19 (76.0)	49 (66.2)	34 (58.6)	28 (73.7)	22 (64.7)	47 (69.1)	37 (59.7)
≥ 1.86 ± 1.02	46	10 (38.5)	13 (31.0)	23 (37.1)	15 (48.4)	6 (24.0)	25 (33.8)	24 (41.4)	10 (26.3)	12 (35.3)	21 (30.9)	25 (40.3)
Invasion												
T1	7	5 (19.2)	2 (4.8)	0[b]	4 (12.9)	1 (4.0)	2 (2.7)	3 (5.2)	3 (7.9)	1 (2.9)	4 (5.9)	3 (4.8)
T2	39	4 (15.4)	15 (35.7)	20 (32.3)	12 (38.7)	8 (32.0)	19 (25.7)	22 (37.9)	9 (23.7)	8 (23.5)	18 (26.5)	21 (33.9)
T3	52	11 (42.3)	15 (35.7)	26 (41.9)	10 (32.3)	6 (24.0)	36 (48.6)	25 (43.1)	16 (42.1)	11 (32.4)	27 (39.7)	25 (40.3)
T4	32	6 (23.1)	10 (23.8)	16 (25.8)	5 (16.1)	10 (40.0)	17 (23.0)	8 (13.8)	10 (26.3)	14 (41.2)	19 (27.9)	13 (21.0)
LNM												
Absent	82	23 (88.5)	29 (69.0)	30 (48.4)[a]	29 (93.5)	16 (64.0)	37 (50.0)[a]	43 (74.1)	23 (60.5)	16 (47.1)[c]	38 (55.9)	44 (71.0)
Present	48	3 (11.5)	13 (31.0)	32 (51.6)	2 (6.5)	9 (36.0)	37 (50.0)	15 (25.9)	15 (39.5)	18 (52.9)	30 (44.1)	18 (29.0)
Metastasis												
Absent	106	26 (100.0)	31 (73.8)	49 (79.0)[c]	21 (67.7)	23 (92.0)	62 (83.8)	49 (84.5)	33 (86.8)	24 (70.6)	55 (80.9)	51 (82.3)
Present	24	-	11 (26.2)	13 (21.0)	10 (32.3)	2 (8.0)	12 (16.2)	9 (15.5)	5 (13.2)	10 (29.4)	13 (19.1)	11 (17.7)
Stage												
I	21	11 (42.3)	9 (21.4)	1 (1.6)[a]	10 (32.3)	6 (24.0)	5 (6.8)[a]	13 (22.4)	6 (15.8)	2 (5.9)	13 (19.1)	8 (12.9)
II	38	6 (23.1)	12 (28.6)	20 (32.3)	8 (25.8)	4 (16.0)	26 (35.1)	20 (34.5)	11 (28.9)	7 (20.6)	18 (26.5)	20 (32.3)
III	48	8 (30.8)	11 (26.2)	29 (46.8)	3 (9.7)	14 (56.0)	31 (41.9)	16 (27.6)	15 (39.5)	17 (50.0)	24 (35.3)	24 (38.7)
IV	23	1 (3.8)	10 (23.8)	12 (19.4)	10 (32.3)	1 (4.0)	12 (16.2)	9 (15.5)	6 (15.8)	8 (23.5)	13 (19.1)	10 (16.1)
Subtype												

Intestinal	100	24 (92.4)	39 (92.9)	37 (59.7)[a]	30 (96.8)	21 (84.0)	49 (66.2)[b]	47 (81.0)	28 (73.7)	25 (73.5)	52 (76.5)	48 (77.4)
Diffuse	19	1 (3.8)	3 (7.1)	15 (24.2)	1 (3.2)	2 (8.0)	16 (21.6)	8 (13.8)	4 (10.5)	7 (20.6)	10 (17.7)	9 (14.5)
Mixed	11	1 (3.8)	0	10 (16.1)	0	2 (8.0)	9 (12.2)	3 (5.2)	6 (15.8)	2 (5.9)	6 (8.8)	5 (8.1)
LVI												
Absent	89	14 (53.8)	28 (66.7)	47 (75.8)	16 (51.6)	19 (76.0)	54 (73.0)	39 (67.2)	24 (63.2)	26 (76.5)	45 (66.2)	44 (71.0)
Present	41	12 (46.2)	14 (33.3)	15 (24.2)	15 (48.4)	6 (24.0)	20 (27.0)	19 (32.8)	14 (36.8)	8 (23.5)	23 (33.8)	18 (29.0)
PNI												
Absent	94	16 (61.5)	28 (66.7)	50 (80.6)	18 (58.1)	19 (76.0)	57 (77.0)	43 (74.1)	25 (65.8)	26 (76.5)	52 (76.5)	42 (67.7)
Present	36	10 (38.5)	14 (33.3)	12 (19.4)	13 (49.0)	6 (24.0)	11 (23.0)	15 (25.9)	13 (34.2)	8 (23.5)	16 (23.5)	20 (32.3)
Survival												
Deceased	100	9 (34.6)	29 (69.0)	62 (100.0)[a]	8 (25.8)	20 (80.0)	72 (97.3)[a]	40 (69.0)	29 (76.3)	31 (91.2)	56 (82.4)	44 (71.0)
Alive	30	17 (65.4)	13 (31.0)	0	23 (74.2)	5 (20.0)	2 (2.7)	18 (31.0)	9 (23.7)	3 (8.8)	12 (17.6)	18 (29.0)
PTB												
PTB1	26	-	-	-	17 (54.8)	3 (12.0)	6 (8.1)[a]	15 (25.9)	8 (21.1)	3 (8.8)[d]	10 (14.7)	16 (25.8)[b]
PTB2	42	-	-	-	14 (45.2)	18 (72.0)	10 (13.5)	24 (41.4)	12 (31.6)	6 (17.7)	17 (25.0)	25 (40.3)
PTB3	62	-	-	-	0	4 (16.0)	58 (78.4)	19 (32.8)	18 (47.4)	25 (73.5)	41 (60.3)	21 (33.9)
ITB												
ITB1	31	-	-	-	-	-	-	21 (36.2)	8 (21.1)	2 (5.9)[d]	9 (13.2)	22 (35.5)[d]
ITB2	25	-	-	-	-	-	-	13 (22.4)	6 (15.8)	6 (17.6)	13 (19.2)	12 (19.4)
ITB3	74	-	-	-	-	-	-	24 (41.4)	24 (63.2)	26 (76.5)	46 (67.6)	28 (45.1)
DR												
DR1	58	-	-	-	-	-	-	-	-	-	22 (32.4)	36 (58.0)[b]
DR2	38	-	-	-	-	-	-	-	-	-	24 (35.2)	14 (22.6)
DR3	34	-	-	-	-	-	-	-	-	-	22 (32.4)	12 (19.4)

[a]$P < 0.001$.
[b]$P < 0.01$.
[c]$P < 0.05$.
[d]$P < 0.01$.
N: Number of cases; PTB: Peritumoral budding; ITB: Intra-tumoral budding; DR: Desmoplastic reaction; TILs: Tumor-infiltrating lymphocytes; LVI: Lymphovascular invasion; PNI: Perineural invasion; LNM: Lymph node metastasis.

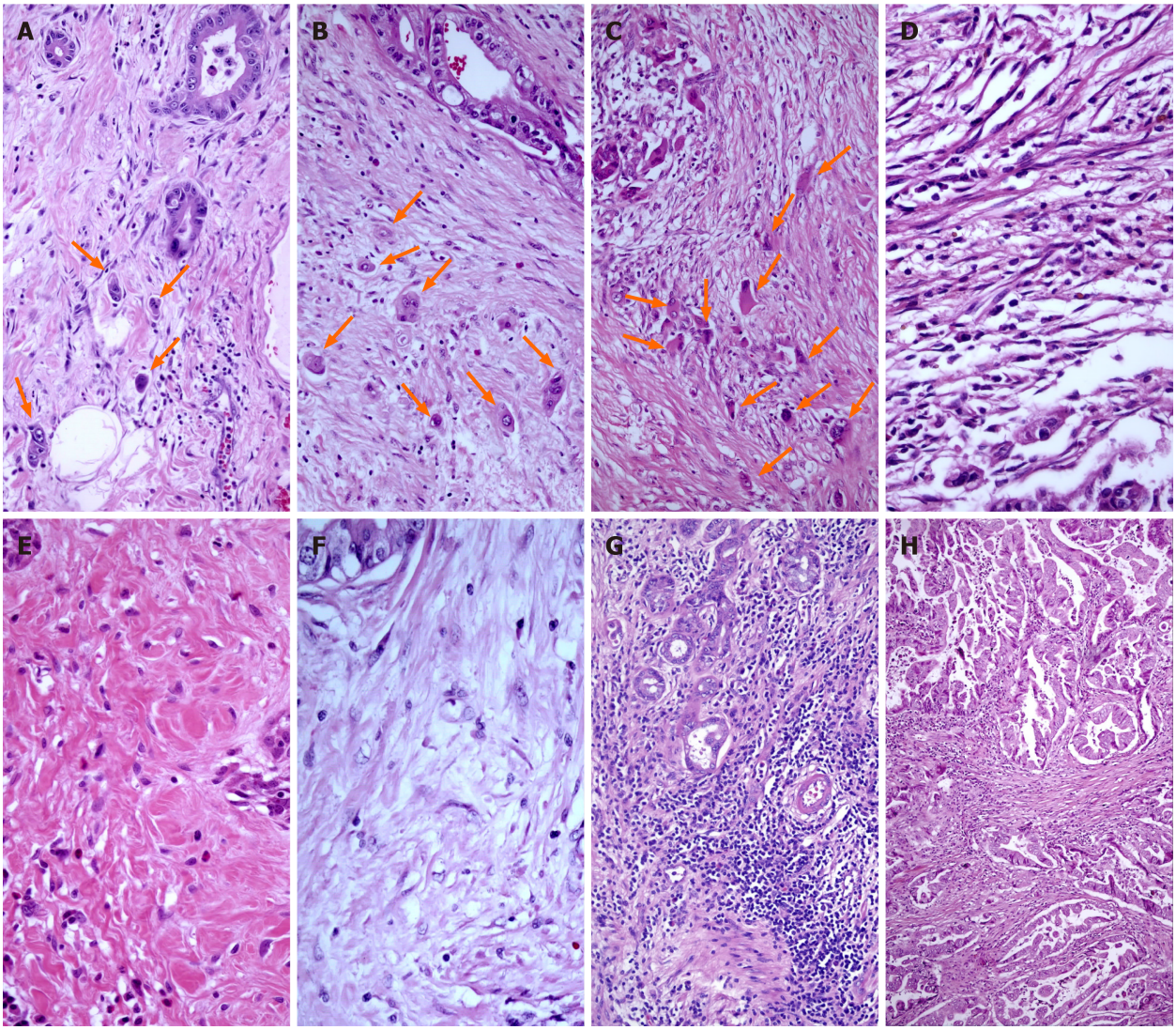

Figure 1 Tumor budding grades assessed according to International Tumor Budding Consensus Conference recommendations, desmoplastic reaction, and tumor-infiltrating lymphocytes. A: TB1 (1-4 tumor bud/hot spot), 200 ×; B: TB2 (5-9 tumor bud/hot spot), 200 ×; C: TB3 (10 tumor bud/hot spot), 200 ×; D: Desmoplastic reaction 1 (DR1), mature stroma composed of tightly packed collagen fibers, 400 ×; E: DR2, intermediate stroma, consisting of areas of collagen that resemble keloids, 400 ×; F: DR3, immature stroma with myxoid alterations; G: Gastric carcinoma with high TILs in tumor stroma (≥ 5%), 100 ×; H: Gastric carcinoma with low TILs (< 5 %), 100 ×. Hematoxylin-eosin, black arrows indicate tumor buds.

(Table 2, Figure 3). The relationships between age, sex, and tumor diameter and these features and outcomes were not significantly different ($P > 0.05$).

According to the multivariate Cox regression analysis, ITB, PTB, and LNM were found to be independent prognostic factors ($P < 0.05$, Table 3).

Clinicopathological and prognostic findings in the intestinal subtype

In this cohort, higher PTB, higher ITB and immature stroma were more common in patients with LNM ($P < 0.003$). DR was also associated with male predominance (Table 4).

There was a positive correlation between PTB and ITB with the stage and grade ($P < 0.01$). In addition, PTB was related to invasion ($P < 0.05$). While PTB was positively associated with DR, an inverse relationship was observed between higher TILs and these parameters ($P < 0.006$, Table 4). Besides, Spearman correlation analysis revealed a strong correlation between PTB and ITB ($r = 0.972$, Figure 2).

In this group, the median survival ranged from 33.8 to 42.1 months (median: 38.0 ± 2.1). Kaplan-Meier analysis revealed that LNM ($P < 0.001$), stage ($P < 0.04$), PTB ($P < 0.001$), ITB ($P < 0.001$), and DR ($P < 0.001$) were powerful indicators of the disease course (Table 4, Figure 4). According to the multivariate analysis, PTB and ITB were found to be independent prognostic parameters ($P < 0.001$, Table 3).

Table 2 Clinicopathological parameters associated with survival in all cases and patients with intestinal tumors (Log-rank test)

Parameters	All cases		Intestinal tumors	
	mean ± SE (95%CI)	Median ± SE (95%CI)	mean ± SE (95%CI)	Median ± SE (95%CI)
Age				
< 62.14 ± 12.00	48.2 ± 4.5 (39.4-56.9)	37.0 ± 1.1 (34.8-39.1)	53 ± 5.9 (41.4-64.5)	36.0 ± 1.2 (33.4-38.5)
≥ 62.14 ± 12.00	50.8 ± 4.8 (41.3-60.3)	39.0 ± 9.5 (20.1-57.8)	55.7 ± 5.7 (44.4-66.9)	60.0 ± 16.2 (28.2-91.7)
Gender				
Male	50.1 ± 4.7 (41.3-59.0)	36.0 ± 2.6 (30.8-41.1)	54.7 ± 5.5 (43.7-65.6)	38.0 ± 3.3 (31.5-44.5)
Female	44.9 ± 5.2 (39.1-58.7)	38.0 ± 1.6 (34.9-41.0)	54.5 ± 6.3 (42-67.1)	38.0 ± 9.6 (19.1-56.8)
Diameter				
< 1.86 ± 1.02	47.3 ± 3.7 (40.0-54.6)	38.0 ± 1.8 (3.4-41.5)	50.2 ± 4.5 (41.3-59.1)	38.0 ± 2.7 (32.6-43.3)
≥ 1.86 ± 1.02	52.3 ± 6.3 (39.8-64.7)	36.0 ± 4.6 (26.8-45.1)	61.4 ± 8.2 (45.2-77.6)	38.0 ± 13.4 (11.6-64.3)
Invasion				
T1	65.1 ± 7.1 (51.2-79.1)	78.0 ± 22.1 (34.5-121.4)	65.1 ± 7.1 (51.1-79.1)	78.0 ± 22.1 (34.5-121.4)
T2	54.5 ± 6.6 (41.5-67.5)	38.0 ± 2.6 (32.9-43.0)	63.5 ± 8.6 (46.6-80.4)	52.0 ± 14.4 (23.7-80.2)
T3	44.8 ± 4.8 (35.4-54.3)	36.0 ± 4.3 (27.5-44.5)	46.4 ± 5.7 (35-57.7)	35.0 ± 5.5 (241.0-45.8)
T4	44.0 ± 5.4 (32.3-53.6)	30.0 ± 2.8 (24.4- 35.5)	48.7 ± 7.0 (35-62.5)	36.0 ± 3.1 (29.7-42.2)
LNM				
Absent	51.6 ± 4.8 (52.1-71.0)	46.0 ± 5.5 (35.1-56.8)[a]	66.1 ± 5.5 (55.3-77.1)	62.0 ± 13.2 (35.8-88.1)[a]
Present	30.6 ± 3.0 (24.6-36.6)	24.0 ± 2.2 (19.5-28.4)	32.1 ± 4.5 (23.3-40.8)	24.0 ± 7.7 (8.7-39.2)
Metastasis				
Absent	48.3 ± 3.2 (41.9-54.8)	38.0 ± 1.5 (34.9-41.0)	53.4 ± 4.1 (45.2-61.6)	39.0 ± 4.8 (29.4-48.5)
Present	51.7 ± 9.7 (32.6-70.7)	24.0 ± 8.3 (7.6-40.3)	55.1 ± 10.8 (33.8-76.5)	36.0 ± 12.0 (12.4-59.5)
Stage				
I	69.1 ± 6.9 (55.5-82.7)	72.0 ± 11.1 (50.3-93.6)[b]	71.5 ± 6.8 (58.1-85.1)	72.0 ± 10.9 (50.5-93.4)[c]
II	45.1 ± 5.3 (34.7-55.5)	37.0 ± 2.2 (32.6-41.3)	52.3 ± 7.2 (38.1-66.5)	39.0 ± 2.5 (34.0-43.9)
III	38.6 ± 3.9 (30.8-46.4)	33.0 ± 3.4 (26.2-39.7)	40.0 ± 5.8 (28.5-51.4)	29.0 ± 4.9 (19.2-38.7)
IV	31.3 ± 10.8 (37.1-59.5)	30.0 ± 12.3 (11.8-60.1)	38.3 ± 10.8 (27.1-48.7)	30.0 ± 12.3 (29.8-42.1)
Subtype				
Intestinal	55.1 ± 4.4 (46.6-63.7)	38.0 ± 2.7 (32.9-42.1)[d]	-	-
Not intestinal	32.5 ± 2.2 (18.8-36.7)	30.0 ± 1.2 (6.2-17.7)	-	-
Grade				
Low	-	-	61.9 ± 5.7 (50.6-73.2)	52.0 ± 11.6 (29.1-74.8)
Moderate	-	-	49.9 ± 10.6 (29.0-70.8)	32.0 ± 4.7 (22.7-41.2)
High	-	-	43.2 ± 6.8 (29.9-56.5)	29.0 ± 12.3 (4.7-53.2)
LVI				
Absent	54.3 ± 5.8 (43.0-65.7)	41.0 ± 2.2 (36.5-45.4)	59.0 ± 6.9 (45.4-72.7)	41.0 ± 7.0 (27.0-54.9)
Present	46.6 ± 4.0 (38.6-54.6)	36.0 ± 3.4 (29.1-42.8)	51.4 ± 5.1 (41.2-61.5)	36.0 ± 2.9 (30.2-41.7)
PNI				
Absent	55.2 ± 6.5 (42.5-67.9)	38.0 ± 3.9 (30.2-45.7)	60.4 ± 7.7 (45.3-75.5)	38.0 ± 9.8 (18.6-57.3)
Present	46.9 ± 3.8 (39.4-54.4)	36.0 ± 2.2 (31.6-40.3)	51.7 ± 4.8 (42.3-61.2)	38.0 ± 2.5 (33-42.9)
PTB				
PTB1	88.3 ± 5.2 (78.0-98.6)	73.5 ± 12.7 (71.6-92.5)[a]	92.4 ± 4.7 (83.3-101.9)	88.2 ± 7.6 (56.9-91.6)[a]

PTB2	61.8 ± 6.4 (48.6-73.6)	38.0 ± 2.8 (33.5-42.5)	63.0 ± 6.5 (50.2-75.8)	41.0 ± 3.1 (34.8-47.1)
PTB3	26.7 ± 2.4 (21.9-31.5)	22.0 ± 1.5 (19.1-24.9)	22.7 ± 3.6 (15.6-29.8)	16.0 ± 2.1 (33.8-42.1)
ITB				
ITB1	101.1 ± 6.7 (87.8-114.4)	91.2 ± 5.2 (81.4-112.5)[a]	103.8 ± 6.4 (91.1-116.4)	63.0 ± 8.3 (88.7-97.4)[a]
ITB2	47.7 ± 4.6 (38.6-56.7)	41.0 ± 4.2 (32.8-49.2)	50.1 ± 5.2 (39.9-60.2)	42.0 ± 6.3 (29.0-52.9)
ITB3	29.9 ± 2.4 (25.3-34.6)	26.0 ± 1.9 (22.2-29.8)	28.5 ± 3.4 (21.8-35.2)	22.0 ± 4.0 (13.9-30.0)
DR				
DR1	60.4 ± 5.4 (49.8-70.9)	41.0 ± 7.9 (25.4-56.6)[d]	67.2 ± 6.2 (55.1-79.3)	72.0 ± 18.1 (36.4-107.5)[a]
DR2	48.0 ± 6.6 (35.1-61.0)	36.0 ± 4.5 (27.3-44.8)	53.5 ± 8.4. (36.9-70.1)	38.0 ± 9.1 (20.0-55.9)
DR3	27.0 ± 4.1 (18.8-35.2)	18.0 ± 3.6 (10.8-25.1)	31.8 ± 5.3 (21.3-42.2)	24.0 ± 6.7 (10.9-37.0)
TILs				
TILs	53.8 ± 4.3 (45.3-62.4)	39.0 ± 4.8 (29.4-48.5)	59.6 ± 5.2 (49.3-70.0)	53.0 ± 14.7 (24.0-81.9)
TILs	43.9 ± 4.6 (34.9-52.9)	35.0 ± 4.5 (26.2-43.7)	47.8 ± 5.7 (36.5-59.1)	35.0 ± 5.1 (24.9-45.0)

[a]$P < 0.001$.
[b]$P < 0.01$.
[c]$P < 0.05$.
[d]$P < 0.01$.
SE: Standard error; CI: Confidence interval; LNM: Lymph node metastasis; LVI: Lymphovascular invasion; PNI: Perineural invasion: PTB: Peritumoral budding; ITB: Intratumoral budding; DR: Desmoplastic reaction; TILs: Tumor-infiltrating lymphocytes.

Table 3 Multivariate analysis including parameters associated with prognosis in the Log-rank test in the entire group and tumors of the intestinal subtype

Parameters	All group			*P* value	Intestinal tumors			*P* value
	HR	Lower (95%CI)	Upper (95%CI)		HR	Lower (95%CI)	Upper (95%CI)	
ITB	2.06	1.40	3.01	< 0.001	3.32	2.34	4.72	< 0.001
PTB	1.83	1.29	2.59	< 0.001	2.01	1.32	3.05	< 0.001
LNM	1.53	1.00	2.33	0.04	1.09	0.59	2.04	0.760
Stage	1.06	0.81	1.40	0.63	1.12	0.80	1.58	0.480
Subtype	0.76	0.50	1.17	0.22	-	-	-	-
DR	1.11	0.87	1.42	0.39	1.17	0.86	1.60	0.290

HR: Hazard ratio; CI: Confidence interval; LNM: Lymph node metastasis; PTB: Peritumoral budding; ITB: Intratumoral budding; DR: Desmoplastic reaction; TILs: Tumor infiltrating lymphocytes.

DISCUSSION

TB has been investigated in numerous studies of CRC and is currently used in pathological reporting protocols due to its prognostic importance in low-grade tumors[31,35]. However, TB has yet to be studied extensively in GAC. This may be because GAC is less frequently observed than CRC, especially in Western countries[26,36]. Moreover, a standard evaluation method for this variable has yet to be determined. For example, studies investigating the role of TB in predicting LNM in early gastric carcinoma (EGC) patients have indicated that detecting the presence of TB may be effective[37-39]. Yim *et al*[40] recently observed a strong association between TB and LNM metastasis with three different evaluation methods in EGC. However, only the presence of TB was an independent prognostic factor. The limited number of EGC patients in our series did not allow a separate analysis of this group. However, these results suggest that the presence of TB is an effective marker for predicting LNM metastasis and patient prognosis, at least in EGC.

Recently, in studies that included gastric cancer (GC) patients of all stages and histopathological subtypes, TB was observed to be an independent prognostic factor, which is consistent with our findings[12-14,41,42]. Interestingly, although different categorizations were used in the statistical analysis to determine the predictive role of TB in the course of the disease, the evaluation methods applied in most of these studies were based on the ITBCC, similar to our research [13,14,28,42]. In our study, the survival of patients with TB3 was significantly lower than that of patients with TB2 or TB1. Taken together, these data point to the value of the ITBCC-recommended evaluation of TB in GAC patients.

Table 4 Tumor budding, desmoplastic reaction, and tumor-infiltrating lymphocytes in relation to clinicopathological parameters in the intestinal group

Parameters	*n*	PTB1 (%)	PTB2 (%)	PTB3 (%)	ITB1 (%)	ITB2 (%)	ITB3 (%)	DR1 (%)	DR2 (%)	DR3 (%)	TILs (%)	TILs (%)
Age, yr		24	39	37	30	21	49	47	28	25	52	48
< 62.14 ± 12.00	47	10 (41.7)	24 (61.5)	13 (35.1)	14 (46.7)	11 (52.4)	22 (44.9)	21 (44.7)	15 (53.6)	11 (44.0)	25 (48.1)	22 (45.8)
≥ 62.14 ± 12.00	53	14 (58.3)	15 (38.5)	24 (63.9)	16 (53.3)	10 (47.6)	27 (55.1)	26 (55.3)	13 (46.4)	14 (56.0)	27 (51.9)	26 (54.2)
Gender												
Male	60	15 (62.5)	24 (61.5)	21 (56.8)	18 (60.0)	15 (71.4)	27 (55.1)	34 (72.3)	16 (57.1)	10 (40.0)[a]	27 (51.9)	33 (68.8)
Female	40	9 (37.5)	15 (38.5)	16 (43.2)	12 (40.0)	6 (28.6)	22 (44.9)	13 (27.7)	12 (42.9)	15 (60.0)	25 (48.1)	15 (31.3)
Diameter												
< 1.86 ± 1.02	67	16 (66.7)	26 (66.7)	25 (67.6)	15 (50.0)	16 (76.2)	36 (73.5)	28 (59.6)	22 (78.6)	17 (68.0)	38 (73.1)	29 (60.4)
≥ 1.86 ± 1.02	33	8 (33.3)	13 (33.3)	12 (32.4)	15 (50.0)	5 (23.8)	13 (26.5)	19 (40.4)	6 (21.4)	8 (32.0)	14 (26.9)	19 (39.6)
Invasion												
T1	7	5 (20.8)	2 (5.1)	0	4 (13.3)	1 (4.8)	2 (4.1)	3 (6.4)	3 (10.7)	1 (4.0)	4 (7.7)	3 (6.3)
T2	28	4 (16.7)	15 (38.5)	9 (24.3)	12 (40.0)	7 (33.3)	9 (18.4)	14 (29.8)	9 (32.1)	5 (20.0)	12 (23.1)	16 (33.3)
T3	42	10 (41.7)	13 (33.3)	19 (51.4)	9 (30.0)	6 (28.6)	27 (55.1)	23 (48.9)	12 (42.9)	7 (28.0)	23 (44.2)	19 (39.6)
T4	23	5 (20.8)	9 (23.1)	9 (24.3)	5 (16.7)	7 (33.3)	11 (22.4)	7 (14.9)	4 (14.3)	12 (48.0)	13 (25.0)	10 (20.8)
LNM												
Absent	69	22 (91.7)	29 (74.4)	18 (48.6)[b]	29 (96.7)	14 (66.7)	26 (53.1)[b]	39 (83.0)	19 (67.9)	11 (44.0)[c]	32 (61.5)	37 (77.1)
Present	31	2 (8.3)	10 (25.6)	19 (51.4)	1 (3.3)	7 (33.3)	23 (46.9)	8 (17.0)	9 (32.1)	14 (56.0)	20 (38.5)	11 (22.9)
Metastasis												
Absent	79	24 (100.0)	29 (74.4)	26 (70.3)[d]	21 (70.0)	20 (95.2)	38 (77.6)	39 (83.0)	23 (82.1)	17 (68.0)	40 (76.9)	39 (81.3)
Present	21	0	10 (25.6)	11 (29.7)	9 (30.0)	1 (4.8)	11 (22.4)	8 (17.0)	5 (17.9)	8 (32.0)	12 (23.1)	9 (18.7)
Stage												
I	20	11 (45.8)	9 (23.2)	0	10 (33.3)	5 (23.8)	5 (10.2)[c]	12 (25.5)	6 (21.4)	2 (8.0)	12 (23.1)	8 (16.7)
II	26	6 (25.0)	10 (25.6)	10 (27.0)	7 (23.3)	4 (19.0)	15 (30.6)	15 (31.9)	9 (32.1)	2 (8.0)	12 (23.1)	14 (29.3)
III	32	6 (25.0)	10 (25.6)	16 (43.2)	3 (10.0)	11 (52.4)	18 (36.7)	12 (25.5)	7 (25.0)	13 (52.0)	15 (28.8)	17 (35.2)
IV	22	1 (4.2)	10 (25.6)	11 (29.7)	10 (33.4)	1 (4.8)	11 (22.4)	8 (17.0)	6 (21.4)	8 (32.0)	13 (25.0)	9 (18.8)
Grade												

Low	54	19 (79.2)	21 (53.8)	14 (37.8)[a]	22 (73.3)	12 (57.1)	20 (40.8)[a]	28 (59.6)	16 (57.1)	10 (40.0)	29 (55.8)	25 (52.1)
Moderate	29	2 (8.3)	13 (33.4)	14 (37.8)	3 (10.0)	6 (28.6)	20 (40.8	11 (23.4)	10 (35.7)	8 (32.0)	15 (28.8)	14 (29.2)
High	17	3 (12.5)	5 (12.8)	9 (24.3)	5 (16.7)	3 (14.3)	9 (18.4)	8 (17.0)	2 (7.2)	7 (28.0)	8 (15.4)	9 (18.7)
LVI												
Absent	67	13 (54.2)	26 (66.7)	28 (75.7)	15 (50.0)	15 (71.4)	37 (75.5)	34 (72.3)	14 (50.0)	19 (76.0)	32 (61.5)	35 (72.9)
Present	33	11 (45.8)	13 (33.3)	9 (24.3)	15 (50.0)	6 (28.6)	12 (24.5)	13 (27.7)	14 (50.0)	6 (24.0)	20 (38.5)	13 (27.1)
PNI												
Absent	71	14 (58.3)	26 (66.7)	31 (83.8)	18 (60.0)	15 (71.4)	38 (77.6)	34 (72.3)	16 (57.1)	21 (84.0)	37 (71.2)	34 (70.8)
Present	29	10 (41.7)	13 (33.3)	6 (16.2)	12 (40.0)	6 (28.6)	11 (22.4)	13 (27.7)	12 (42.9)	4 (16.0)	15 (28.8)	14 (29.2)
Survival												
Deceased	71	7 (29.2)	27 (69.2)	37 (100.0)[b]	7 (23.3)	16 (76.2)	48 (98.0)[b]	29 (61.7)	20 (71.4)	22 (88.0)	41 (78.8)	30 (62.5)
Alive	29	17 (70.8)	12 (30.8)	0	23 (76.7)	5 (23.8)	1 (2.0)	18 (38.3)	8 (28.6)	3 (12.0)	11 (21.2)	18 (37.5)
PTB												
PTB1	24	-	-	-	17 (56.7)	3 (14.3)	4 (8.2)[b]	14 (29.8)	8 (28.6)	2 (8.0)[c]	10 (19.2)	14 (29.2)[d]
PTB2	39	-	-	-	13 (43.3)	18 (85.7)	8 (16.3)	23 (48.9)	11 (39.3)	5 (20.0)	16 (30.8)	23 (47.9)
PTB3	37	-	-	-	0	0	37 (75.5)	10 (21.3)	9 (32.1)	18 (72.0)	26 (50.0)	11 (22.9)
ITB												
ITB1	30	-	-	-	-	-	-	21 (44.7)	8 (28.6)	1 (4.0)[c]	9 (17.3)	21 (43.8)[c]
ITB2	21	-	-	-	-	-	-	12 (25.5)	3 (10.7)	6 (24.0)	9 (17.3)	12 (25.0)
ITB3	49	-	-	-	-	-	-	14 (29.8)	17 (60.7)	18 (72.0)	34 (65.4)	15 (31.2)
DR												
DR1	47	-	-	-	-	-	-	-	-	-	17 (32.7)	30 (62.5)[d]
DR2	28	-	-	-	-	-	-	-	-	-	17 (32.7)	11 (22.9)
DR3	25	-	-	-	-	-	-	-	-	-	18 (34.6)	7 (14.6)

[a]$P < 0.05$.
[b]$P < 0.001$.
[c]$P < 0.008$.
[d]$P < 0.01$.
N: Number of cases; PTB: Peritumoral budding; ITB: Intratumoral budding; DR: Desmoplastic reaction; TILs: Tumor-infiltrating lymphocytes; LNM: Lymph node metastasis.

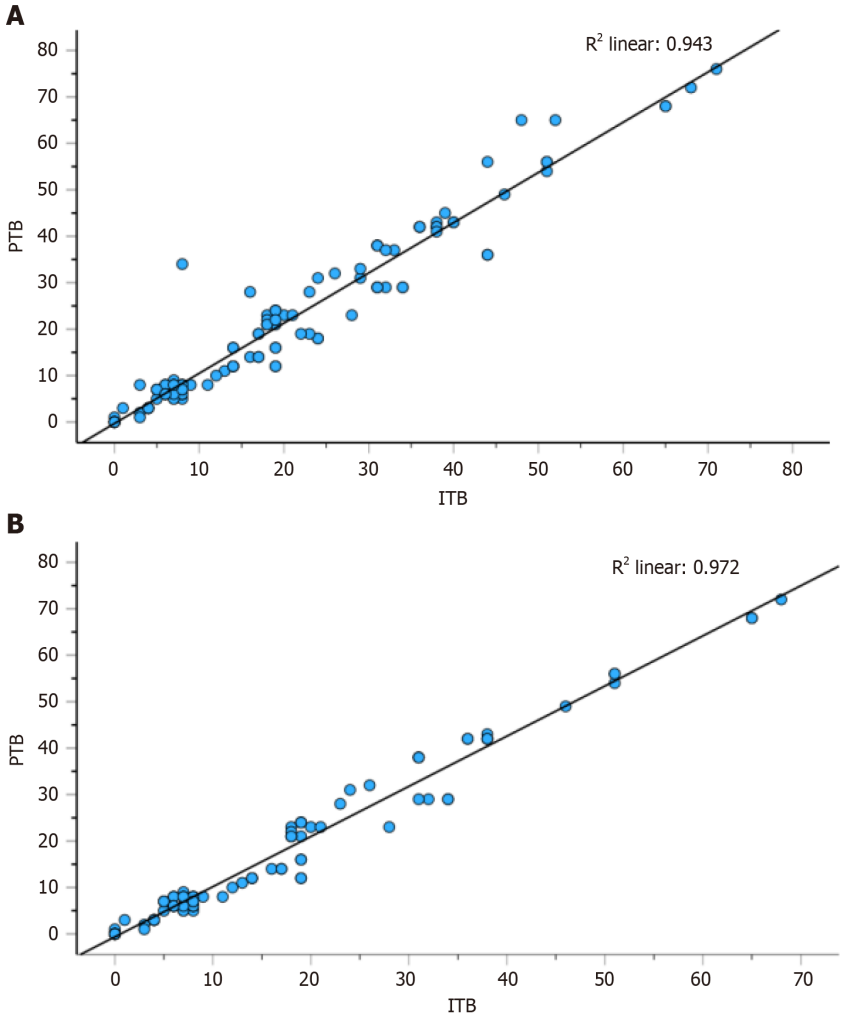

Figure 2 Scatter plot of Spearman's rank correlation between peritumoral budding (vertical axis) and intratumoral budding (horizontal axis). A: Whole cohort B: Intestinal subgroup. ITB: Intratumoral budding.

When adenocarcinoma subtypes in GC were considered separately, TB was observed to be associated with tumor behavior in the intestinal type of GAC but not in diffuse tumors. Although no further analysis of this subtype could be performed in our study group due to the limited number of patients with nonintestinal tumors, TB was observed to be related to survival in patients with intestinal-type GAC according to the log-rank analysis. Moreover, multivariate analysis revealed that the TB score is an independent prognostic parameter. Although TB incidence has been correlated with intestinal-type GAC behavior and survival in many studies, the results of multivariate analyses have yet to be consistent. While in some studies, the evaluation of TB was observed to be a decisive parameter in determining the course of the disease[13,42,43], such an effect was not noted in others[15,16,26]. These different findings may be due to diversity in the number of cases and data categorization among studies. Our findings are consistent with those of studies in which TB was observed to be a strong prognostic parameter in intestinal-type GAC patients and emphasize the need for additional research to establish the value of TB in GAC reporting guidelines.

Another notable finding of our study was that in addition to the whole cohort, PTB and ITB were found to be independent prognostic factors for the intestinal subtype, and their correlation with each other was strong. To our knowledge, only one study has evaluated TB separately in intestinal GAC patients. Qi *et al*[43] observed a strong association between ITB and PTB; both were found to be independent prognostic parameters for predicting survival. Although these findings need to be supported by further studies, the independent prognostic value of TB in both topographic areas support the idea that TB can be evaluated to stratify patients with intestinal-type GAC for prognosis[4,5]. Furthermore, given the substantial correlation between the ITB and PTB, TB could be used as a predictive parameter for determining tumor behavior, especially in patients who are unsuitable for surgical resection.

Although DR in GAC was associated with survival according to univariate analysis in this study, it was not an independent prognostic factor when other parameters related to tumor behavior and prognosis were analyzed. To our knowledge, very few studies have investigated the effectiveness of DR in determining the survival of patients with GAC [24-26]. In these tumors, examination of the thickness of collagen fibers by second-generation harmonic imaging indicated that the presence of large desmoplastic collagen fibers was associated with poor prognosis[24]. In an elegant study in which DR was categorized into two groups (mature and immature), Kemi *et al*[25] reported that DR was an independent parameter for determining the course of disease in patients with GAC. They also noted that DR was associated with 5-

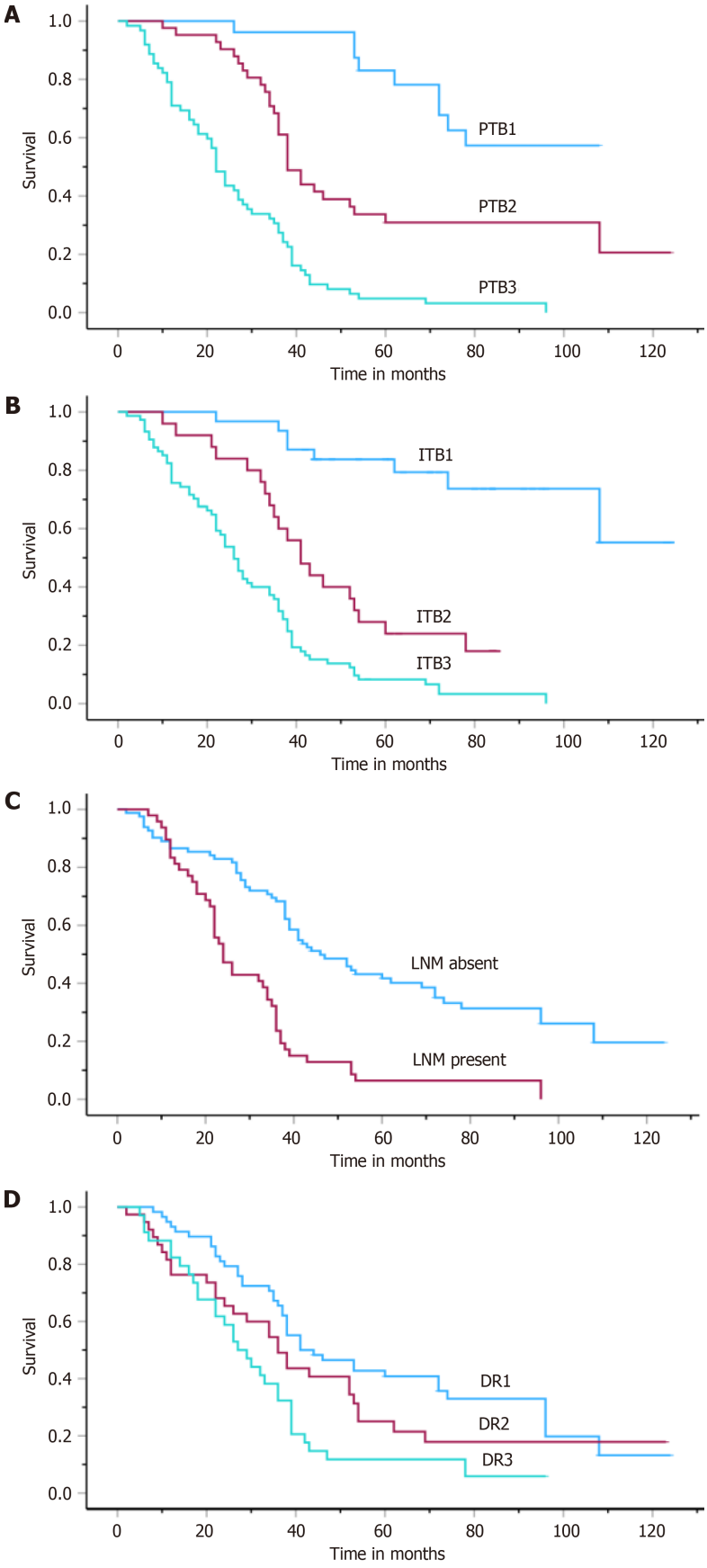

Figure 3 Kaplan-Meier curves of survival analyses in the total cohort. A: Peritumoral budding (log-rank test $P < 0.001$); B: Intratumoral budding (log-rank test $P < 0.001$); C: The presence of lymph node metastasis (log-rank test $P < 0.002$): Desmoplastic reaction (log-rank test $P < 0.002$). PTB: Peritumoral budding; ITB: Intratumoral budding; LNM: Lymph node metastasis; DR: Desmoplastic reaction.

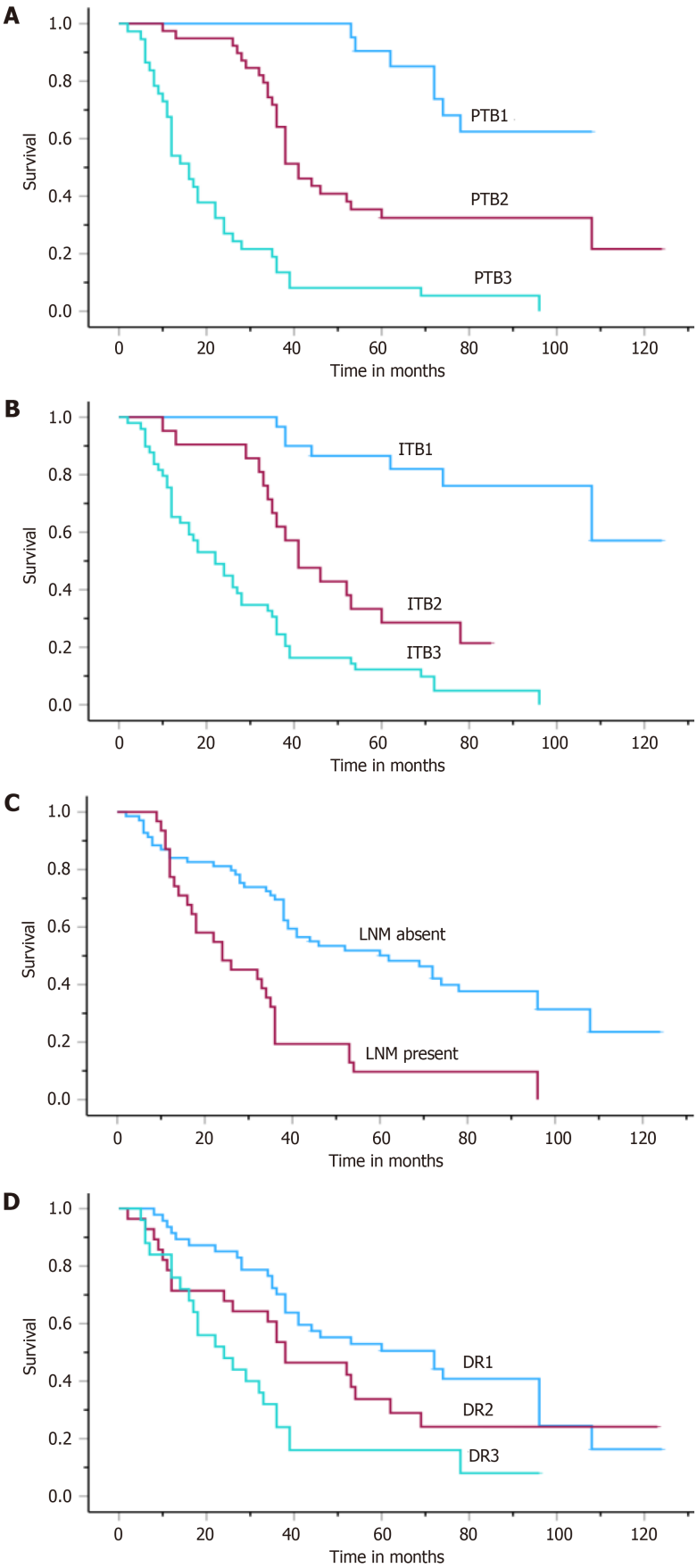

Figure 4 Kaplan-Meier curves of survival analyses in patients with an intestinal subtype of gastric adenocarcinoma. A: Peritumoral budding (log-rank test, $P < 0.001$); B: Intratumoral budding (log-rank test, $P < 0.001$); C: Desmoplastic reaction (log-rank test, $P < 0.001$); D: The presence of lymph node metastasis (log-rank test, $P < 0.001$). PTB: Peritumoral budding; ITB: Intratumoral budding; LNM: Lymph node metastasis; DR: Desmoplastic reaction.

year survival in the intestinal subgroup, whereas no such association was observed for diffuse carcinomas.

On the other hand, Pun *et al*[26] did not detect such a relationship in intestinal-type adenocarcinomas. In both studies, DR was evaluated both in the invasive tumor area and in the main tumor mass. In our study, we investigated DR only on invasive edges according to the method applied in the assessment of DR in many studies, and we found that DR was not an effective prognostic parameter in either the whole group or intestinal tumors. These results emphasize that a different method should be applied to investigate the role of DR in GAC. Recently, Hacking *et al*[44] suggested a different approach for evaluating stromal maturity in patients with CRC. However, the prognostic impact of DR in GAC remains to be investigated *via* this method. In brief, further studies comparing different evaluation methods and categorizations in large patient series are needed to determine the value of DR as a parameter in pathology protocols for these tumors.

We observed a strong positive relationship between DR and TB in the study group. In parallel with these data, a recent study demonstrated the association of high TB with immature stroma in GAC[26]. Moreover, our research revealed an inverse correlation between DR and TILs. To our knowledge, no study has investigated the relationship between these three parameters in patients with GAC. Our findings support studies highlighting the importance of DR in the TME. Although it has not been determined to be an independent prognostic marker, further studies are needed to determine the potential of DR as a marker in GAC.

TILs, an essential component of the tumor environment, have been studied extensively in GAC, but the results are still controversial, even when evaluating lymphocyte subsets by immunohistochemistry. In this study, we did not observe TILs to be a significant predictive parameter for GAC prognosis. There are studies in which TILs were semiquantitatively investigated by H&E staining, similar to our method. A substantial correlation between TILs and survival has been noted [45,46]. Unfortunately, the topographical differences in the evaluation of TILs (intratumoral *vs* stromal) in these studies and the investigation of different types of GACs, such as EBV-associated GCs, limit the comparison of our data[47,48].

Regarding immunohistochemical studies on TILs in GAC, while one study linked higher CD8+ T-cell density in GAC to poor prognosis[49], another noted that higher numbers of CD8+ T cells and TILs improved overall survival (OS)[50]. Similarly, there is disagreement over the predictive importance of CD4+ T-cell tumor infiltration[27,51]. Different data were also obtained in past meta-analyses of GAG[51-53]. The presence of CD3+ lymphocytes was the highest predictive factor for OS (HR = 0.52)[51]. A significant relationship between CD8+ TILs and survival was demonstrated in another analysis[53]. The results also indicated that high intratumoral T-cell infiltration levels were associated with improved survival in GAC patients, and a high density of intratumoral FOXP3+ T cells was not closely associated with poor prognosis[28].

In our study, the strong association between TILs and TB suggested the potential role of TILs in tumor behavior in GAC. Parallel to this observation, in a recent study, Zhang *et al*[28], by double immunohistochemical staining, noted an inverse correlation between TILs and TB, predicting a favorable outcome. On the other hand, we did not observe TILs to be a significant predictive factor. The present study suggests that the method employed for assessing TILs has certain limitations. In other words, it is essential to emphasize that the finding that TILs were unrelated to survival in our study does not exclude the importance of recent research that has primarily investigated various lymphocyte subtypes by immunohistochemistry.

To our knowledge, the relationship between TILs and DR has yet to be described in GAC, and the present study revealed the inverse relationship between TILs and DR, suggesting that DR is an important component of tumor immune surveillance. Moreover, these data merit further investigations into the association of DR with different subsets of lymphocytes to better understand its role in the prediction of survival in GAC.

This study has several limitations. It is conducted within a single center, limiting the sample size to remain relatively small, which might restrict the power to detect more nuanced associations or differences, particularly when stratifying the analysis by adenocarcinoma subtypes or evaluating the interaction between different prognostic factors. Moreover, potential selection biases cannot be excluded due to the retrospective nature of the study, limiting the generalizability of the results to other populations and settings. Therefore, multicenter prospective studies and external validation are needed to confirm the findings.

Another limitation is the need for a standardized evaluation method for assessing TB, DR, and TILs in GAC, which might lead to variability in the results. Although we have employed methods consistent with current literature and guidelines, the need for universally accepted criteria for these histopathological features may affect the reproducibility and comparison of our findings with those of other studies. Additionally, the heterogeneous behavior of GAC necessitates a multifactorial analysis incorporating a wide range of potential prognostic markers. Our study focused on a select few, which, while important, do not encompass all the factors that could influence patient outcomes.

Despite these limitations, our study contributes valuable insights into the prognostic significance of TB, DR, and TILs in GAC, supporting the need for their consideration in future research and potential inclusion in pathological reporting protocols.

CONCLUSION

The findings support that the assessment of TB based on the ITBCC criteria can be used to categorize patients with GAC for treatment and prognosis. Although the strong relationship between PTB and ITB also suggests that these two variables can be used in determining the course of the disease in patients for whom surgical resection is not feasible, especially for those with the intestinal subtype, further studies are needed to delineate their role.

Although DR was related to TB in our series, it was not an independent parameter for predicting survival, suggesting that its value in determining GAC behavior merits further research.

Within the context of our findings, despite the emergence of recent discoveries, we did not notice TILs to be a significant predictive component in GAC. The present study suggested that the method employed for assessing TILs in these tumors has certain limitations. However, it is essential to note that this does not diminish the importance of recent research investigating various lymphocyte subtypes.

The relationships among TB, DR, and TILs in the tumor area observed in our study warrant further investigations with a more extensive patient cohort to determine the role of a scoring system consisting of these three parameters in determining the behavior of GC.

FOOTNOTES

Author contributions: Yavuz A and Elpek GOE designed and performed the research and wrote the paper; Simsek K, Alpsoy A, Altunay B, Gedik EO, Unal B, and Bassorgun CI are involved in the data curation, investigation, and resources; Tatli AM provided clinical advice; and all authors equally contributed to the analysis of the data.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of the Akdeniz University (Approval No. 13.12.2023/915).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous data obtained after each patient agreed to treatment by written permission. One of these forms is presented below.

Conflict-of-interest statement: There is no conflict of interest.

Data sharing statement: No additional data are available.

Country/Territory of origin: Türkiye

ORCID number: Ayşen Yavuz 0000-0001-9991-5515; Kubra Simsek 0009-0008-7731-109X; Anil Alpsoy 0000-0003-4978-7652; Busra Altunay 0000-0001-6534-6078; Elif Ocak Gedik 0000-0003-2618-498X; Betul Unal 0000-0002-9572-3601; Cumhur Ibrahim Bassorgun 0000-0003-2440-511X; Ali Murat Tatli 0000-0001-9696-1102; Gulsum Ozlem Elpek 0000-0002-1237-5454.

S-Editor: Chen YL
L-Editor: A
P-Editor: Zhao YQ

REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Orditura M**, Galizia G, Sforza V, Gambardella V, Fabozzi A, Laterza MM, Andreozzi F, Ventriglia J, Savastano B, Mabilia A, Lieto E, Ciardiello F, De Vita F. Treatment of gastric cancer. *World J Gastroenterol* 2014; **20**: 1635-1649 [PMID: 24587643 DOI: 10.3748/wjg.v20.i7.1635]

3 **Luu C**, Thapa R, Woo K, Coppola D, Almhanna K, Pimiento JM, Chen DT, Marquez DD, Hodul PJ. Does histology really influence gastric cancer prognosis? *J Gastrointest Oncol* 2017; **8**: 1026-1036 [PMID: 29299363 DOI: 10.21037/jgo.2017.09.08]

4 **Sano T**, Coit DG, Kim HH, Roviello F, Kassab P, Wittekind C, Yamamoto Y, Ohashi Y. Proposal of a new stage grouping of gastric cancer for TNM classification: International Gastric Cancer Association staging project. *Gastric Cancer* 2017; **20**: 217-225 [PMID: 26897166 DOI: 10.1007/s10120-016-0601-9]

5 **Joshi SS**, Badgwell BD. Current treatment and recent progress in gastric cancer. *CA Cancer J Clin* 2021; **71**: 264-279 [PMID: 33592120 DOI: 10.3322/caac.21657]

6 **Song K**, Ma C, Gu B, Wang B, Ma H, Deng X, Chen H. Molecular mechanism underlying epithelial-mesenchymal transformation and cisplatin resistance in esophageal squamous cell carcinoma. *Thorac Cancer* 2023; **14**: 3069-3079 [PMID: 37718469 DOI: 10.1111/1759-7714.15094]

7 **Kozak J**, Forma A, Czeczelewski M, Kozyra P, Sitarz E, Radzikowska-Büchner E, Sitarz M, Baj J. Inhibition or Reversal of the Epithelial-Mesenchymal Transition in Gastric Cancer: Pharmacological Approaches. *Int J Mol Sci* 2020; **22** [PMID: 33383973 DOI: 10.3390/ijms22010277]

8 **Lu J**, Kornmann M, Traub B. Role of Epithelial to Mesenchymal Transition in Colorectal Cancer. *Int J Mol Sci* 2023; **24** [PMID: 37834263 DOI: 10.3390/ijms241914815]

9 **Lugli A**, Zlobec I, Berger MD, Kirsch R, Nagtegaal ID. Tumour budding in solid cancers. *Nat Rev Clin Oncol* 2021; **18**: 101-115 [PMID: 32901132 DOI: 10.1038/s41571-020-0422-y]

10 **Haddad TS**, Lugli A, Aherne S, Barresi V, Terris B, Bokhorst JM, Brockmoeller SF, Cuatrecasas M, Simmer F, El-Zimaity H, Fléjou JF, Gibbons D, Cathomas G, Kirsch R, Kuhlmann TP, Langner C, Loughrey MB, Riddell R, Ristimäki A, Kakar S, Sheahan K, Treanor D, van der

Laak J, Vieth M, Zlobec I, Nagtegaal ID. Improving tumor budding reporting in colorectal cancer: a Delphi consensus study. *Virchows Arch* 2021; **479**: 459-469 [PMID: 33650042 DOI: 10.1007/s00428-021-03059-9]

11 **Zlobec I**, Lugli A. Tumour budding in colorectal cancer: molecular rationale for clinical translation. *Nat Rev Cancer* 2018; **18**: 203-204 [PMID: 29376521 DOI: 10.1038/nrc.2018.1]

12 **Che K**, Zhao Y, Qu X, Pang Z, Ni Y, Zhang T, Du J, Shen H. Prognostic significance of tumor budding and single cell invasion in gastric adenocarcinoma. *Onco Targets Ther* 2017; **10**: 1039-1047 [PMID: 28255247 DOI: 10.2147/OTT.S127762]

13 **Szalai L**, Jakab Á, Kocsmár I, Szirtes I, Kenessey I, Szijártó A, Schaff Z, Kiss A, Lotz G, Kocsmár É. Prognostic Ability of Tumor Budding Outperforms Poorly Differentiated Clusters in Gastric Cancer. *Cancers (Basel)* 2022; **14** [PMID: 36230653 DOI: 10.3390/cancers14194731]

14 **Dao TV**, Nguyen CV, Nguyen QT, Vu HTN, Phung HT, Bui OT, Nguyen DK, Luong BV, Tran TV. Evaluation of Tumor Budding in Predicting Survival for Gastric Carcinoma Patients in Vietnam. *Cancer Control* 2020; **27**: 1073274820968883 [PMID: 33136444 DOI: 10.1177/1073274820968883]

15 **Tanaka K**, Shimura T, Kitajima T, Kondo S, Ide S, Okugawa Y, Saigusa S, Toiyama Y, Inoue Y, Araki T, Uchida K, Mohri Y, Kusunoki M. Tropomyosin-related receptor kinase B at the invasive front and tumour cell dedifferentiation in gastric cancer. *Br J Cancer* 2014; **110**: 2923-2934 [PMID: 24853179 DOI: 10.1038/bjc.2014.228]

16 **Olsen S**, Jin L, Fields RC, Yan Y, Nalbantoglu I. Tumor budding in intestinal-type gastric adenocarcinoma is associated with nodal metastasis and recurrence. *Hum Pathol* 2017; **68**: 26-33 [PMID: 28428104 DOI: 10.1016/j.humpath.2017.03.021]

17 **Ulase D**, Heckl S, Behrens HM, Krüger S, Röcken C. Prognostic significance of tumour budding assessed in gastric carcinoma according to the criteria of the International Tumour Budding Consensus Conference. *Histopathology* 2020; **76**: 433-446 [PMID: 31538348 DOI: 10.1111/his.13997]

18 **Patriarca C**, Pini GM, Conti G. Invasion and metastasis: a historical perspective. *Pathologica* 2020; **112**: 229-233 [PMID: 33393528 DOI: 10.32074/1591-951X-111]

19 **Chan TS**, Shaked Y, Tsai KK. Targeting the Interplay Between Cancer Fibroblasts, Mesenchymal Stem Cells, and Cancer Stem Cells in Desmoplastic Cancers. *Front Oncol* 2019; **9**: 688 [PMID: 31417869 DOI: 10.3389/fonc.2019.00688]

20 **Kong BT**, Fan QS, Wang XM, Zhang Q, Zhang GL. Clinical implications and mechanism of histopathological growth pattern in colorectal cancer liver metastases. *World J Gastroenterol* 2022; **28**: 3101-3115 [PMID: 36051338 DOI: 10.3748/wjg.v28.i26.3101]

21 **Hu Q**, Wang Y, Yao S, Mao Y, Liu L, Li Z, Chen Y, Zhang S, Li Q, Zhao Y, Fan X, Cui Y, Zhao K, Liu Z. Desmoplastic Reaction Associates with Prognosis and Adjuvant Chemotherapy Response in Colorectal Cancer: A Multicenter Retrospective Study. *Cancer Res Commun* 2023; **3**: 1057-1066 [PMID: 37377615 DOI: 10.1158/2767-9764.CRC-23-0073]

22 **Yoshida Y**, Nakanishi Y, Mitsuhashi T, Yamamoto H, Hayashi MO, Oba M, Nitta T, Ueno T, Yamada T, Ono M, Kuwabara S, Hatanaka Y, Hirano S. Postoperative Prognosis According to Pathologic Categorization of Desmoplastic Reaction in Patients with Extrahepatic Cholangiocarcinoma. *Ann Surg Oncol* 2023; **30**: 7348-7357 [PMID: 37528304 DOI: 10.1245/s10434-023-13867-9]

23 **Sato H**, Hara T, Meng S, Tsuji Y, Arao Y, Saito Y, Sasaki K, Kobayashi S, Doki Y, Eguchi H, Ishii H. Multifaced roles of desmoplastic reaction and fibrosis in pancreatic cancer progression: Current understanding and future directions. *Cancer Sci* 2023; **114**: 3487-3495 [PMID: 37480223 DOI: 10.1111/cas.15890]

24 **Zhou ZH**, Ji CD, Xiao HL, Zhao HB, Cui YH, Bian XW. Reorganized Collagen in the Tumor Microenvironment of Gastric Cancer and Its Association with Prognosis. *J Cancer* 2017; **8**: 1466-1476 [PMID: 28638462 DOI: 10.7150/jca.18466]

25 **Kemi NA**, Eskuri M, Pohjanen VM, Karttunen TJ, Kauppila JH. Histological assessment of stromal maturity as a prognostic factor in surgically treated gastric adenocarcinoma. *Histopathology* 2019; **75**: 882-889 [PMID: 31173384 DOI: 10.1111/his.13934]

26 **Pun C**, Luu S, Swallow C, Kirsch R, Conner JR. Prognostic Significance of Tumour Budding and Desmoplastic Reaction in Intestinal-Type Gastric Adenocarcinoma. *Int J Surg Pathol* 2023; **31**: 957-966 [PMID: 35726174 DOI: 10.1177/10668969221105617]

27 **Díaz Del Arco C**, Ortega Medina L, Estrada Muñoz L, García Gómez de Las Heras S, Fernández Aceñero MJ. Is there still a place for conventional histopathology in the age of molecular medicine? Laurén classification, inflammatory infiltration and other current topics in gastric cancer diagnosis and prognosis. *Histol Histopathol* 2021; **36**: 587-613 [PMID: 33565601 DOI: 10.14670/HH-18-309]

28 **Zhang N**, Wang D, Duan Y, Ayarick VA, Cao M, Wang Y, Zhang G. The special immune microenvironment of tumor budding and its impact on prognosis in gastric adenocarcinoma. *Pathol Res Pract* 2020; **216**: 152926 [PMID: 32327282 DOI: 10.1016/j.prp.2020.152926]

29 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675 DOI: 10.1111/apm.1965.64.1.31]

30 **Amin MB**, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin* 2017; **67**: 93-99 [PMID: 28094848 DOI: 10.3322/caac.21388]

31 **Lugli A**, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Fléjou JF, Hansen TP, Hartmann A, Kakar S, Langner C, Nagtegaal I, Puppa G, Riddell R, Ristimäki A, Sheahan K, Smyrk T, Sugihara K, Terris B, Ueno H, Vieth M, Zlobec I, Quirke P. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. *Mod Pathol* 2017; **30**: 1299-1311 [PMID: 28548122 DOI: 10.1038/modpathol.2017.46]

32 **Ueno H**, Kanemitsu Y, Sekine S, Ishiguro M, Ito E, Hashiguchi Y, Kondo F, Shimazaki H, Mochizuki S, Kajiwara Y, Shinto E, Yamamoto J. Desmoplastic Pattern at the Tumor Front Defines Poor-prognosis Subtypes of Colorectal Cancer. *Am J Surg Pathol* 2017; **41**: 1506-1512 [PMID: 28877064 DOI: 10.1097/PAS.0000000000000946]

33 **Zhang D**, He W, Wu C, Tan Y, He Y, Xu B, Chen L, Li Q, Jiang J. Scoring System for Tumor-Infiltrating Lymphocytes and Its Prognostic Value for Gastric Cancer. *Front Immunol* 2019; **10**: 71 [PMID: 30761139 DOI: 10.3389/fimmu.2019.00071]

34 **Green SB**. How Many Subjects Does It Take To Do A Regression Analysis. *Multivariate Behav Res* 1991; **26**: 499-510 [PMID: 26776715 DOI: 10.1207/s15327906mbr2603_7]

35 **Jesinghaus M**, Schmitt M, Lang C, Reiser M, Scheiter A, Konukiewitz B, Steiger K, Silva M, Tschurtschenthaler M, Lange S, Foersch S, Becker KF, Saur D, Friess H, Halfter K, Engel J, Boxberg M, Pfarr N, Wilhelm D, Weichert W. Morphology Matters: A Critical Reappraisal of the Clinical Relevance of Morphologic Criteria From the 2019 WHO Classification in a Large Colorectal Cancer Cohort Comprising 1004 Cases. *Am J Surg Pathol* 2021; **45**: 969-978 [PMID: 34105518 DOI: 10.1097/PAS.0000000000001692]

36 **Rahman R**, Asombang AW, Ibdah JA. Characteristics of gastric cancer in Asia. *World J Gastroenterol* 2014; **20**: 4483-4490 [PMID: 24782601 DOI: 10.3748/wjg.v20.i16.4483]

37 **Gulluoglu M**, Yegen G, Ozluk Y, Keskin M, Dogan S, Gundogdu G, Onder S, Balik E. Tumor Budding Is Independently Predictive for

Lymph Node Involvement in Early Gastric Cancer. *Int J Surg Pathol* 2015; **23**: 349-358 [PMID: 25911564 DOI: 10.1177/1066896915581200]
38 **Du M**, Chen L, Cheng Y, Wang Y, Fan X, Zhang Y, Zhou X, Guo L, Xu G, Zou X, Huang Q. Tumor Budding and Other Risk Factors of Lymph Node Metastasis in Submucosal Early Gastric Carcinoma: A Multicenter Clinicopathologic Study in 621 Radical Gastrectomies of Chinese Patients. *Am J Surg Pathol* 2019; **43**: 1074-1082 [PMID: 31094925 DOI: 10.1097/PAS.0000000000001276]
39 **Yao G**, Fang Y, Fu Y, Xu J, Song H, Zhu H, Gu M, Ding X. Tumor budding as an indicator for lymph node metastasis and prognosis of early gastric cancer. *J Cancer Res Clin Oncol* 2023; **149**: 5603-5616 [PMID: 36512103 DOI: 10.1007/s00432-022-04522-z]
40 **Yim K**, Jang WM, Lee SH. Modified Tumor Budding as a Better Predictor of Lymph Node Metastasis in Early Gastric Cancer: Possible Real-World Applications. *Cancers (Basel)* 2021; **13** [PMID: 34298621 DOI: 10.3390/cancers13143405]
41 **Gabbert HE**, Meier S, Gerharz CD, Hommel G. Tumor-cell dissociation at the invasion front: a new prognostic parameter in gastric cancer patients. *Int J Cancer* 1992; **50**: 202-207 [PMID: 1730514 DOI: 10.1002/ijc.2910500208]
42 **Kemi N**, Eskuri M, Ikäläinen J, Karttunen TJ, Kauppila JH. Tumor Budding and Prognosis in Gastric Adenocarcinoma. *Am J Surg Pathol* 2019; **43**: 229-234 [PMID: 30334831 DOI: 10.1097/PAS.0000000000001181]
43 **Qi B**, Liu L, Pan Y, Xu S, Li J. Prognostic significance of peritumoural and intratumoural budding in intestinal-type gastric adenocarcinoma. *Arab J Gastroenterol* 2020; **21**: 111-116 [PMID: 32423856 DOI: 10.1016/j.ajg.2020.04.005]
44 **Hacking S**, Ebare K, Angert M, Lee L, Vitkovski T, Thomas R, Chavarria H, Jin C, Nasim M. Immature Stroma and Prognostic Profiling in Colorectal Carcinoma: Development and Validation of Novel Classification Systems. *Pathol Res Pract* 2020; **216**: 152970 [PMID: 32534718 DOI: 10.1016/j.prp.2020.152970]
45 **Kang BW**, Kim JG, Lee IH, Bae HI, Seo AN. Clinical significance of tumor-infiltrating lymphocytes for gastric cancer in the era of immunology. *World J Gastrointest Oncol* 2017; **9**: 293-299 [PMID: 28808502 DOI: 10.4251/wjgo.v9.i7.293]
46 **Zhang N**, Zhang G, Wang D, Liu H, Zhang Y, Ayarick VA, Han X, Lv Y, Wang Y. The relationship of the tertiary lymphoid structures with the tumor-infiltrating lymphocytes and its prognostic value in gastric cancer. *Arch Med Sci* 2024; **20**: 255-266 [PMID: 38414448 DOI: 10.5114/aoms/140622]
47 **Kang BW**, Seo AN, Yoon S, Bae HI, Jeon SW, Kwon OK, Chung HY, Yu W, Kang H, Kim JG. Prognostic value of tumor-infiltrating lymphocytes in Epstein-Barr virus-associated gastric cancer. *Ann Oncol* 2016; **27**: 494-501 [PMID: 26673353 DOI: 10.1093/annonc/mdv610]
48 **Grogg KL**, Lohse CM, Pankratz VS, Halling KC, Smyrk TC. Lymphocyte-rich gastric cancer: associations with Epstein-Barr virus, microsatellite instability, histology, and survival. *Mod Pathol* 2003; **16**: 641-651 [PMID: 12861059 DOI: 10.1097/01.MP.0000076980.73826.C0]
49 **Thompson ED**, Zahurak M, Murphy A, Cornish T, Cuka N, Abdelfatah E, Yang S, Duncan M, Ahuja N, Taube JM, Anders RA, Kelly RJ. Patterns of PD-L1 expression and CD8 T cell infiltration in gastric adenocarcinomas and associated immune stroma. *Gut* 2017; **66**: 794-801 [PMID: 26801886 DOI: 10.1136/gutjnl-2015-310839]
50 **Yu PC**, Long D, Liao CC, Zhang S. Association between density of tumor-infiltrating lymphocytes and prognoses of patients with gastric cancer. *Medicine (Baltimore)* 2018; **97**: e11387 [PMID: 29979429 DOI: 10.1097/MD.0000000000011387]
51 **Zhang N**, Cao M, Duan Y, Bai H, Li X, Wang Y. Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: a meta-analysis and experimental validation. *Arch Med Sci* 2020; **16**: 1092-1103 [PMID: 32863998 DOI: 10.5114/aoms.2019.86101]
52 **Zheng X**, Song X, Shao Y, Xu B, Chen L, Zhou Q, Hu W, Zhang D, Wu C, Tao M, Zhu Y, Jiang J. Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: a meta-analysis. *Oncotarget* 2017; **8**: 57386-57398 [PMID: 28915679 DOI: 10.18632/oncotarget.18065]
53 **Lee JS**, Won HS, Sun S, Hong JH, Ko YH. Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: A systematic review and meta-analysis. *Medicine (Baltimore)* 2018; **97**: e11769 [PMID: 30095632 DOI: 10.1097/MD.0000000000011769]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2024 April 22; 15(1): 92085

DOI: 10.4291/wjgp.v15.i1.92085

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Retrospective Study

Sepsis during short bowel syndrome hospitalizations: Identifying trends, disparities, and clinical outcomes in the United States

Dushyant Singh Dahiya, Jennifer Wachala, Shantanu Solanki, Dhanshree Solanki, Asim Kichloo, Samantha Holcomb, Uvesh Mansuri, Khwaja Saad Haq, Hassam Ali, Manesh Kumar Gangwani, Yash R Shah, Teresa Varghese, Hafiz Muzaffar Akbar Khan, Simon Peter Horslen, Thomas D Schiano, Syed-Mohammed Jafri

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Sanayeh EB, United States

Received: January 15, 2024
Peer-review started: January 15, 2024
First decision: February 3, 2024
Revised: February 10, 2024
Accepted: March 25, 2024
Article in press: March 25, 2024
Published online: April 22, 2024

Dushyant Singh Dahiya, Division of Gastroenterology, Hepatology & Motility, The University of Kansas School of Medicine, Kansas City, KS 66160, United States

Jennifer Wachala, Asim Kichloo, Samantha Holcomb, Department of Internal Medicine, Samaritan Medical Center, Watertown, NY 13601, United States

Shantanu Solanki, Division of Gastroenterology Hepatology & Nutrition, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, United States

Dhanshree Solanki, Department of Medicine, Institute for Foreign Medical Graduate Education, Houston, TX 77030, United States

Uvesh Mansuri, Department of Internal Medicine, MedStar Harbor Hospital, Baltimore, MD 21225, United States

Khwaja Saad Haq, Teresa Varghese, Department of Internal Medicine, WellStar Spalding Regional Hospital, Griffin, GA 30224, United States

Hassam Ali, Division of Gastroenterology, Hepatology and Nutrition, East Carolina University/Brody School of Medicine, Greenville, NC 27858, United States

Manesh Kumar Gangwani, Department of Gastroenterology and Hepatology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, United States

Yash R Shah, Department of Internal Medicine, Trinity Health Oakland/Wayne State University, Pontiac, MI 48341, United States

Hafiz Muzaffar Akbar Khan, Division of Gastroenterology and Hepatology, SUNY Upstate Medical University, Syracuse, NY 13210, United States

Simon Peter Horslen, Department of Pediatrics, School of Medicine and UPMC Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA 15219, United States

Thomas D Schiano, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, NY 10029, United States

Syed-Mohammed Jafri, Division of Gastroenterology and Hepatology, Henry Ford Hospital, Detroit, MI 48202, United States

Corresponding author: Dushyant Singh Dahiya, MD, Doctor, Division of Gastroenterology, Hepatology & Motility, The University of Kansas School of Medicine, 2000 Olathe Blvd, Kansas City, KS 66160, United States. dush.dahiya@gmail.com

Abstract

BACKGROUND

Short bowel syndrome (SBS) hospitalizations are often complicated with sepsis. There is a significant paucity of data on adult SBS hospitalizations in the United States and across the globe.

AIM

To assess trends and outcomes of SBS hospitalizations complicated by sepsis in the United States.

METHODS

The National Inpatient Sample was utilized to identify all adult SBS hospitalizations between 2005-2014. The study cohort was further divided based on the presence or absence of sepsis. Trends were identified, and hospitalization characteristics and clinical outcomes were compared. Predictors of mortality for SBS hospitalizations complicated with sepsis were assessed.

RESULTS

Of 247097 SBS hospitalizations, 21.7% were complicated by sepsis. Septic SBS hospitalizations had a rising trend of hospitalizations from 20.8% in 2005 to 23.5% in 2014 (P trend < 0.0001). Compared to non-septic SBS hospitalizations, septic SBS hospitalizations had a higher proportion of males (32.8% *vs* 29.3%, P < 0.0001), patients in the 35-49 (45.9% *vs* 42.5%, P < 0.0001) and 50-64 (32.1% *vs* 31.1%, P < 0.0001) age groups, and ethnic minorities, *i.e.*, Blacks (12.4% *vs* 11.3%, P < 0.0001) and Hispanics (6.7% *vs* 5.5%, P < 0.0001). Furthermore, septic SBS hospitalizations had a higher proportion of patients with intestinal transplantation (0.33% *vs* 0.22%, P < 0.0001), inpatient mortality (8.5% *vs* 1.4%, P < 0.0001), and mean length of stay (16.1 d *vs* 7.7 d, P < 0.0001) compared to the non-sepsis cohort. A younger age, female gender, White race, and presence of comorbidities such as anemia and depression were identified to be independent predictors of inpatient mortality for septic SBS hospitalizations.

CONCLUSION

Septic SBS hospitalizations had a rising trend between 2005-2014 and were associated with higher inpatient mortality compared to non-septic SBS hospitalizations.

Key Words: Short bowel syndrome; Sepsis; Outcomes; Mortality; Trends

Core Tip: Short bowel syndrome (SBS) is a well-known complication of small bowel surgical resection. Sepsis is a well-documented complication of SBS, particularly in infants and children. However, there is limited data on adult SBS hospitalizations complicated by sepsis in the United States. In this study, we noted that about one-fifth of SBS hospitalizations were complicated by sepsis. There was a higher proportion of men, individuals in the 35-64 age group, and ethnic minorities (Blacks and Hispanics) in the septic SBS cohort compared to the non-sepsis cohort. Septic SBS hospitalizations also had a higher length of stay and inpatient mortality compared to the non-sepsis cohort. Furthermore, younger age, female gender, White race, anemia, and depression were identified to be independent predictors of inpatient mortality for septic SBS hospitalizations.

Citation: Dahiya DS, Wachala J, Solanki S, Solanki D, Kichloo A, Holcomb S, Mansuri U, Haq KS, Ali H, Gangwani MK, Shah YR, Varghese T, Khan HMA, Horslen SP, Schiano TD, Jafri SM. Sepsis during short bowel syndrome hospitalizations: Identifying trends, disparities, and clinical outcomes in the United States. *World J Gastrointest Pathophysiol* 2024; 15(1): 92085
URL: https://www.wjgnet.com/2150-5330/full/v15/i1/92085.htm
DOI: https://dx.doi.org/10.4291/wjgp.v15.i1.92085

INTRODUCTION

Short bowel syndrome (SBS) is a well-known complication of surgical resection of the small bowel[1]. Nonsurgical causes include inflammatory bowel disease, cancer of the intestine, or ischemic and hemorrhagic vascular diseases of the gut[1,2]. Sepsis is a well-documented complication of SBS in infants and children, and these recurrent bloodstream infections (BSI) have been associated with higher rates of childhood morbidity and mortality[3,4]. The primary pathophysiologic mechanism implicated in the development of BSI is bacterial translocation from the gut to the bloodstream during enteral

feeding in children with SBS[5].

Most patients with SBS derive their nutrition *via* parenteral routes using indwelling venous catheters. Hence, these patients are at a greater-than-average risk of BSI from skin flora, especially if the indwelling catheter has been placed for a prolonged duration[4]. Moreover, parenteral nutrition leads to the impairment of immunological barriers (altered inflammatory responses) and physical barriers (secondary to villous atrophy) which may increase the risk of small bowel bacterial overgrowth (SBBO)[4,6].

SBS has established itself to be one of the strongest predictors of BSI in the pediatric population. However, there is a significant paucity of data on adult SBS hospitalizations complicated by sepsis, both in the United States and across the globe. Hence, this study was designed to investigate trends, hospitalization characteristics, predictors, racial disparities of Elixhauser co-morbidities, and gender disparities of Elixhauser co-morbidities for septic SBS hospitalizations in the United States. Furthermore, we also performed a comparative analysis for trends, hospitalization characteristics, and predictors between septic SBS and non-septic SBS hospitalizations.

MATERIALS AND METHODS

Data source

The National Inpatient Sample (NIS) is the largest, publicly available, multi-ethnic, all-payer inpatient database which is a part of the healthcare cost and utilization project (HCUP)[7,8]. HCUP is a family of healthcare databases and related software tools developed through a unique Federal-State-Industry partnership and sponsored by the Agency for Healthcare Research and Quality[9]. The NIS, when weighted, estimates more than 35 million hospitalizations nationally [7]. HCUP databases are limited data sets, which can be used to generate United States regional and national estimates[7].

Study population, design, and outcomes

We used the International Classification of Diseases, Clinical Modification (ICD-9-CM) codes to identify all adults (≥ 18 years of age) with SBS from the NIS database for the 2005-2014 period. The precedence of utilization of ICD-9-CM codes for SBS hospitalizations has already been established in previous studies[10,11]. Individuals < 18 years of age were excluded from the analysis. This study population was further divided based on the presence and absence of sepsis using the Clinical Classification Software diagnosis code "2", which has been used previously in multiple NIS-based studies[12, 13]. We then compared hospitalization characteristics (age, race, and gender), hospital-level characteristics (bed size, location, and admission type), and clinical outcomes [length of stay (LOS), all-cause inpatient mortality, and disposition status] between septic and non-septic SBS hospitalizations. Furthermore, the rates of comorbidities were calculated using Elixhauser Comorbidity Index codes provided by HCUP[14].

Statistical analysis

Statistical Analysis Software 9.3 (SAS Institute, Cary, NC, United States) was used for univariate and multivariate analyses. We used weighted values provided by HCUP to produce nationally representative estimates for all variables [15]. Categorical variables like gender, race, and comorbidities were compared using the Chi-squared (χ^2) test, and continuous variables like age and LOS were compared using the Wilcoxon rank-sum test. We also created a multivariate logistic regression model to determine predictors of inpatient mortality for SBS hospitalizations complicated by sepsis. P value ≤ 0.05 was considered statistically significant.

Ethical considerations

A review by our institutional review board was not required as the NIS database is Health Insurance Portability and Accountability Act protected and does not contain identifiable patient and hospital-level data[10].

RESULTS

Trends of septic SBS hospitalizations

Between 2005-2014, there were 247097 adult SBS hospitalizations in the United States. Of these, 53550 (21.7%) were complicated by sepsis. We noted a rising trend of SBS hospitalizations complicated by sepsis from 20.8% in 2005 to 23.5% in 2014 (P < 0.0001) (Table 1).

Hospitalization characteristics and clinical outcomes

Compared to non-septic SBS hospitalizations, septic SBS hospitalizations had a higher proportion of males (32.8% *vs* 29.3%, P < 0.0001), and patients in the 35-49 (45.9% *vs* 42.5%, P < 0.0001), and 50-64 (32.1% *vs* 31.1%, P < 0.0001) age groups. However, within the septic SBS cohort, a female predominance was noted (Table 2).

Racial disparities were also prevalent as we noted a higher proportion of ethnic minorities such as Blacks (12.4% *vs* 11.3%, P < 0.0001) and Hispanics (6.7% *vs* 5.5%, P < 0.0001) in the septic SBS cohort, while there was a higher proportion of Whites (80.0% *vs* 77.3%, P < 0.0001) in the non-septic cohort (Table 2).

From a hospital perspective, large (69.4% *vs* 64.6%, P < 0.0001), urban teaching (57.3% *vs* 51.8%, P < 0.0001) hospitals had a higher proportion of septic SBS hospitalizations compared to the non-septic cohort. Furthermore, there was a

Table 1 Trends for short bowel syndrome hospitalizations and septic short bowel syndrome hospitalizations in the United States from 2005-2014

Years	Short bowel syndrome hospitalizations	Septic short bowel syndrome hospitalizations	% of sepsis in short bowel syndrome hospitalizations
2005	4199	20198	20.8
2006	3908	20206	19.3
2007	4083	20206	20.2
2008	5762	26411	21.8
2009	5869	25810	22.7
2010	5640	25423	22.2
2011	5805	27647	21.0
2012	5780	27015	21.4
2013	6105	26900	22.7
2014	6400	27280	23.5
Total	53550	247097	21.7

higher proportion of emergent or urgent (19.3 *vs* 18.9%, $P < 0.0001$) septic SBS hospitalizations compared to the non-septic SBS cohort (Table 2).

The all-cause inpatient mortality was significantly higher for septic SBS hospitalizations (8.5% *vs* 1.4%, $P < 0.0001$) compared to the non-sepsis cohort. Additionally, we noted a longer mean LOS for septic SBS hospitalizations (16.1 d ± 0.4 d *vs* 7.7 d ± 0.1 d, $P < 0.0001$) compared to the non-septic cohort (Table 2).

Predictors of inpatient mortality for septic SBS hospitalizations

For septic SBS hospitalizations, the 18-44 age group had a 5.85 times higher risk of inpatient mortality compared to the ≥ 85 age group [odds ratio (OR): 5.85; 95% confidence interval (95%CI): 3.95-8.66, $P < 0.0001$; Table 3]. Hence, the risk of inpatient mortality decreased with increasing age. Additionally, women had a higher risk of inpatient mortality as compared to men (OR: 1.18; 95%CI: 1.02-1.38, $P = 0.03$). Race was also identified to be an independent predictor of all-cause inpatient mortality. Whites were noted to have a higher mortality risk (OR: 1.65; 95%CI: 1.17-2.33, $P = 0.005$) compared to other races. Furthermore, the presence of comorbidities such as deficiency anemias and depression were associated with a significantly higher risk of inpatient mortality for septic SBS hospitalizations (Table 3).

Intestinal transplantation and septic SBS hospitalizations

We noted a higher proportion of patients who had transplant of the intestine (TOI) for septic SBS hospitalizations (0.33% *vs* 0.22%, $P < 0.0001$) compared to the non-septic cohort (Table 1). However, due to limitations of the NIS database, we were unable to ascertain whether these hospitalizations took place within a year of undergoing TOI or later.

Racial disparities in exhauster comorbidities for septic SBS hospitalizations

A significantly higher proportion of septic SBS hospitalizations for Whites had comorbidities such as congestive heart failure, chronic pulmonary diseases, hypothyroidism, depression, and other psychiatric disorders (Table 4). Meanwhile, Blacks had significantly higher rates of comorbidities such as deficiency anemias, drug abuse, hypertension, obesity, and renal failure. Furthermore, Hispanics had the highest rates of comorbidities like coagulopathy, uncomplicated diabetes, and liver disease (Table 4).

Gender disparities in exhauster comorbidities for septic SBS hospitalizations

Septic SBS hospitalizations for women were noted to have higher rates of comorbidities such as deficiency anemias, depression, rheumatic disorders, chronic pulmonary disease, hypothyroidism, and obesity; however, men had higher rates of diabetes, drug abuse, liver disease, and renal failure (Table 5).

DISCUSSION

After surgical resection of the bowel in adults, there is a significant alteration of the nutritional, fluid, and electrolyte homeostasis, along with significant changes in the gut microbiome. These changes may lead to malabsorptive diarrhea, micronutrient deficiency, malnutrition, and SBBO[16]. Disruption of the intestinal microbiome seen in patients with SBBO impacts the production of antimicrobial peptides and immunomodulatory cells[17]. Furthermore, commensal bacteria dysbiosis may disrupt intestinal permeability leading to bacterial translocation in surrounding areas, thereby increasing

Table 2 Baseline hospitalization characteristics and clinical outcomes for septic and non-septic short bowel syndrome hospitalizations in the United States from 2005-2014

Variable	Non-septic short bowel syndrome hospitalizations	%	Septic short bowel syndrome hospitalizations	%	*P* value
Number of obs. (*n*)	193547	78.30	53550	21.70	
Age, yr (mean ± SE)	58.0 ± 0.3	-	57.9 ± 0.2	-	
Age, yr					< 0.0001
18-34	41979	21.70	10000	18.70	
35-49	82308	42.50	24561	45.90	
50-64	60113	31.10	17333	32.40	
≥ 65	9149	4.70	1655	3.10	
Gender					< 0.0001
Male	56601	29.30	17545	32.80	
Female	136916	70.80	36005	67.20	
Race					< 0.0001
White	130004	80.00	35660	77.30	
Black	18436	11.30	5730	12.40	
Hispanic	8866	5.50	3091	6.70	
Others	5274	3.20	1651	3.60	
Hospital location					< 0.0001
Rural	22155	11.50	4576	8.60	
Urban nonteaching	70692	36.70	18187	34.10	
Urban teaching	99780	51.80	30509	57.30	
Hospital bed size					< 0.0001
Small	22978	11.90	5107	9.60	
Medium	45289	23.50	11219	21.10	
Large	124360	64.60	36946	69.40	
Admission type					0.0100
Elective	157070	81.20	43201	80.70	
Emergent or Urgent	36477	18.90	10349	19.30	
Intestinal Transplant	430	0.22	178	0.33	< 0.0001
Disposition status					< 0.0001
Home	156802	81.10	34439	64.40	
Facility	33898	17.50	14497	27.10	
Inpatient mortality	2724	1.40	4537	8.50	< 0.0001
LOS, d (mean ± SE)	7.7 ± 0.1	-	16.1 ± 0.4	-	< 0.0001

LOS: Length of stay.

the risk of sepsis[17]. Despite known alteration of the intestine, current literature lacks data to support the routine use of antibiotics in SBS patients to prevent inflammatory gut changes[16]. Management for these patients is primarily focused on nutrition. However, patients with SBS who rely on parenteral nutrition are at increased risk of sepsis from catheter-associated infections, leading to increased hospitalizations[16]. This finding was highlighted in our study as we noted a rising trend of septic SBS hospitalizations from 20.8% in 2005 to 23.5% in 2014.

The utilization of prebiotics, probiotics, or antibiotics in SBS patients is controversial[16]. Intestinal transplantation is indicated in SBS patients with recurrent sepsis or those who are unable to receive total parenteral nutrition due to end-stage liver disease or end-stage loss of venous access[18]. Intestinal transplant significantly improves intestinal transit

Table 3 Predictors of mortality for septic short bowel syndrome hospitalizations in the United States from 2005-2014

Characteristics/co-morbidities	Odds ratio (95% confidence interval)	*P* value
Age (yr)		
18-44	5.85 (3.95-8.66)	< 0.0001
45-64	3.30 (2.37-4.59)	< 0.0001
65-84	1.75 (1.27-2.41)	0.001
≥ 85	Reference	
Gender		
Male	Reference	
Female	1.18 (1.02-1.38)	0.030
Race		
White	1.65 (1.17-2.33)	0.005
Black	1.28 (0.86-1.90)	0.220
Hispanic	1.22 (0.79-1.88)	0.360
Others	Reference	
Hospital bed size		
Small	1.16 (0.90-1.51)	0.250
Medium	1.10 (0.92-1.31)	0.320
Large	Reference	
Hospital type		
Rural	1.05 (0.79-1.38)	0.360
Urban non-teaching	0.94 (0.80-1.10)	0.420
Teaching	Reference	
Median household income		
Quartile 1	Reference	
Quartile 2	0.96 (0.78-1.18)	0.690
Quartile 3	1.03 (0.83-1.27)	0.800
Quartile 4	1.18 (0.94-1.47)	0.150
Co-morbidities		
Deficiency anemias	1.58 (1.35-1.85)	< 0.0001
Rheumatic disorders	1.20 (0.80-1.79)	0.380
Depression	1.66 (1.30-2.13)	< 0.0001
Drug abuse	1.51 (0.95-2.40)	0.090
Hypertension	1.16 (0.98-1.36)	0.080
Hypothyroidism	1.06 (0.83-1.34)	0.650
Lymphoma	2.48 (0.99-6.21)	0.050
Valvular disease	1.02 (0.73-1.41)	0.920

time, peristalsis, and the absorptive functions of the gut[19]. However, like any transplant, post-operative care for these patients requires lifelong immunosuppression, which imminently increases the risk for subsequent infections and sepsis. Expectedly, in our study, we noted a higher proportion of patients who had TOI in septic SBS hospitalizations compared to the non-sepsis cohort (Table 1).

Traditionally, sepsis tends to affect the elderly. However, a multicenter longitudinal cohort study in California from 2008-2015 noted that the highest overall increase in rates of sepsis and severe sepsis were for patients 18-44 years of age [20]. Although there were higher incidence rates of sepsis in the elderly, there was a notable increase in the relative risk of sepsis among young adults[20]. This was consistent with the findings in our study. In the septic SBS cohort, the 35-49 age

Table 4 Racial distribution of Elixhauser co-morbidities for septic short bowel syndrome hospitalizations in the United States from 2005-2014

Elixhauser co-morbidity	Whites	Blacks	Hispanics	Others	*P* value
Acquired immunodeficiency syndrome	0.0	1.0	0.3	0.0	< 0.0001
Alcohol abuse	1.6	2.4	0.8	1.4	< 0.0001
Deficiency anemias	38.8	49.0	45.4	37.6	< 0.0001
Rheumatic disorders	4.3	4.1	2.9	4.0	0.0040
Chronic blood loss anemia	2.1	2.1	2.3	3.2	0.0200
Congestive heart failure	11.2	10.8	8.6	9.8	< 0.0001
Chronic pulmonary disease	19.7	14.6	10.6	18.4	< 0.0001
Coagulopathy	15.4	16.9	19.1	16.9	< 0.0001
Depression	18.0	10.9	14.2	11.5	< 0.0001
Uncomplicated diabetes	11.4	14.4	17.7	17.0	< 0.0001
Diabetes with chronic complications	2.8	5.8	5.4	5.0	< 0.0001
Drug abuse	4.8	6.6	4.4	5.0	< 0.0001
Hypertension	32.4	42.5	34.5	34.6	< 0.0001
Hypothyroidism	13.5	10.4	8.8	8.6	< 0.0001
Liver disease	5.8	7.0	9.9	6.9	< 0.0001
Lymphoma	1.1	1.1	1.2	0.9	0.7500
Fluid and electrolyte disorders	57.5	62.5	61.2	58.2	< 0.0001
Metastatic cancer	4.5	4.4	5.2	8.6	< 0.0001
Neurological disorders	8.8	8.3	6.7	7.9	0.001
Obesity	5.6	6.3	5.7	6.9	0.0300
Paralysis	2.3	3.3	4.2	4.9	< 0.0001
Peripheral vascular disorders	6.8	7.4	7.0	6.5	0.4300
Psychiatric disorder	6.1	5.5	5.6	4.1	0.0030
Pulmonary circulation disorders	4.0	5.5	2.3	3.4	< 0.0001
Renal failure	21.2	30.7	16.7	18.8	< 0.0001
Solid tumor without metastasis	2.6	2.8	2.9	5.2	< 0.0001
Peptic ulcer disease excluding bleeding	0.1	0.1	0.1	0.0	0.3400
Valvular disease	5.2	4.8	3.8	5.3	0.0050
Weight loss	40.4	40.9	39.3	47.9	< 0.0001

group had the highest proportion of patients, while the ≥ 65 age group had the lowest proportion of patients (Table 2). On comparative analysis, septic SBS hospitalizations had a higher proportion of patients between the ages of 35-64 compared to the non-septic cohort. The exact reasons for the higher hospitalization rates of septic SBS younger patients are currently unknown, but may, in part, be due to a greater degree of awareness of sepsis in this subset population prompting them to seek immediate care or due to complications from comorbidities not previously common in this age group[20]. Nonetheless, additional prospective studies are needed to further investigate these findings.

A prospective observational cohort study of critically ill patients from 2011-2014 showed similar rates of sepsis and mortality between men and women[21]. However, there was a greater degree of endothelial cell activation in young women compared to men[21]. Increased gut permeability, often seen in patients with SBS, coupled with increased endothelial disruption of the vasculature may lead to the transportation of antigens and commensal gut microbiota from the intestine to the blood, making females even more prone to sepsis[22]. The findings of our study aligned with this current literature as females made up more than two-thirds of the total septic SBS hospitalizations in the United States.

The study by Siddiqui *et al*[2] reported that Whites made up 78% of all SBS hospitalizations in the United States. We report similar findings as septic SBS hospitalizations had a higher proportion of Whites (77.3%) compared to other ethnic minorities such as Blacks or Hispanics (Table 2). However, on comparative analysis, septic SBS hospitalizations had a higher proportion of Blacks (12.4% *vs* 11.3%, $P < 0.0001$) and Hispanics (6.7% *vs* 5.5%, $P < 0.0001$) compared to the non-

Table 5 Gender distribution of elixhauser co-morbidities for septic short bowel syndrome hospitalizations in the United States from 2005-2014

Elixhauser co-morbidity	Male	Female	*P* value
Acquired immunodeficiency syndrome	0.3	0.1	< 0.0001
Alcohol abuse	2.9	1.0	< 0.0001
Deficiency anemias	36.8	39.7	< 0.0001
Rheumatic disorders	2.0	5.1	< 0.0001
Chronic blood loss anemia	1.9	2.1	0.0800
Congestive heart failure	10.7	10.8	0.9000
Chronic pulmonary disease	16.7	19.0	< 0.0001
Coagulopathy	16.8	15.0	< 0.0001
Depression	12.4	18.1	< 0.0001
Uncomplicated diabetes	12.6	11.6	0.0010
Diabetes with chronic complications	3.9	2.7	< 0.0001
Drug abuse	5.1	4.5	0.0010
Hypertension	33.6	32.1	0.0010
Hypothyroidism	6.0	14.9	< 0.0001
Liver disease	7.4	5.3	< 0.0001
Lymphoma	1.4	0.9	< 0.0001
Fluid and electrolyte disorders	56.6	57.9	0.0030
Metastatic cancer	4.9	4.4	0.0020
Neurological disorders	7.0	8.8	< 0.0001
Obesity	3.8	6.4	< 0.0001
Paralysis	3.5	2.1	< 0.0001
Peripheral vascular disorders	8.1	5.9	< 0.0001
Psychiatric disorder	4.7	6.3	< 0.0001
Pulmonary circulation disorders	4.2	3.8	0.0400
Renal failure	26.3	18.8	< 0.0001
Solid tumor without metastasis	2.6	2.7	0.5900
Peptic ulcer disease excluding bleeding	0.0	0.1	< 0.0001
Valvular disease	5.2	4.7	0.0100
Weight loss	39.1	40.9	< 0.0001

septic cohort. This may, in part, be due to lack of healthcare facilities, and awareness about SBS among ethnic minorities leading to a progression of their disease and further complications by sepsis. Hence, we advocate for the need for urgent interventions to improve healthcare access, increase awareness about sepsis in SBS, and improve outpatient follow-up for these high-risk populations.

The all-cause inpatient mortality for SBS hospitalizations was found to be 1.4%. However, when these hospitalizations were complicated by sepsis, the all-cause inpatient mortality increased to 8.5%. This was in line with current literature which reports increased inpatient mortality for septic SBS hospitalizations[2]. Moreover, the presence of a greater number of comorbidities is also associated with higher rates of mortality in patients with sepsis[23]. A cohort study evaluating adult sepsis survivors identified age, sex, race, severe comorbidities, and site of initial infection to be long-term risk factors for mortality[24]. Similarly, in our study, independent risk factors that increased inpatient mortality for septic SBS hospitalizations are as follows.

Age

In our study, the risk of inpatient mortality was almost six times higher in the 18-44 age group compared to individuals ≥ 85 years of age. In 2013, a Quality and Cost of Primary Care study reported that younger patients were more likely to visit a healthcare provider for both mental and physical health conditions compared to the elderly population[25]. The

increased health awareness, highly accurate provider evaluation in younger adults without non-specific baseline symptoms, and presence of additional comorbidities which were previously not common in this age group may have led to increased diagnosis and associated inpatient mortality.

Gender

In 2011, a prospective clinical trial of intensive care unit patients reported that females with sepsis had higher mortality rates than males with sepsis[26]. Another study by Wilcox *et al*[27] noted similar gender outcomes. Similarly, in our study, septic SBS female patients had higher rates of all-cause inpatient mortality compared to males. Furthermore, we noted higher rates of deficiency anemias and depression in females compared to males, which was also independently associated with higher mortality rates in septic SBS hospitalizations.

Race

We noted that White septic SBS hospitalizations had a 1.65 times greater risk of inpatient mortality compared to other races. A study of Caucasians with acute respiratory distress syndrome (ARDS) secondary to pneumonia evaluated the FER rs4957796 TT genotype as a means of determining 90-d mortality risk[28]. Although this study specifically assessed ARDS patients and their survival in the setting of pneumonia, interestingly the FER gene is known to play a key role in the regulation of intestinal barrier function[29]. White septic SBS hospitalizations may have a higher mortality risk due to the absence of FER protein which may exacerbate intestinal dysfunction and increase bacterial translocation into the bloodstream leading to a greater severity of sepsis. However, additional prospective studies are needed to further validate our findings.

Comorbidities

In our study, deficiency anemias and depression were found to coincide with significantly greater mortality risk in septic SBS hospitalizations. Deficiency anemias are highly prevalent in SBS patients due to altered anatomy[30-33]. In septic SBS hospitalizations, deficiency anemia can negatively impact the host's defense and immunomodulatory response, increasing the severity of sepsis and overall mortality risk. Furthermore, severe depression has a known association with increased BSI, leading to sepsis and higher mortality rates[34,35]. Depression ultimately leads to decreased immune function and disruption of the brain-gut-microbiome axis, which may increase the host's risk for sepsis and adverse clinical outcomes.

Limitations

A key strength of this study is the study population which has been derived from one of the biggest, national, diverse, multi-ethnic databases in the United States. Through our analysis over 10 years, we are able to provide meaningful information on the trends of septic SBS hospitalizations. Furthermore, we also perform a unique comparative analysis between septic and non-septic SBS hospitalizations and identify predictors of inpatient mortality for septic SBS hospitalizations to give gastroenterologists real-world data on the patients at the highest risk of adverse clinical outcomes. However, we do acknowledge all the limitations associated with our study. We were unable to perform a detailed analysis after the 2014 study period as the NIS changed from ICD-9-CM to ICD-10-CM coding at the beginning of October 1, 2015. Converting ICD-9 to ICD-10 is an extremely challenging process as there are differences in the structure and granularity of codes. Hence, it would be impossible to find the exact matches for the codes with a high level of confidence Additionally, the NIS database lacks information on the time from hospitalization to diagnosis of sepsis, hospital course, treatment aspects, inpatient procedures, and pharmacological aspects of management. Lastly, the NIS is an administrative database. Therefore, the possibility of coding errors cannot be excluded. Despite these limitations, we believe that our study helps fill the current knowledge gaps for SBS hospitalizations complicated by sepsis in the US as this entity has not been studied extensively. We hope that the findings of our study can serve as a foundation for future prospective studies and randomized controlled trials.

CONCLUSION

In conclusion, SBS is a well-known complication of surgical resection of the intestine. Due to their dependence on parenteral nutrition using indwelling venous catheters, these patients are at increased risk of BSI and sepsis from bacterial translocation from the gut to the bloodstream during enteral feeding. In the United States from 2005-2014, we noted a rising trend of septic SBS hospitalizations with a significant female predominance. Compared to the non-septic cohort, septic SBS hospitalizations had a higher proportion of patients with TOI, higher all-cause inpatient mortality, and longer mean LOS. Independent predictors of mortality for septic SBS hospitalizations included White race, female gender, younger age, and those with associated comorbidities such as deficiency anemias and depression.

FOOTNOTES

Author contributions: Dahiya DS, Wachala J, Solanki S, and Jafri SM contributed to the conception and design; Dahiya DS, Solanki S, Kichloo A, and Jafri SM contributed to the administrative support; Dahiya DS, Wachala J, Solanki S, Solanki D, Kichloo A, Holcomb S, Mansuri U, Haq KS, Ali H, Gangwani MK, and Shah YR contributed to the provision, collection, and assembly of data; Dahiya DS,

Wachala J, Solanki S, Solanki D, Kichloo A, Holcomb S, Mansuri U, Haq KS, Ali H, Gangwani MK, Shah YR, Varghese T, Khan HMA, Horslen S, Schiano TD, and Jafri SM contributed to the review of literature and drafting the manuscript; Dahiya DS, Wachala J, Solanki S, Solanki D, Kichloo A, Holcomb S, Mansuri U, Haq KS, Ali H, Gangwani MK, Shah YR, Varghese T, Khan HMA, Horslen S, Schiano TD, and Jafri SM contributed to the revision of key components of the manuscript and final approval of manuscript; Dahiya DS, Wachala J, Solanki S, Solanki D, Kichloo A, Holcomb S, Mansuri U, Haq KS, Ali H, Gangwani MK, Shah YR, Varghese T, Khan HMA, Horslen S, Schiano TD, and Jafri SM are accountable for all aspects of the work.

Institutional review board statement: The National Inpatient Sample lacks specific patient and hospital identifiers. Hence, our analysis did not require institutional review board (IRB) approval as per guidelines put forth by our institutional IRB for the analysis of national databases.

Informed consent statement: The National Inpatient Sample lacks specific patient identifiers. Hence, informed consent was not required.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Data sharing statement: The National Inpatient Sample (NIS) is a large, publicly available, all-payer inpatient care database in the United States containing data on millions of hospital stays per year. The large sample size derived from the NIS database provides sufficient data for analysis of common diseases, uncommon disorders, and procedures. The NIS is publicly available at: https://www.hcup-us.ahrq.gov/

Country/Territory of origin: United States

ORCID number: Dushyant Singh Dahiya 0000-0002-8544-9039; Shantanu Solanki 0000-0001-9563-2470; Hassam Ali 0000-0001-5546-9197; Manesh Kumar Gangwani 0000-0002-3931-6163; Thomas D Schiano 0000-0003-1878-5101; Syed-Mohammed Jafri 0000-0001-8108-7408.

S-Editor: Chen YL
L-Editor: A
P-Editor: Zhao YQ

REFERENCES

1 **Massironi S**, Cavalcoli F, Rausa E, Invernizzi P, Braga M, Vecchi M. Understanding short bowel syndrome: Current status and future perspectives. *Dig Liver Dis* 2020; **52**: 253-261 [PMID: 31892505 DOI: 10.1016/j.dld.2019.11.013]
2 **Siddiqui MT**, Al-Yaman W, Singh A, Kirby DF. Short-Bowel Syndrome: Epidemiology, Hospitalization Trends, In-Hospital Mortality, and Healthcare Utilization. *JPEN J Parenter Enteral Nutr* 2021; **45**: 1441-1455 [PMID: 33233017 DOI: 10.1002/jpen.2051]
3 **Kurkchubasche AG**, Smith SD, Rowe MI. Catheter sepsis in short-bowel syndrome. *Arch Surg* 1992; **127**: 21-4; discussion 24 [PMID: 1734846 DOI: 10.1001/archsurg.1992.01420010027004]
4 **Miko BA**, Kamath SS, Cohen BA, Jeon C, Jia H, Larson EL. Epidemiologic Associations Between Short-Bowel Syndrome and Bloodstream Infection Among Hospitalized Children. *J Pediatric Infect Dis Soc* 2015; **4**: 192-197 [PMID: 26336089 DOI: 10.1093/jpids/piu079]
5 **Weber TR**. Enteral feeding increases sepsis in infants with short bowel syndrome. *J Pediatr Surg* 1995; **30**: 1086-8; discussion 1088 [PMID: 7472938 DOI: 10.1016/0022-3468(95)90347-x]
6 **Terra RM**, Plopper C, Waitzberg DL, Cukier C, Santoro S, Martins JR, Song RJ, Gama-Rodrigues J. Remaining small bowel length: association with catheter sepsis in patients receiving home total parenteral nutrition: evidence of bacterial translocation. *World J Surg* 2000; **24**: 1537-1541 [PMID: 11193720 DOI: 10.1007/s002680010274]
7 **Healthcare Cost and Utilization Project (HCUP)**. Overview of National (Nationwide) Inpatient Sample (NIS). [cited 1 March 2024]. Available from: https://hcup-us.ahrq.gov/nisoverview.jsp
8 **Healthcare Cost and Utilization Project (HCUP)**. NIS Database Documentation. Volume 2020: Healthcare Cost and Utilization Project (HCUP). [cited 1 March 2024]. Available from: https://hcup-us.ahrq.gov/nisoverview.jsp
9 **Hoffman RD**, Danos DM, Lau FH. National health disparities in incisional hernia repair outcomes: An analysis of the Healthcare Cost and Utilization Project National Inpatient Sample (HCUP-NIS) 2012-2014. *Surgery* 2021; **169**: 1393-1399 [PMID: 33422347 DOI: 10.1016/j.surg.2020.11.028]
10 **Healthcare Cost and Utilization Project (HCUP)**. Healthcare Cost and Utilization Project Data Use Agreement Course. [cited 1 March 2024]. Available from: https://hcup-us.ahrq.gov/tech_assist/dua.jsp
11 **Limketkai BN**, Parian AM, Chen PH, Colombel JF. Treatment With Biologic Agents Has Not Reduced Surgeries Among Patients With Crohn's Disease With Short Bowel Syndrome. *Clin Gastroenterol Hepatol* 2017; **15**: 1908-1914.e2 [PMID: 28666947 DOI: 10.1016/j.cgh.2017.06.040]
12 **Papolos A**, Narula J, Bavishi C, Chaudhry FA, Sengupta PP. U.S. Hospital Use of Echocardiography: Insights From the Nationwide Inpatient Sample. *J Am Coll Cardiol* 2016; **67**: 502-511 [PMID: 26846948 DOI: 10.1016/j.jacc.2015.10.090]
13 **Mansuri U**, Patel A, Shah H, Chauhan K, Poojary P, Saha A, Dave M, Hazra A, Mishra T, Annapureddy N, Nadkarni G, Chan L. Trends and outcomes of sepsis hospitalizations complicated by acute kidney injury requiring hemodialysis. *J Crit Care* 2017; **38**: 353-355 [PMID: 27986342 DOI: 10.1016/j.jcrc.2016.10.012]

14 **Healthcare Cost and Utilization Project (HCUP)**. Elixhauser Comorbidity Software, Version 3.7. Volume 2021. [cited 1 March 2024]. Available from: https://hcup-us.ahrq.gov/toolssoftware/comorbidity/comorbidity.jsp

15 **Healthcare Cost and Utilization Project (HCUP)**. Trend Weights for HCUP NIS Data. Volume 2020. [cited 1 March 2024]. Available from: https://hcup-us.ahrq.gov/db/nation/nis/trendwghts.jsp

16 **Parrish CR**, DiBaise JK. Managing the Adult Patient With Short Bowel Syndrome. *Gastroenterol Hepatol (N Y)* 2017; **13**: 600-608 [PMID: 29230136]

17 **Fay KT**, Ford ML, Coopersmith CM. The intestinal microenvironment in sepsis. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 2574-2583 [PMID: 28286161 DOI: 10.1016/j.bbadis.2017.03.005]

18 **Höllwarth ME**. Surgical strategies in short bowel syndrome. *Pediatr Surg Int* 2017; **33**: 413-419 [PMID: 28039510 DOI: 10.1007/s00383-016-4043-6]

19 **Kaufman SS**, Avitzur Y, Beath SV, Ceulemans LJ, Gondolesi GE, Mazariegos GV, Pironi L. New Insights Into the Indications for Intestinal Transplantation: Consensus in the Year 2019. *Transplantation* 2020; **104**: 937-946 [PMID: 31815899 DOI: 10.1097/TP.0000000000003065]

20 **Wardi G**, Tainter CR, Ramnath VR, Brennan JJ, Tolia V, Castillo EM, Hsia RY, Malhotra A, Schmidt U, Meier A. Age-related incidence and outcomes of sepsis in California, 2008-2015. *J Crit Care* 2021; **62**: 212-217 [PMID: 33429114 DOI: 10.1016/j.jcrc.2020.12.015]

21 **van Vught LA**, Scicluna BP, Wiewel MA, Hoogendijk AJ, Klein Klouwenberg PMC, Ong DSY, Cremer OL, Horn J, Franitza M, Toliat MR, Nürnberg P, Bonten MMJ, Schultz MJ, van der Poll T; MARS Consortium. Association of Gender With Outcome and Host Response in Critically Ill Sepsis Patients. *Crit Care Med* 2017; **45**: 1854-1862 [PMID: 28806220 DOI: 10.1097/CCM.0000000000002649]

22 **Assimakopoulos SF**, Triantos C, Thomopoulos K, Fligou F, Maroulis I, Marangos M, Gogos CA. Gut-origin sepsis in the critically ill patient: pathophysiology and treatment. *Infection* 2018; **46**: 751-760 [PMID: 30003491 DOI: 10.1007/s15010-018-1178-5]

23 **Rhee C**, Jones TM, Hamad Y, Pande A, Varon J, O'Brien C, Anderson DJ, Warren DK, Dantes RB, Epstein L, Klompas M; Centers for Disease Control and Prevention (CDC) Prevention Epicenters Program. Prevalence, Underlying Causes, and Preventability of Sepsis-Associated Mortality in US Acute Care Hospitals. *JAMA Netw Open* 2019; **2**: e187571 [PMID: 30768188 DOI: 10.1001/jamanetworkopen.2018.7571]

24 **Shankar-Hari M**, Harrison DA, Ferrando-Vivas P, Rubenfeld GD, Rowan K. Risk Factors at Index Hospitalization Associated With Longer-term Mortality in Adult Sepsis Survivors. *JAMA Netw Open* 2019; **2**: e194900 [PMID: 31150081 DOI: 10.1001/jamanetworkopen.2019.4900]

25 **Thompson AE**, Anisimowicz Y, Miedema B, Hogg W, Wodchis WP, Aubrey-Bassler K. The influence of gender and other patient characteristics on health care-seeking behaviour: a QUALICOPC study. *BMC Fam Pract* 2016; **17**: 38 [PMID: 27036116 DOI: 10.1186/s12875-016-0440-0]

26 **Nachtigall I**, Tafelski S, Rothbart A, Kaufner L, Schmidt M, Tamarkin A, Kartachov M, Zebedies D, Trefzer T, Wernecke KD, Spies C. Gender-related outcome difference is related to course of sepsis on mixed ICUs: a prospective, observational clinical study. *Crit Care* 2011; **15**: R151 [PMID: 21693012 DOI: 10.1186/cc10277]

27 **Elizabeth Wilcox M**, Donnelly JP, Lone NI. Understanding gender disparities in outcomes after sepsis. *Intensive Care Med* 2020; **46**: 796-798 [PMID: 32072302 DOI: 10.1007/s00134-020-05961-3]

28 **Hinz J**, Büttner B, Kriesel F, Steinau M, Frederik Popov A, Ghadimi M, Beissbarth T, Tzvetkov M, Bergmann I, Mansur A. The FER rs4957796 TT genotype is associated with unfavorable 90-day survival in Caucasian patients with severe ARDS due to pneumonia. *Sci Rep* 2017; **7**: 9887 [PMID: 28851893 DOI: 10.1038/s41598-017-08540-7]

29 **Qi W**, Ebbert KV, Craig AW, Greer PA, McCafferty DM. Absence of Fer protein tyrosine kinase exacerbates endotoxin induced intestinal epithelial barrier dysfunction in vivo. *Gut* 2005; **54**: 1091-1097 [PMID: 16009680 DOI: 10.1136/gut.2004.061887]

30 **Liu Q**, Wu J, Zhang X, Wu X, Zhao Y, Ren J. Iron homeostasis and disorders revisited in the sepsis. *Free Radic Biol Med* 2021; **165**: 1-13 [PMID: 33486088 DOI: 10.1016/j.freeradbiomed.2021.01.025]

31 **Sun H**, Weaver CM. Decreased Iron Intake Parallels Rising Iron Deficiency Anemia and Related Mortality Rates in the US Population. *J Nutr* 2021; **151**: 1947-1955 [PMID: 33834234 DOI: 10.1093/jn/nxab064]

32 **Alker W**, Haase H. Zinc and Sepsis. *Nutrients* 2018; **10** [PMID: 30060473 DOI: 10.3390/nu10080976]

33 **Wolffenbuttel BHR**, Heiner-Fokkema MR, Green R, Gans ROB. Relationship between serum B12 concentrations and mortality: experience in NHANES. *BMC Med* 2020; **18**: 307 [PMID: 33032595 DOI: 10.1186/s12916-020-01771-y]

34 **Askim Å**, Gustad LT, Paulsen J, Reitan SK, Mehl A, Mohus RM, Dewan A, Damås JK, Solligård E, Åsvold BO. Anxiety and Depression Symptoms in a General Population and Future Risk of Bloodstream Infection: The HUNT Study. *Psychosom Med* 2018; **80**: 673-679 [PMID: 29923889 DOI: 10.1097/PSY.0000000000000619]

35 **Thorkildsen MS**, Laugsand LE, Nilsen TIL, Mohus RM, Høvik LH, Rogne T, Solligård E, Damås JK, Gustad LT. Insomnia symptoms and risk of bloodstream infections: prospective data from the prospective population-based Nord-Trøndelag Health Study (HUNT), Norway. *J Sleep Res* 2023; **32**: e13696 [PMID: 36068650 DOI: 10.1111/jsr.13696]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2024 April 22; 15(1): 90893

DOI: 10.4291/wjgp.v15.i1.90893

ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Observational Study

Des-gamma-carboxy prothrombin and alpha-fetoprotein levels as biomarkers for hepatocellular carcinoma and their correlation with radiological characteristics

Muhammad Ali Qadeer, Zaigham Abbas, Shaima Amjad, Bushra Shahid, Abeer Altaf, Mehreen Siyal

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Nasrallah O, Syria

Received: December 16, 2023
Peer-review started: December 16, 2023
First decision: January 31, 2024
Revised: February 13, 2024
Accepted: March 26, 2024
Article in press: March 26, 2024
Published online: April 22, 2024

Muhammad Ali Qadeer, Zaigham Abbas, Abeer Altaf, Mehreen Siyal, Department of Hepatogastroenterology, Dr. Ziauddin University Hospital Clifton, Karachi 75600, Pakistan

Shaima Amjad, Family Medicine, Dr. Ziauddin University Hospital Clifton, Karachi 75600, Pakistan

Bushra Shahid, Internal Medicine, Dr. Ziauddin University Hospital Clifton, Karachi 75600, Pakistan

Corresponding author: Zaigham Abbas, AGAF, FACG, FACP, FCPS, FRCP, MBBS, Professor, Department of Hepatogastroenterology, Dr. Ziauddin University Hospital, 4/B Shahrah-e-Ghalib Rd, Block 6, Clifton, Karachi 75600, Pakistan. drzabbas@gmail.com

Abstract

BACKGROUND

Alpha-fetoprotein (AFP), a commonly used biomarker for hepatocellular carcinoma (HCC), is normal in up to one-third of patients.

AIM

To evaluate the diagnostic performance of des-gamma-carboxy-prothrombin (DCP) alone and in combination with AFP.

METHODS

In this study, 202 patients with radiologically proven HCC were enrolled, and their DCP and AFP levels were evaluated for their diagnostic performance.

RESULTS

The mean age of the enrolled patients was 58.5 years; 72.0% were male. DCP was elevated in 86.6% (n = 175) of all patients, 100.0% (n = 74) of patients with portal vein thrombus, and 87.4% (n = 111) of patients with multicentric HCC. AFP was elevated in 64.3% (n = 130) of all the patients, 74% (n = 55) of the patients with portal vein thrombus, and 71.6% (n = 91) of the patients with multicentric HCC (P = 0.030, 0.001, and 0.015, respectively). In tumors less than 2 cm in size (n = 46), DCP was increased in 32 (69.5%) patients, and AFP was increased in 25 (54.3%) patients (P = 0.801). There was good pairing between DCP and AFP for HCCs of 2 cm size or larger (P < 0.001); however, the pairing among tumors < 2 cm size was

not significant (P = 0.210). In 69 of the patients (34.1%), only one of the tumor markers was positive; DCP was elevated alone in 57/202 (28.2%) of all patients, and AFP alone was elevated in 12/202 (5.9%) of the patients. The areas under receiver operating characteristic curves (AUROC) for tumors > 2 cm was 0.74 for DCP and 0.59 for AFP; combining both markers resulted in an AUROC of 0.73. For tumors < 2 cm, the AUROC was 0.25 for DCP and 0.40 for AFP.

CONCLUSION

DCP, as an individual marker, had a better diagnostic performance in many cases of HCC. Hence, DCP may replace AFP as the primary HCC biomarker.

Key Words: Des-gamma-carboxy prothrombin; Protein induced by vitamin K absence-II; Cirrhosis; Alpha-fetoprotein; Biomarkers; Hepatocellular carcinoma; Portal vein thrombus

Core Tip: In this prospective study, the performance of des-gamma-carboxy prothrombin (DCP) relative to alpha-fetoprotein (AFP) was assessed in 202 patients diagnosed with hepatocellular carcinoma (HCC). DCP, when used as a standalone marker, exhibited superior diagnostic performance compared to AFP. Combining both tumor markers increased the overall detection rate of HCC, particularly in tumors less than 2 cm in length. Nevertheless, it is recommended that, if a single tumor marker is used, DCP is preferred. The role of DCP as a screening biomarker should be incorporated into the HCC guidelines.

Citation: Qadeer MA, Abbas Z, Amjad S, Shahid B, Altaf A, Siyal M. Des-gamma-carboxy prothrombin and alpha-fetoprotein levels as biomarkers for hepatocellular carcinoma and their correlation with radiological characteristics. *World J Gastrointest Pathophysiol* 2024; 15(1): 90893

URL: https://www.wjgnet.com/2150-5330/full/v15/i1/90893.htm

DOI: https://dx.doi.org/10.4291/wjgp.v15.i1.90893

INTRODUCTION

Hepatocellular carcinoma (HCC), the predominant liver malignancy, is the sixth most commonly diagnosed cancer[1]. Radiological modalities have been at the forefront of screening and diagnosing HCC, but tumor markers have also contributed to its early detection[2]. Early detection of HCC not only dictates treatment modality but also influences expected survival[3]. Tumor markers such as alpha-fetoprotein (AFP) and the protein induced by vitamin K absence-II (PIVKA-II) also known as des-gamma-carboxy-prothrombin (DCP), if elevated beyond certain limits, can influence the HCC recurrence rate after liver transplantation[4].

Various studies have noted the inadequacy of AFP for screening for HCC, with almost 40% of tumors flagged as non-AFP-producing[5]. PIVKA II/DCP, an abnormal prothrombin precursor, has also been described as a potential screening tool for liver cancer[6]. A large body of evidence supports that it has better sensitivity than AFP, with reported sensitivities reaching up to 84%[7-9]. DCP has been shown to be effective not only for detecting HCC but also for predicting its radiological and histological characteristics[9]. However, despite this supportive literature, conflicting data have also been published that have limited the use of DCP in clinical practice. Hence, we evaluated the diagnostic performance of DCP alone and in combination with AFP and analyzed its correlation with radiographic parameters such as size, lobe involvement, and vascular invasion. The diagnostic performance of DCP for AFP-negative HCC was also analyzed.

MATERIALS AND METHODS

Patient data

A total of 202 patients with radiologically confirmed HCC were included in this prospective study at Dr. Ziauddin University Hospital from January 2019 to March 2022. This study was approved by Dr. Ziauddin University's ethical review board. Informed consent was obtained from all the participants following the ethical standards of the 1964 Helsinki Declaration.

HCC was diagnosed based on the Liver Imaging Reporting and Data System v2017. Liver lesions falling within the LIRAD-V were considered acceptable for inclusion in this study. Patients aged less than 18 years with a history of HCC treatment, use of vitamin K antagonists, or obstructive jaundice were excluded.

Analysis of PIVKA and AFP

The ARCHITECT PIVKA-II assay3C10 (Abbott Laboratories, IL, United States) using chemiluminescent technology was used for the quantification of PIVKA-II. DCP was considered normal if the value fell below 46 mAU/mL. The AFP

concentration was analyzed using an ARCHITECT AFP 3P36 (Abbott Laboratories, IL, United States) kit, which uses a two-step immunoassay for quantitative measurement. AFP was considered normal if the value was less than 8.78 ng/mL.

Statistical analysis

SPSS version 26 was used for the statistical analysis. For all dichotomous variables, the data are summarized as percentages. Categorical variables were compared using Fisher's exact test. The Mann-Whitney *U* test was used for continuous variables. The McNemar test was used to assess the performance of the two tumor markers, and the areas under receiver operating characteristic curves (AUROC) was also calculated.

RESULTS

A total of 202 HCC patients with a mean age of 58.5 years ± 10.3 years were enrolled. Seventy-two percent (n = 146) of the enrolled patients were male. The main causes of HCC were hepatitis C virus (HCV) in 51.0% (n = 103) and hepatitis B virus (HBV) in 25% (n = 52), while non-alcoholic fatty liver disease (NAFLD) was diagnosed in 13.9% (n = 28) of patients. A decompensated CLD was found in 77% (n = 157) of the patients. A total of 36.6% (n = 74) of the included patients had portal vein thrombosis. A total of 22.8% (n = 46) of the HCCs were less than 2 cm in length. HCC was classified as multicentric in 62.9% (n = 127) of patients. Satellite lesions were identified in 15.3% (n = 31) of the patients. A total of 63.9% (n = 129) of the HCCs involved one lobe, while 36.1% (n = 73) had bilobar involvement (Table 1).

A total of 86.6% (n = 175) of the total HCC patients enrolled had elevated DCP levels (> 45 mAU/L), while 64.3% (n = 130) of the total patients had elevated AFP levels (> 10 ng/mL; P = 0.03). For small HCCs, less than 2 cm in size (n = 46), the DCP was increased in 69.5% (n = 32) and the AFP was increased in 54.3% (n = 25; P = 0.801). Among a total of 127 patients with multicentric HCC, 87.4% (n = 111) had increased DCP, while 71.6% (n = 91) had increased AFP (P = 0.015). Radiographic evidence of satellite lesions was observed in 31 patients, of which DCP and AFP were elevated in 87% (n = 27) and 54.8% (n = 17; P = 0.835), respectively. Thrombi in the portal vein were observed in 74 patients; all had increased DCP, and 74.3% (n = 55) had increased AFP (P < 0.001).

In patients with HCC caused by hepatitis C (n = 103), the DCP concentration increased by 92.2% (n = 95), while the AFP concentration increased by 66.9% (n = 69; P = 0.009). Of the 52 HCC patients with underlying hepatitis B etiology, 76.9% (n = 40) had a positive DCP result, and 67.3% (n = 35) had a positive AFP result (P = 0.095). Among the 28 patients with NAFLD and HCC, DCP was elevated in 82.1% (n = 23), and AFP was elevated in 57.1% (n = 16; P = 0.887).

Among the 202 patients, 58.4% had elevated both tumor marker levels (n = 118). In 34.1% (n = 69) of the patients, one of the tumor markers was positive, and in 7.4% of the patients, both tumor markers were negative (n = 15). In the group in which one of the tumor biomarker markers was positive, DCP alone was elevated in 57 (28.2% of all patients), whereas AFP alone was elevated in 12 (5.9%) patients. There was a strong pairing between DCP and AFP levels for HCCs of all sizes (P < 0.001) and for HCCs of 2 cm or larger (P < 0.001), but the pairing was weaker for smaller HCCs (P = 0.210).

There was a correlation between the DCP and AFP according to Spearman's correlation test (P < 0.001; Figure 1). ROC plots were drawn to analyze the magnitude of the increase in DCP and AFP levels. For tumors larger than 2 cm in size, the log10 values of DCP exceeded the log10 values of AFP, with areas under the curve of 0.74 and 0.59, respectively (Figure 2). Combining the values of two markers to detect HCC did not improve diagnostic ability, with an AUROC of 0.739. For tumors less than 2 cm in length, the area under the curve for the log value of DCP was 0.250 *vs* 0.409 for AFP. Therefore, DCP elevation may be modest in smaller localized tumors, although it crossed the positivity threshold in more patients than did AFP elevation.

DISCUSSION

HCC, a leading liver cancer, not only has a considerable mortality rate but also imposes an enormous economic burden[1,10]. The false-negative rate of AFP, the most widely used tumor marker for HCC, is 30%-40%, motivating researchers to discover a more potent tumor marker with better diagnostic performance[11-13].

In this study, we examined the performance of DCP compared to AFP and correlated the values with radiological features. Our study showed that DCP performed better as a single marker than AFP for detecting HCC, but the combination of both markers did not improve the diagnostic capability. These observations agree with those reported by Xing *et al*[14], who showed that DCP was superior to AFP regardless of primary tumor size and underlying etiology, and the combination of the two markers resulted in increased sensitivity but decreased specificity, resulting in a decrease in overall diagnostic power[14,15].

Consistent with previous data from Pakistan, HCV appears to be a major cause of HCC development[16]. A subgroup analysis showed that DCP performed better in the HCV group. Similar findings were made in a Chinese study by Liu *et al*[17]. A statistically significant difference was not found between the two tumor markers in the HBV group in our study, possibly due to the small sample size, but DCP was still able to outcompete AFP in terms of diagnostic performance because of its detectability in a larger number of HCC patients. Several studies conducted to date on patients with HBV have shown that DCP alone and in combination with AFP yield better results than AFP alone[7,18].

It has been reported that DCP, when elevated, acts as a predictor of microvascular invasion, even in the absence of radiological evidence[9,19]. Although we did not assess microvascular invasion histologically, we did evaluate the diagnostic ability of these two markers for tumor portal vein thrombosis (PVT). Interestingly, our findings showed that

Table 1 Characteristics of study patients

Variables	Values
Age (yr)	58.5 ± 10.3
Gender: Males	146 (72.3)
Etiology	
HCV	103 (51.0)
HBV	52 (25.7)
Alcohol	8 (4.0)
NAFLD	28 (13.9)
Autoimmune liver disease	7 (3.5)
Cryptogenic	9 (4.5)
Lab parameters	
Hemoglobin (g/dL)	11.3 ± 2.1
White dell count (× 10^9/L)	8.3 ± 5.5
Platelets (× 10^9/L)	148.0 ± 102.0
Total bilirubin (mg/dL)	3.3 ± 5.6
Alanine aminotransferase (IU/L)	63.9 ± 51.4
Aspartate aminotransferase (IU/L)	113.0 ± 206.0
Alkaline phosphatase (IU/L)	203.0 ± 227.0
International normalization ratio	1.3 ± 0.4
Tumor parameters	
HCC size 2 cm or more	156 (77.2)
HCC less than 2 cm	46 (22.8)
Portal vein thrombus	74 (36.6)
Unilobed	129 (63.9)
Bilobed	73 (36.1)
Satellite lesions	31 (15.3)
Multicentric tumor	127 (62.9)
Des-gamma carboxyprothrombin (mAU/L)	669.4 (16.7-300000.0)
Alpha-fetoprotein (ng/mL)	32 (1-20000)

Values are mean ± SD or *n* (%).
HCV: Hepatitis C virus; HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease; HCC: Hepatocellular carcinoma.

DCP was elevated in all subjects with portal vein thrombus. Similar results were reported by Xu *et al*[20]. In their study, all 65 participants with PVT had elevated DCP levels.

Since patients with small HCCs (< 2 cm) have a good prognosis, it is important to detect them early in the disease course[3]. Interestingly, although a greater percentage of small HCC lesions exceeded the positivity threshold for DCP, the log values of AFP were much greater. The McNemar test did not reveal good agreement between the two markers, underscoring the value of testing both tumor markers in patients with small HCC. Data from other studies also have similar conclusions regarding sensitivity[21].

The GALAD scoring system, which consists of these two tumor markers in addition to sex, age, and AFP-L3, has been proposed and validated for determining the risk of HCC[22,23]. We did not test for AFP-L3. Recent studies have shown that sex, age, AFP, and DCP combination, the "GAAD" score, can be used to predict the presence of HCC effectively when the value is greater than 2.57[24]. AFP and DCP (PIVKA II) assays were performed on the Elecsys platform with an AFP cutoff of 20 ng/mL and a PIVKA II cutoff of 28.4 ng/mL. We used a cutoff AFP of 10 ng/mL to increase the sensitivity, and a cutoff DCP of 45.0 mAU/mL was used. As our assays were performed on the Architect platform, calculation, and implementation of GAAD scoring were not possible.

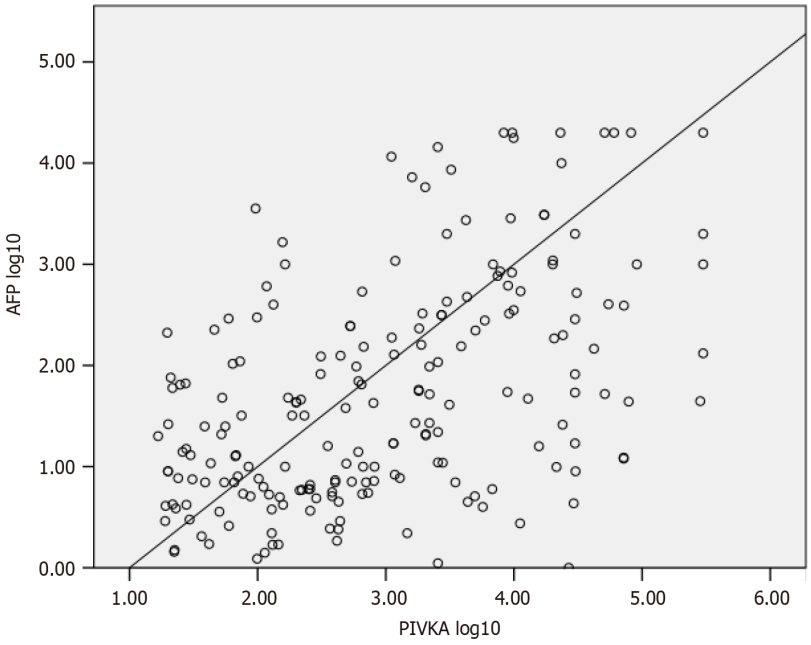

Figure 1 Correlation of Des-gamma-carboxy prothrombin (DCP) and alpha fetoprotein in hepatocellular carcinoma patients using Spearman's rank correlation analysis. $P < 0.001$. AFP: Alpha fetoprotein; PIVKA: protein induced by vitamin K absence-II also known as DCP.

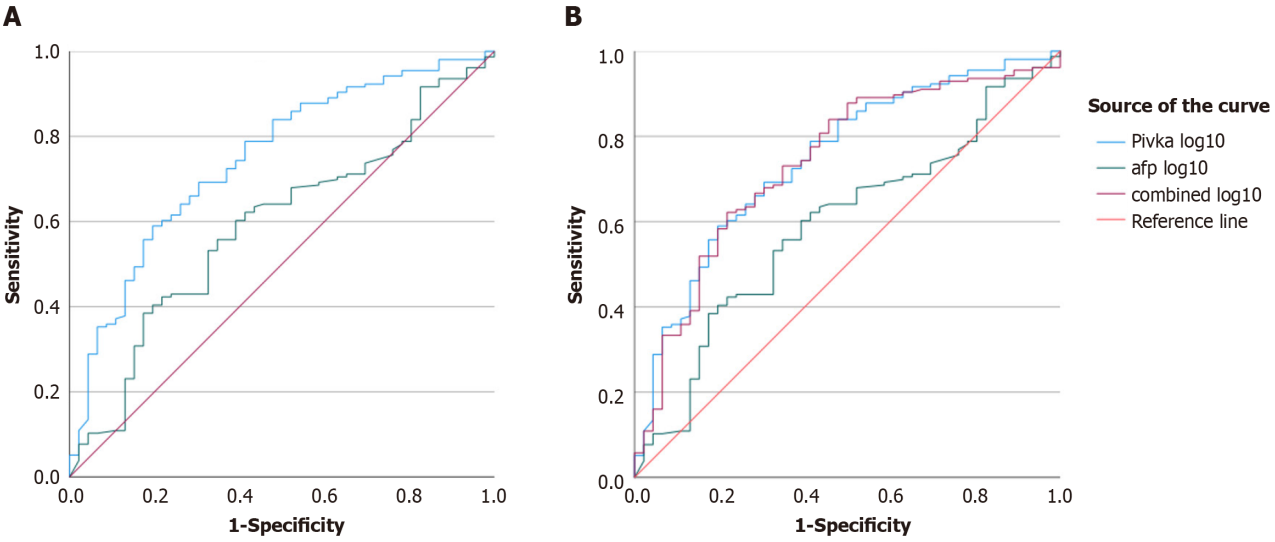

Figure 2 Receiver operating characteristic curve. A and B: Receiver operating characteristic curve comparing the values of two hepatocellular carcinoma biomarkers, des-gamma-carboxy-prothrombin, and alpha fetoprotein alone (A) or in combination (B) in a tumour size of 2 cm or more. Pivka: Protein induced by vitamin K absence-II [same as des-gamma-carboxy-prothrombin (DCP)]; afp: Alpha fetoprotein; combined: Combining DCP and AFP.

This study has several notable strengths that are worth acknowledging. First, our research, which focused on the effectiveness of DCP and AFP as biomarkers for HCC, is relevant and significant, given the increasing incidence of this cancer globally. Second, we employed rigorous statistical analyses, such as McNemar's test and AUROC analysis, to evaluate the correlation between DCP and AFP levels in patients with HCC. Third, the study illustrates the complementarity of DCP and AFP as biomarkers for diagnosing HCC in patients with a tumor diameter of less than 2 cm. Fourth, the study contributes to the mounting evidence supporting the use of DCP and AFP as biomarkers for HCC, which could result in better patient outcomes through earlier detection and treatment. Finally, the study offers valuable insights into the potential use of DCP as a biomarker for patients with HCC with portal vein thrombosis, which may guide future research in this field.

The current study has some limitations that must be considered when interpreting the results. First, the sample size was relatively small, and the study was conducted in a single center, which may limit the generalizability of the findings to other populations. Second, the cross-sectional design of the study makes it difficult to establish a causal relationship between DCP and AFP levels and the development of HCC. Additionally, the lack of a control group limits the ability to compare the results to individuals without HCC or other liver diseases. Finally, due to the small sample size, there was

limited statistical power to obtain statistically significant differences in DCP and AFP levels for tumors smaller than 2 cm, which may limit the generalizability of the results to individuals with early-stage HCC. Overall, these limitations must be taken into consideration when interpreting the findings of this study.

CONCLUSION

According to the results of our study, DCP was found to be a better biomarker than AFP for HCC detection, especially in patients with portal vein thrombosis. DCP, as an individual marker, performed better in many categories of HCC. Hence, DCP may replace AFP as the primary HCC biomarker. The findings also suggest that DCP and AFP may have complementary roles in the diagnosis of small HCC, and the combination of both markers could be considered for early detection of HCC, highlighting the importance of utilizing multiple biomarkers in the diagnosis of small HCC, as relying on a single biomarker may not be sufficient. The role of DCP as a screening biomarker should be incorporated into the HCC guidelines.

FOOTNOTES

Co-first authors: Muhammad Ali Qadeer and Zaigham Abbas.

Author contributions: Qadeer MA and Abbas Z contributed equally to the manuscript; Qadeer MA drafted the manuscript, supervised data collection, and assisted in data analysis; Abbas Z conceived the study, performed data analysis, supervised manuscript writing, and revised manuscript according to the reviewers' comments; Amjad S, Shahid B, Altaf A, and Siyal M were involved in the data collection; and all authors read the final manuscript and approved it.

Institutional review board statement: The study was reviewed and approved by Dr. Ziauddin University's Ethical Review Board.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All authors declare that they have no conflict of interest.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

Country/Territory of origin: Pakistan

ORCID number: Zaigham Abbas 0000-0002-9513-5324.

S-Editor: Chen YL
L-Editor: A
P-Editor: Zhao YQ

REFERENCES

1 **Ferlay J**, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. 2020. [cited 10 July 2023]. Available from: https://gco.iarc.fr/today

2 **Singal AG**, Llovet JM, Yarchoan M, Mehta N, Heimbach JK, Dawson LA, Jou JH, Kulik LM, Agopian VG, Marrero JA, Mendiratta-Lala M, Brown DB, Rilling WS, Goyal L, Wei AC, Taddei TH. AASLD Practice Guidance on prevention, diagnosis, and treatment of hepatocellular carcinoma. *Hepatology* 2023; **78**: 1922-1965 [PMID: 37199193 DOI: 10.1097/HEP.0000000000000466]

3 **Reig M**, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, Kelley RK, Galle PR, Mazzaferro V, Salem R, Sangro B, Singal AG, Vogel A, Fuster J, Ayuso C, Bruix J. BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. *J Hepatol* 2022; **76**: 681-693 [PMID: 34801630 DOI: 10.1016/j.jhep.2021.11.018]

4 **Kim SH**, Moon DB, Kim WJ, Kang WH, Kwon JH, Jwa EK, Cho HD, Ha SM, Chung YK, Lee SG. Preoperative prognostic values of α-fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) in patients with hepatocellular carcinoma for living donor liver transplantation. *Hepatobiliary Surg Nutr* 2016; **5**: 461-469 [PMID: 28124000 DOI: 10.21037/hbsn.2016.11.05]

5 **Chi X**, Jiang L, Yuan Y, Huang X, Yang X, Hochwald S, Liu J, Huang H. A comparison of clinical pathologic characteristics between alpha-fetoprotein negative and positive hepatocellular carcinoma patients from Eastern and Southern China. *BMC Gastroenterol* 2022; **22**: 202

[PMID: 35461226 DOI: 10.1186/s12876-022-02279-w]

6 **Liebman HA**, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, Coleman MS, Furie B. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984; **310**: 1427-1431 [PMID: 6201741 DOI: 10.1056/NEJM198405313102204]

7 **Seo SI**, Kim HS, Kim WJ, Shin WG, Kim DJ, Kim KH, Jang MK, Lee JH, Kim JS, Kim HY, Lee MS, Park CK. Diagnostic value of PIVKA-II and alpha-fetoprotein in hepatitis B virus-associated hepatocellular carcinoma. *World J Gastroenterol* 2015; **21**: 3928-3935 [PMID: 25852278 DOI: 10.3748/wjg.v21.i13.3928]

8 **Zakhary NI**, Khodeer SM, Shafik HE, Abdel Malak CA. Impact of PIVKA-II in diagnosis of hepatocellular carcinoma. *J Adv Res* 2013; **4**: 539-546 [PMID: 25685463 DOI: 10.1016/j.jare.2012.10.004]

9 **Poté N**, Cauchy F, Albuquerque M, Voitot H, Belghiti J, Castera L, Puy H, Bedossa P, Paradis V. Performance of PIVKA-II for early hepatocellular carcinoma diagnosis and prediction of microvascular invasion. *J Hepatol* 2015; **62**: 848-854 [PMID: 25450201 DOI: 10.1016/j.jhep.2014.11.005]

10 **Likhitsup A**, Parikh ND. Economic Implications of Hepatocellular Carcinoma Surveillance and Treatment: A Guide for Clinicians. *Pharmacoeconomics* 2020; **38**: 5-24 [PMID: 31573053 DOI: 10.1007/s40273-019-00839-9]

11 **Trevisani F**, D'Intino PE, Morselli-Labate AM, Mazzella G, Accogli E, Caraceni P, Domenicali M, De Notariis S, Roda E, Bernardi M. Serum alpha-fetoprotein for diagnosis of hepatocellular carcinoma in patients with chronic liver disease: influence of HBsAg and anti-HCV status. *J Hepatol* 2001; **34**: 570-575 [PMID: 11394657 DOI: 10.1016/s0168-8278(00)00053-2]

12 **Nikolova D**. Alpha Fetoprotein and Hepatocellular Carcinoma: An Opinion. *BJSTR* 2019; **12** [DOI: 10.26717/BJSTR.2019.12.002316]

13 **Gupta S**, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. *Ann Intern Med* 2003; **139**: 46-50 [PMID: 12834318 DOI: 10.7326/0003-4819-139-1-200307010-00012]

14 **Xing H**, Zheng YJ, Han J, Zhang H, Li ZL, Lau WY, Shen F, Yang T. Protein induced by vitamin K absence or antagonist-II versus alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: A systematic review with meta-analysis. *Hepatobiliary Pancreat Dis Int* 2018; **17**: 487-495 [PMID: 30257796 DOI: 10.1016/j.hbpd.2018.09.009]

15 **Hafeez Bhatti AB**, Dar FS, Waheed A, Shafique K, Sultan F, Shah NH. Hepatocellular Carcinoma in Pakistan: National Trends and Global Perspective. *Gastroenterol Res Pract* 2016; **2016**: 5942306 [PMID: 26955390 DOI: 10.1155/2016/5942306]

16 **Parkash O**, Hamid S. Next big threat for Pakistan Hepatocellular Carcinoma (HCC). *J Pak Med Assoc* 2016; **66**: 735-739 [PMID: 27339578]

17 **Liu S**, Sun L, Yao L, Zhu H, Diao Y, Wang M, Xing H, Lau WY, Guan M, Pawlik TM, Shen F, Xu M, Tong X, Yang T. Diagnostic Performance of AFP, AFP-L3, or PIVKA-II for Hepatitis C Virus-Associated Hepatocellular Carcinoma: A Multicenter Analysis. *J Clin Med* 2022; **11** [PMID: 36079006 DOI: 10.3390/jcm11175075]

18 **Perne MG**, Sitar-Tăut AV, Alexescu TG, Ciumărnean L, Milaciu MV, Coste SC, Vlad CV, Cozma A, Sitar-Tăut DA, Orăşan OH, Crăciun A. Diagnostic Performance of Extrahepatic Protein Induced by Vitamin K Absence in the Hepatocellular Carcinoma: A Systematic Review and Meta-Analysis. *Diagnostics (Basel)* 2023; **13** [PMID: 36899960 DOI: 10.3390/diagnostics13050816]

19 **Masuda T**, Beppu T, Okabe H, Nitta H, Imai K, Hayashi H, Chikamoto A, Yamamoto K, Ikeshima S, Kuramoto M, Shimada S, Baba H. Predictive factors of pathological vascular invasion in hepatocellular carcinoma within 3 cm and three nodules without radiological vascular invasion. *Hepatol Res* 2016; **46**: 985-991 [PMID: 26670198 DOI: 10.1111/hepr.12637]

20 **Xu F**, Zhang L, He W, Song D, Ji X, Shao J. The Diagnostic Value of Serum PIVKA-II Alone or in Combination with AFP in Chinese Hepatocellular Carcinoma Patients. *Dis Markers* 2021; **2021**: 8868370 [PMID: 33628341 DOI: 10.1155/2021/8868370]

21 **Saitta C**, Raffa G, Alibrandi A, Brancatelli S, Lombardo D, Tripodi G, Raimondo G, Pollicino T. PIVKA-II is a useful tool for diagnostic characterization of ultrasound-detected liver nodules in cirrhotic patients. *Medicine (Baltimore)* 2017; **96**: e7266 [PMID: 28658121 DOI: 10.1097/MD.0000000000007266]

22 **Johnson PJ**, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, Morse J, Hull D, Patman G, Kagebayashi C, Hussain S, Graham J, Reeves H, Satomura S. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 144-153 [PMID: 24220911 DOI: 10.1158/1055-9965.EPI-13-0870]

23 **Berhane S**, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S, Schweitzer N, Vogel A, Manns MP, Benckert J, Berg T, Ebker M, Best J, Dechêne A, Gerken G, Schlaak JF, Weinmann A, Wörns MA, Galle P, Yeo W, Mo F, Chan SL, Reeves H, Cox T, Johnson P. Role of the GALAD and BALAD-2 Serologic Models in Diagnosis of Hepatocellular Carcinoma and Prediction of Survival in Patients. *Clin Gastroenterol Hepatol* 2016; **14**: 875-886.e6 [PMID: 26775025 DOI: 10.1016/j.cgh.2015.12.042]

24 **Piratvisuth T**, Hou J, Tanwandee T, Berg T, Vogel A, Trojan J, De Toni EN, Kudo M, Eiblmaier A, Klein HG, Hegel JK, Madin K, Kroeniger K, Sharma A, Chan HLY. Development and clinical validation of a novel algorithmic score (GAAD) for detecting HCC in prospective cohort studies. *Hepatol Commun* 2023; **7** [PMID: 37938100 DOI: 10.1097/HC9.0000000000000317]

World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Pathophysiol 2024 April 22; 15(1): 91100

DOI: 10.4291/wjgp.v15.i1.91100

ISSN 2150-5330 (online)

SYSTEMATIC REVIEWS

Prevalence and outcome of sarcopenia in non-alcoholic fatty liver disease

Suprabhat Giri, Prajna Anirvan, Sumaswi Angadi, Ankita Singh, Anurag Lavekar

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Moriyama K, Japan

Received: December 21, 2023
Revised: February 9, 2024
Accepted: April 1, 2024
Published online: April 22, 2024

Suprabhat Giri, Department of Gastroenterology and Hepatology, Kalinga Institute of Medical Sciences, Bhubaneswar 751024, Odisha, India

Prajna Anirvan, Department of Gastroenterology, Kalinga Gastroenterology Foundation, Cuttack, 753001, Odisha, India

Sumaswi Angadi, Department of Gastroenterology, Nizam's Institute of Medical Sciences, Hyderabad 500082, Telangana, India

Ankita Singh, Department of Gastroenterology, Seth GS Medical College and KEM Hospital, Mumbai 400012, Maharashtra, India

Anurag Lavekar, Department of Gastroenterology, Sagar Hospital, Bengaluru 560041, Karnataka, India

Corresponding author: Anurag Lavekar, MD, Consultant Gastroenterologist, Department of Gastroenterology, Sagar Hospital, No. 44/54, 30th Cross Road, Extension, 4th T Block East, Tilak Nagar, Jayanagar, Bengaluru 560041, Karnataka, India. anuraglavekar@gmail.com

Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of conditions, progressing from mild steatosis to advanced fibrosis. Sarcopenia, characterized by decreased muscle strength and mass, shares common pathophysiological traits with NAFLD. An association exists between sarcopenia and increased NAFLD prevalence. However, data on the prevalence of sarcopenia in NAFLD and its impact on the outcomes of NAFLD remain inconsistent.

AIM

To analyze the prevalence and outcomes of sarcopenia in patients with NAFLD.

METHODS

We conducted a comprehensive search for relevant studies in MEDLINE, Embase, and Scopus from their inception to June 2023. We included studies that focused on patients with NAFLD, reported the prevalence of sarcopenia as the primary outcome, and examined secondary outcomes, such as liver fibrosis and other adverse events. We also used the Newcastle-Ottawa scale for quality assessment.

RESULTS

Of the 29 studies included, the prevalence of sarcopenia in NAFLD varied widely

(1.6% to 63.0%), with 20 studies reporting a prevalence of more than 10.0%. Substantial heterogeneity was noted in the measurement modalities for sarcopenia. Sarcopenia was associated with a higher risk of advanced fibrosis (odd ratio: 1.97, 95% confidence interval: 1.44-2.70). Increased odds were consistently observed in fibrosis assessment through biopsy, NAFLD fibrosis score/body mass index, aspartate aminotransferase to alanine aminotransferase ratio, diabetes (BARD) score, and transient elastography, whereas the fibrosis-4 score showed no such association. Sarcopenia in NAFLD was associated with a higher risk of steatohepatitis, insulin resistance, cardiovascular risks, and mortality.

CONCLUSION

This systematic review highlights the critical need for standardized diagnostic criteria and measurement methods for sarcopenia in NAFLD patients. The variability in study designs and assessment methods for sarcopenia and liver fibrosis may account for the inconsistent findings. This review demonstrates the multidimensional impact of sarcopenia on NAFLD, indicating its importance beyond liver-related events to include cardiovascular risks, mortality, and metabolic complications.

Key Words: Non-alcoholic fatty liver disease; Sarcopenia; Hepatic fibrosis; Low muscle mass; Hand grip strength; Bioelectric impedance analysis; Dual X-ray absorptiometry

Core Tip: The prevalence of sarcopenia in nonalcoholic fatty liver disease (NAFLD) varies widely. Sarcopenia in NAFLD is consistently associated with a higher risk of advanced fibrosis. In addition to liver-related events, sarcopenia in NAFLD is associated with adverse outcomes, including an increased risk of nonalcoholic steatohepatitis, mortality, cardiovascular risks, and metabolic complications. The heterogeneity in prevalence and associations highlights the importance of accurately defining measurement modalities and cutoff criteria. Establishing consensus guidelines is crucial for advancing research and enhancing clinical management in the complex relationship between sarcopenia and NAFLD.

Citation: Giri S, Anirvan P, Angadi S, Singh A, Lavekar A. Prevalence and outcome of sarcopenia in non-alcoholic fatty liver disease. *World J Gastrointest Pathophysiol* 2024; 15(1): 91100
URL: https://www.wjgnet.com/2150-5330/full/v15/i1/91100.htm
DOI: https://dx.doi.org/10.4291/wjgp.v15.i1.91100

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) includes a spectrum of liver conditions, beginning with mild steatosis and potentially advancing through steatohepatitis, fibrosis, and cirrhosis[1]. Sarcopenia, prevalent in aging populations, is defined by a reduction in muscle strength and/or function, often evidenced by a decrease in muscle mass observed in cross-sectional imaging[2]. Sarcopenic obesity is characterized by the concurrent presence of sarcopenia and increased fat mass, typically measured by body mass index (BMI) or waist circumference[3]. Factors such as hyperammonemia, endotoxemia, and endocrine disturbances, including insulin resistance and decreased testosterone levels, contribute to the increased prevalence of sarcopenia in individuals with liver cirrhosis[4]. Several pathophysiological similarities exist between NAFLD and sarcopenia, including insulin resistance, myostatin and adiponectin dysregulation, hormonal imbalances, chronic inflammation, impaired glucose uptake, and myosteatosis[5,6].

Studies have reported an association of sarcopenia with a higher prevalence of NAFLD and more severe liver damage in individuals with NAFLD. An increased fat mass in patients with NAFLD is associated with a higher incidence of sarcopenic obesity. A meta-analysis of five cross-sectional studies involving 27804 patients identified an increased risk of NAFLD in individuals with sarcopenia[7]. However, data regarding the prevalence of sarcopenia among patients with NAFLD are inconsistent. Moreover, the effects of sarcopenia on the outcomes of patients with NAFLD remain unclear. Thus, this systematic review aimed to analyze the prevalence and impact of sarcopenia in individuals with NAFLD.

MATERIALS AND METHODS

The current systematic review was conducted in accordance with the updated Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[8].

Database search

We searched for relevant studies in MEDLINE, Embase, and Scopus from the inception of these databases until June 31, 2023, by using the following keywords: (NAFLD OR Fatty liver OR Steatotic liver disease OR MAFLD OR NASH) AND

(Sarcopenia OR sarcopenic OR Muscle wasting). The titles and abstracts of the retrieved studies were screened by two independent reviewers, who then assessed the full texts for eligibility before inclusion. Furthermore, the bibliographies of the included studies were reviewed to identify additional relevant studies. Any disagreements between the two independent reviewers were resolved by a third reviewer.

Study inclusion

Both prospective and retrospective studies that met the following criteria were included in this systematic review: (1) Studies including patients with NAFLD as determined by serology, ultrasonography (USG), transient elastography (TE), or magnetic resonance imaging (MRI); (2) Studies examining the prevalence of sarcopenia as the primary outcome; and (3) Studies evaluating the effect of sarcopenia on the risk of liver fibrosis or other adverse outcomes as secondary outcomes. Editorials, correspondences, case reports, case series, and review articles were excluded. Moreover, studies with insufficient or irrelevant clinical data were excluded.

Data extraction and quality assessment

Two reviewers independently extracted the data, and a third reviewer resolved any disagreements. The extracted information for each study included the title, first author, year of publication, country, number of patients, age and sex distribution, BMI, prevalence of metabolic syndrome parameters, diagnostic method used for fatty liver diagnosis, assessment method for sarcopenia, prevalence of sarcopenia, and the effect of sarcopenia on the outcomes of patients with NAFLD. The quality of the included studies was assessed by two independent reviewers by using the Newcastle-Ottawa Scale for cohort studies[9,10]. In case of a disagreement, a third reviewer was consulted.

RESULTS

Study characteristics and quality assessment

A total of 2134 records were identified using the predefined search strategy, with 29 studies ultimately included in the systematic review. Figure 1 illustrates the PRISMA flowchart detailing the study selection and inclusion process. Table 1 presents the baseline characteristics and outcomes of the included studies. The majority of the studies were from Asia, followed by North America. The mean age of participants in the included studies ranged from 41.9 to 67.8 years, and the proportion of male participants varied from 19.5% to 89.8%. Only three studies included biopsy-proven NAFLD cases[11-13], whereas the remaining studies used noninvasive methods for NAFLD diagnosis. Among the studies using noninvasive modalities, four used serological tests[10,14-16], 16 used USG[17-32], two used MRI[33,34], and four used TE with controlled attenuation parameters[35-38]. Bioimpedance analysis was the most common modality used for the assessment of sarcopenia (16 studies)[11,12,17,18,20-23,28,30-37], followed by dual X-ray absorptiometry (DEXA; 7 studies)[10,19,14,25,26,28,29], computed tomography (1 study)[13], hand-grip strength (1 study)[15], and MRI (1 study)[33]. Both the studies by Wijarnpreecha *et al*[18] and Kim *et al*[23] analyzed the data from the third National Health and Nutrition Examination Survey conducted from 1988 to 1994 but analyzed different outcomes. Six studies were of good quality[1-13,30,35,37], 20 were of fair quality[10,14,15,17-23,24-29,31-34,38], and three were of poor quality[16,24,35].

Prevalence of sarcopenia in NAFLD

A total of 24 studies reported the prevalence of sarcopenia in NAFLD. The overall prevalence varied significantly, from 1.6% when determined using MRI[33] to 63.0% when assessed using DEXA[24]. Four studies reported a prevalence of less than 10.0%[17,20,33,34], 14 reported a prevalence of 10.0%-30.0%[10,11,13,14,19,22,26-31,35,38], four reported a prevalence of 30.0%-50.0%[12,14,33,38], and two reported a prevalence of more than 50.0%[24,36]. In studies using DEXA, the prevalence of sarcopenia ranged from 12.2% when using an appendicular skeletal muscle mass (ASM)/BMI (cutoff of 0.789 in men and 0.521 in women)[10] to 63.0% when using ASM/weight (cutoff of 29.0 in men and 22.9 in women[25]). In studies using BIA, the prevalence of sarcopenia ranged from 4.4% by using a combination of ASM/weight, ASM/height^2, and ASM/BMI[34] to 54.8% using ASM/height^2 (cutoff of 7.0 in men and 5.7 in women)[32].

Risk of advanced fibrosis in NAFLD with sarcopenia

Ten studies examined the correlation between advanced fibrosis and sarcopenia in NAFLD[11-13,18,22,29,35,37]. The combined analysis of these studies revealed that sarcopenia was associated with a higher risk of advanced fibrosis, with an odds ratio (OR) of 1.97 [95% confidence interval (95%CI): 1.44-2.70; I^2 = 79.8%]. When considering individual modalities for the assessment of fibrosis, including biopsy, NAFLD fibrosis scores/BMI, the ratio of aspartate aminotransferase to alanine aminotransferase, diabetes (NFS/BARD) scores, and TE, sarcopenia was consistently associated with an increased risk of advanced fibrosis with ORs of 1.98 (95%CI: 1.39-2.82; I^2 = 0.0%), 2.09 (95%CI: 1.55-2.81; I^2 = 0.0%), and 3.71 (95%CI: 2.62-5.24; I^2 = 0.0%), respectively, indicating no heterogeneity (Figure 2). However, when using the fibrosis-4 (FIB-4) score, no association was observed between sarcopenia and advanced fibrosis with an OR of 1.38 (95%CI: 0.96-1.99; I^2 = 63.3%), indicating moderate heterogeneity.

Risk of other events in NAFLD with sarcopenia

Eight studies explored the outcome of NAFLD with sarcopenia in addition to the increased risk of advanced hepatic fibrosis. Koo *et al*[11] reported a higher risk of nonalcoholic steatohepatitis (NASH) in NAFLD with sarcopenia (aOR: 2.59; 95%CI: 1.22-5.48). Petta *et al*[12] reported that the prevalence of NASH was higher in the presence of sarcopenia (88.7% *vs*.

Table 1 Baseline characteristics of the included studies showing the prevalence and outcome of sarcopenia in patients with non-alcoholic fatty liver disease

Ref.	Country, study design	Population and size	Age, in years, male sex, in %	Comorbidities	Definition of NAFLD	Definition and prevalence of sarcopenia	Outcome	Study quality
Lee *et al*[10], 2016	South Korea, retrospective	Korean National Health and Nutrition Examination Surveys 2008-2011, *n* = 2761	55.8 ± 14.3, 45%	BMI: 25.8 ± 3.1; MS: 81%; DM: 30%	NAFLD liver fat score	DEXA was used for the calculation of SI = ASM/BMI. Sarcopenia was defined using a cut-off point of SI < 0.789 in men and < 0.521 in women; *n* = 337 (12.2%)	Significant fibrosis was defined as FIB-4 ≥ 2.67. After adjusting for all covariates, a higher value of SI was associated with a lower risk of significant fibrosis with aOR: 0.67 (95%CI: 0.49-0.91)	Fair
Koo *et al*[11], 2017	South Korea, prospective	Boramae NAFLD registry, *n* = 240	53.3 ± 14.3, 48.7%	BMI: 27.4 ± 3.5; DM: 39.6%; HTN: 40.4%; smoking: 22.5%	≥ 5% macrovesicular steatosis on liver biopsy	BIA was used to calculate ASM, which was divided by weight = ASM%. ASM% < 29.0 in men or < 22.9 in women was considered as sarcopenia. *n* = 64 (26.7%) (21/117 in NAFLD and 43/123 in NASH)	Among patients with NAFLD, sarcopenia was associated with a higher risk of NASH (aOR: 2.59; 95%CI: 1.22-5.48). Sarcopenia was also associated with the presence of significant fibrosis (F2-F4) on liver biopsy (aOR: 2.21; 95%CI: 1.10-4.44)	Good
Petta *et al*[12], 2017	Italy, prospective	Consecutive patients with NAFLD at a single center, *n* = 225	48.3 ± 13.4, 62.7%	BMI: 30.3 ± 5.2; DM: 45.3%; HTN: 32.9%; obesity: 71.1%	≥ 5% macrovesicular steatosis on liver biopsy	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. Sarcopenia was defined as an SMI ≤ 37 in males and ≤ 28 in females. *n* = 98 (43.6%)	Sarcopenia was also associated with the presence of advanced fibrosis (F3-F4) on liver biopsy (aOR: 2.36; 95%CI: 1.16-4.77). The prevalence of NASH was higher in the presence of sarcopenia (88.7% *vs* 76.3% in nonsarcopenic cases, *P* = 0.01)	Good
Kang *et al*[17], 2019	South Korea, retrospective	Adults undergoing comprehensive health screening at a single center from 2010-2017, *n* = 10711	47.9 ± 11.6, 52.8%	BMI: 23.9 ± 2.9; MS: 12.5%; DM: 5.9%; HTN: 11.6%; obesity: 34.1%	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight = ASM/BW%; ASM/BW% < 29.0 in men or < 22.9 in women was considered as sarcopenia; *n* = 615 (5.7%)	Advanced fibrosis was defined as NFS ≥ 0.676 and FIB-4 ≥ 2.670. Sarcopenia was also associated with the presence of advanced fibrosis (F3-F4) as defined by NFS with aOR: 2.68 (95%CI: 1.28-5.59), but not using FIB-4 (aOR: 1.58, 95%CI: 0.87-2.85)	Fair
Wijarnpreecha *et al*[18], 2019	United States, retrospective	Analysis of the third National Health and Nutrition Examination Survey (NHANES), conducted from 1988 to 1994, *n* = 4188	45.4 ± 0.4[2], 50.4%	BMI: 28.9 ± 0.2[2]; HTN: 31.6%; DM: 7.5%	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. Sarcopenia was defined as an SMI ≤ 37 in males and ≤ 28 in females; *n* = 2023 (48.3%)	Advanced fibrosis was defined as NFS ≥ 0.676; sarcopenia was significantly associated with advanced fibrosis (aOR: 2.39, 95%CI: 1.50-3.84)	Fair
Gan *et al*[19], 2020	China, prospective	Lanxi cohort, a community-based prospective cohort with a focus on obesity-related diseases, *n* = 1088	55.2 ± 11.5, 32.9%	BMI: 25.9 ± 2.9; MS: 59.5%; DM: 12.9%; HTN: 48.1%	Abdominal ultrasound[1]	DEXA was used for the calculation of SMI = total appendicular lean mass (ALM)/weight. The cut-off points for sarcopenia were 28.64% for men and 24.12% for women; *n* = 246 (22.6%)	-	Fair
Golabi *et al*[14], 2020	United State, retrospective	Analysis of the National Health and Nutrition Examination	50.7 ± 0.7[2],	BMI: 32.5 ± 0.3[2]; obesity: 60.6%; HTN:	Fatty liver index (FLI) ≥ 30 based on age,	DEXA was used to calculate SI = ASM/BMI. Sarcopenia was defined using a cut-off point of	Sarcopenia was an independent predictor of mortality in NAFLD with	Fair

		Survey (NHANES), from 1999 to 2004, *n* = 1351	60.0%	68.4%; MS: 63.9%; DM: 20.7%	race/ethnicity, waist circumference, GGT, activity, fasting insulin, and fasting glucose	SI < 0.789 in men and < 0.521 in women. *n* = 239 (17.7%)	aHR 1.78 (95%CI: 1.16-2.73)	
Hsieh *et al*[13], 2021	Taiwan, prospective	Boramae NAFLD cohort, *n* = 521	52.0 ± 15.0, 50.9%	BMI: 27.8 ± 3.8; DM: 39.3%; HTN: 42.4%	≥ 5% macrovesicular steatosis on liver biopsy	Cross-sectional CT images at L3 was used to calculate SMI; Sarcopenia defined by L3-SMI < 50 cm^2/m^2 for men and < 39 cm^2/m^2 for women. *n* = 122 (23.4%)	Sarcopenia was also associated with the presence of significant fibrosis (F2-F4) on liver biopsy (aOR: 1.72; 95%CI: 1.05-2.84)	Good
Kang *et al*[15], 2020	South Korea, retrospective	Korean National Health and Nutrition Examination Surveys 2014-2016 with age 35-65 yr, *n* = 2092	45.6 ± 0.2[2], 42.4%	BMI: 23.8 ± 0.0[2]; DM: 10.7%; HTN: 24.1%; obesity: 33.6%	HIS was calculated based on ALT, AST, BMI, DM, sex, NAFLD defined by HIS > 36	Hand grip strength was calculated using a dynamometer, and sarcopenia was defined for individuals in the 1st quartile (Q1) of muscle strength	Advanced fibrosis was defined as either a FIB-4 score ≥ 1.30 or a BARD score ≥ 2.00. Sarcopenia was also associated with the presence of advanced fibrosis as defined by BARD with aOR: 1.68 (95%CI: 1.07-2.62), but not using FIB-4 (aOR: 1.35, 95%CI: 0.75-2.45)	Fair
Park *et al*[20], 2020	South Korea, retrospective	Patients attending annual health examination at a single center, *n* = 747	48.9 ± 10.8, 68.1%	BMI: 24.9 ± 3.1	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. ASM/BW% < 29.1 in men or < 23.0 in women was considered as sarcopenia. *n* = 66 (8.8%)	-	Fair
Seo *et al*[21], 2020	South Korea, retrospective	Seoul Metabolic Syndrome Cohort, *n* = 1278	55.8 ± 10.8, 53.6%	BMI: 26.5 ± 3.3; DM: 100%	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight = ASM/BW%. ASM/BW% < 29.0 in men or < 22.9 in women was considered as sarcopenia. *n* = 528 (41.3%)	-	Fair
Kang *et al*[22], 2021	South Korea, retrospective	Patients undergoing carotid ultrasound at a single center, *n* = 683	49.1 ± 10.0, 86.1%	BMI: 26.4 ± 2.6; DM: 15.2%; obesity: 67.0%; HTN: 29.1%; MS: 43.6%	Abdominal ultrasound[1]	BIA was used to calculate SI = ASM/BMI. Sarcopenia was defined using a cut-off point of SI < 0.789 in men and < 0.521 in women. *n* = 75 (11.0%)	Sarcopenia was an independent predictor of increased intima-media thickness (OR: 2.26, (95%CI: 1.26-4.04) and carotid plaque (OR: 2.74, 95%CI: 1.30-5.78)	Fair
Kim *et al*[23], 2021	United States, retrospective	Analysis of the third National Health and Nutrition Examination Survey (NHANES), conducted from 1988 to 1994, *n* = 3773	45.5 ± 0.45[2], 50.5%	BMI: 29.0 ± 0.23[2]; DM: 12.1%; HTN: 30.9%	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. Sarcopenia was defined as an SMI ≤ 37 in males and ≤ 28 in females. *n* = 1822 (48.3%)	Sarcopenia was an independent predictor of mortality in NAFLD with aHR 1.44 (95%CI: 1.16-1.80)	Fair
Lee *et al*[24], 2021	South Korea, retrospective	Gangnam Severance Hospital Check-up (GSHC) dataset from 2016 to 2019, *n* = 4168	51.2 ± 11.5, 65.5%	BMI: 26.1 ± 3.5	Abdominal ultrasound[1]	*n* = 1288 (30.9%)	-	Poor
Lee *et al*[25], 2021	South Korea, retrospective	Korean Genome and Epidemiology Study on Atherosclerosis Risk of Rural Areas in the Korean General Population data, *n* = 320	65.7 ± 7.6, 63.6%	BMI: 26.9 ± 2.9; DM: 67.9%; HTN: 60.5%	Abdominal ultrasound[1]	57 (39.6%), 107 (59.8%), and 148 (63.0%) participants had low muscle mass adjusted for height, BMI, and body weight in the NAFLD group, respectively	Appendicular muscle mass adjusted for body weight only was associated with hepatic fibrosis but not when adjusted for height and BMI	Fair
Linge *et al*[33], 2021	United Kingdom, retrospective	Participants of United Kingdom Biobank study, aged 40-69 yr at recruitment in 2006-2010, *n* = 1204	62.9 ± 7.4, 53.5%	BMI: 30.1 ± 4.8	MRI liver PDFF > 5%	Sarcopenia, defined as low hand grip strength [< 16/27 kg (females/males)] and low muscle quantity [MRI threshold of 3.0 and 3.6 L/m^2 for thigh FFMV/height^2 (females/males)]. *n* =	-	Fair

						19 (1.6%)		
Wang *et al*[26], 2021	China, prospective	Patients attending annual health examination at a single center in 2019, *n* = 154	67.8 ± 9.3, 19.5%	BMI: 24.9 ± 2.9	Abdominal ultrasound[1]	Sarcopenia, defined as low hand grip strength (< 18 kg in women and < 26 kg in men), a gait speed < 0.8 m/s, and DEXA-based ASM/height^2 < 5.4 in women and < 7.0 kg/m^2 in men. *n* = 25 (16.2%)	-	Fair
Almeida *et al* [27], 2022	Brazil, prospective	Consecutive patients with NAFLD at a single center, *n* = 57	52.7 ± 11.3, 24.6%	-	Abdominal ultrasound[1]	Probable sarcopenia, defined as low hand grip strength [< 16/27 kg (females/males)]. *n* = 15 (26.3%)	-	Fair
Guo *et al*[35], 2022	China, prospective	Patients undergoing health checkup at a single center from 2020-2021, *n* = 1830	47.4 ± 10.5, 80.2%	BMI: 27.1 ± 3.0	Transient elastography with fat attenuation parameter > 240 dB/m	BIA was used to calculate ASM, which was divided by height × 100 = SMI. SMI gradually decreased in a stepwise manner as the severity of hepatic steatosis increased	LSM values > 7.3 kPa were classified as having liver fibrosis. Participants in the tertile 1 of SMI had significantly higher odds of liver fibrosis (aOR: 3.7, 95%CI: 2.6-5.3) compared to tertile 3	Good
Seo *et al*[36], 2022	South Korea, retrospective	Patients undergoing health checkup at a single center from 2017-2019, *n* = 3198	54.2 ± 9.6, 89.8%	BMI: 26.2 ± 2.9; HTN: 40.2%; DM: 20.1%	Transient elastography with controlled attenuation parameter > 248 dB/m	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. ASM/BW% < 29.1 in men or < 23.0 in women was considered as sarcopenia. *n* = 517 (16.2%)	-	Poor
Song *et al*[37], 2023	South Korea, retrospective	Patients undergoing health checkup at a single center from 2007-2018, *n* = 1180	53.3 ± 10.3, 71.5%	BMI: 26.7 ± 3.67; DM: 20.7%	Transient elastography with fat attenuation parameter > 260 dB/m	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. ASM/BW% < 30.0 in men or < 26.8 in women was considered as sarcopenia	LSM values ≥ 7.5 kPa (≥ F2) were classified as having liver fibrosis. Sarcopenia was not a predictor of fibrosis in NAFLD with aOR: 3.80 (95%CI: 0.86-16.75)	Good
Zhang *et al*[28], 2022	China, retrospective	T2DM patients with BMI < 25 kg/m^2 were enrolled from a single center from 2017 to 2021, *n* = 1112	53.4 ± 10.7, 57.6%	BMI: 22.6; DM: 100%	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight × 100 = SMI. ASM/BW% < 32.2 in men or < 25.5 in women was considered as sarcopenia. *n* = 290 (26.1%)	-	Fair
Zhu *et al*[29], 2023	China, prospective	Participants of Shanghai Changfeng Study, a community-based prospective cohort study of multiple chronic diseases Jun 2009 to Dec 2012, with age > 45 yr, *n* = 1305	62.6 ± 8.9, 33.1%	BMI: 25.7 ± 3.2	Fatty liver was diagnosed when liver fat content by ultrasound exceeded the cut-off value of 9.15%	DEXA was used to calculate SI = ASM/height^2. The cut-off SI for sarcopenia were 6.88 kg/m^2 in male and 5.67 kg/m^2 in female. *n* = 260 (19.9%)	Significant fibrosis was defined as FIB-4 ≥ 2.67. The presence of sarcopenia was associated with increased risk of carotid plaque (aOR: 2.22; 95%CI: 1.23-4.02) and liver fibrosis (aOR: 2.07; 95%CI: 1.24-3.44)	Fair
Cho *et al*[30], 2023	South Korea, retrospective	Patients with T2DM from the Seoul Metabolic Syndrome Cohort, *n* = 456	55.0 ± 9.4, 46.3%	BMI: 25.7 ± 2.8; DM: 100%; HTN: 36.0%	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was divided by weight = ASM/BW%; ASM/BW% < 29.0 in men or < 22.9 in women was considered as sarcopenia. *n* = 123 (27.0%)	Sarcopenia was an independent predictor carotid plaque progression (OR: 2.02, 95%CI: 1.32-3.08)	Good
Choe *et al*[16], 2023	South Korea, retrospective	Korean Genome and Epidemiology Study (KoGES) Ansung-Ansan cohort, *n* = 1442	51.7 ± 8.5, 40.0%	BMI: 27.9 ± 2.5; DM: 28.4%; HTN: 34.7%; MS: 69.7%	Hepatic steatosis index (HSI) based on ALT, AST, BMI, DM, sex. NAFLD defined by HSI > 36	-	Fibrosis was defined as FIB-4 ≥ 1.3 and APRI ≥ 0.5. In the adjusted model, low muscle mass (lowest quartile) did not contribute to progression to hepatic fibrosis (HR: 1.02, 95%CI: 0.85-1.22)	Poor
Chun *et al*[31],	South Korea,	Patients undergoing health	50.0 ±	BMI: 25.9 ± 3.3; DM:	Abdominal ultrasound[1]	BIA was used to calculate ASM, which was	-	Fair

2023	retrospective	checkup at a three center from 2014-2020, *n* = 23889	11.0, 69.5%	14.4%; HTN: 37%; obesity: 56.9%; MS: 47.1%		divided by weight = ASM/BW%; ASM/BW% < 29.0 in men or < 22.9 in women was considered as sarcopenia. *n* = 3092 (12.9%). Sarcopenia was defined using a cut-off point of ASM/BMI = SI < 0.789 in men and < 0.521 in women. *n* = 1577 (6.6%)		
Harring *et al* [38], 2023	United States, retrospective	Analysis of the third National Health and Nutrition Examination Survey (NHANES), from 2017 to 2018, *n* = 1056	41.9 ± 0.42[2], 54.8%	BMI: 33.5 ± 0.37[2]; obesity: 78.7%; DM: 18.1%; HTN: 53.9%; MS: 64.8%	Transient elastography with fat attenuation parameter > 263 dB/m	DEXA was used to calculate SI = ASM/BMI. Sarcopenia was defined using a cut-off point of SI < 0.789 in men and < 0.512 in women. *n* = 303 (28.7%)	-	Good
Lu *et al*[32], 2023	China, retrospective	Patients diagnosed with obesity during health checkup at a single center from 2020-2021, *n* = 476	51.0 ± 13.7, 52.7%	BMI: 27.9 ± 3.3; obesity: 100%	Abdominal ultrasound[1]	BIA was used to calculate SMI = appendicular skeletal mass/height^2. Sarcopenia defined as SMI ≤ 7.0 kg/m^2 for males and ≤ 5.7 kg/m^2 for females; *n* = 261 (54.8%)	-	Fair
Zhou *et al*[34], 2023	China, prospective	Consecutively enrolled subjects who underwent BIA at a single center, between May 2017 and July 2022, *n* = 1123	37.8 ± 10.6, 58.7%	BMI: 28.9 ± 5.1; DM: 17.6%	MRI liver PDFF > 5%	BIA was used to calculate the appendicular skeletal mass (ASM). Sarcopenia was defined as ASM/height^2 or ASM/weight or ASM/BMI less than 2 SD. *n* = 50 (4.4%)	The MAFLD patients with lower quartiles of ASM/W had a higher risk OR for insulin resistance, both in male and female (OR: 2.14, 95%CI: 1.16-3.97), and OR: 4.26, 95%CI: 1.29, 14.02) for Q4 *vs* Q1	Fair

[1]Abdominal ultrasound showing at least two of the following three abnormal findings: (1) Diffusely increased echogenicity in liver near field ('bright liver') with greater liver echogenicity than kidney or spleen; (2) vascular blurring; and (3) poor visualization of the posterior portion of the right lobe because of deep attenuation.

[2]Standard error.

ALT/AST/GGT: Alanine transaminase/aspartate transaminase/gamma-glutamyltransferase; aOR: Adjusted odds ratio; 95%CI: 95% confidence interval; ASM: Appendicular skeletal muscle mass; BIA: Bioelectrical impedance analysis; BMI: Body mass index; BW: Body weight; DM/HTN/MS: Diabetes mellitus/hypertension/metabolic syndrome; DEXA: Dual X-ray absorptiometry; LSM: Liver stiffness measurement; MRI-PDFF: Magnetic resonance imaging proton density fat fraction; SMI: Skeletal muscle mass index; HIS: Hepatic steatosis index; FIB-4: Fibrosis-4; NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease.

76.3% in nonsarcopenic cases, *P* = 0.01). Two studies identified sarcopenia as a predictor of mortality in patients with NAFLD, with adjusted hazard ratios (aHRs) of 1.78 (95%CI: 1.16-2.73)[14] and 1.44 (95%CI: 1.16-1.80)[23]. Kang *et al*[22] reported sarcopenia as an independent predictor of increased intima-media thickness with an OR of 2.26 (95%CI: 1.26-4.04). Furthermore, Kang *et al*[22] and Zhu *et al*[29] reported that sarcopenia in NAFLD was associated with a higher risk of carotid plaque, with aORs of 2.74 (95%CI: 1.30-5.78) and 2.22 (95%CI: 1.23-4.02), respectively. However, Cho *et al*[30] reported higher odds of carotid plaque development with sarcopenia in those without carotid plaque at baseline (OR: 2.02, 95%CI: 1.32-3.08). Finally, Zhou *et al*[34] reported that NAFLD patients with sarcopenia had a higher risk of insulin resistance in both men and women (OR: 2.14, 95%CI: 1.16-3.97; OR: 4.26, 95%CI: 1.29-14.02).

DISCUSSION

An increasing number of studies have indicated the association between sarcopenia and NAFLD. However, the exact prevalence of sarcopenia in the NAFLD population remains unclear. This systematic review is the first to summarize the current evidence on the prevalence of sarcopenia in NAFLD patients. Of the 24 studies reporting the prevalence, fourteen,

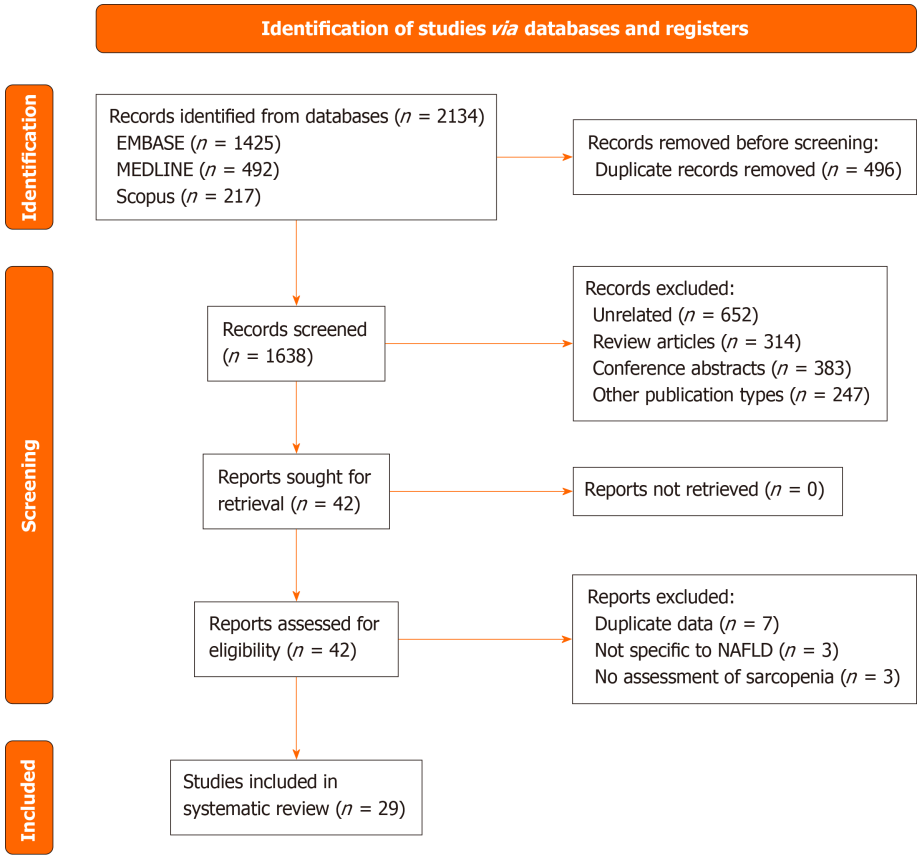

Figure 1 PRISMA flowchart for study identification, selection, and inclusion process. NAFLD: Nonalcoholic fatty liver disease.

four, and two studies reported prevalence rates of 10%-30%, 30%-50%, and more than 50%, respectively, whereas only four studies demonstrated a prevalence rate of less than 10%. This finding indicates that a considerable number of patients with NAFLD develop sarcopenia. In addition, sarcopenia was associated with an increased risk of advanced fibrosis in NAFLD, with an OR of 1.97 (95%CI: 1.44-2.70). Furthermore, sarcopenia in patients with NAFLD was associated with increased risks of NASH, insulin resistance, carotid plaque, and mortality.

Our systematic review highlighted a considerable variation in the reported prevalence of sarcopenia among patients with NAFLD. This variation is attributed to the diagnostic modality used, from 1.6% using MRI to 63.0% with DEXA. This discrepancy is amplified by using different cutoff values and indices within the same diagnostic modality, such as the normalization of ASM to BMI, weight, or height squared. This substantial variability in sarcopenia prevalence emphasizes the need for standardized diagnostic criteria and measurement techniques for sarcopenia in NAFLD patients. The European Working Group on Sarcopenia in Older People has proposed criteria and cutoffs for the three essential components of sarcopenia: muscle mass, muscle strength, and physical performance[2]. The choice of diagnostic modality and cutoff criteria markedly affects the reported prevalence rates, highlighting the necessity for consensus guidelines to ensure consistency across studies and populations. The variation in prevalence across different studies is primarily influenced by the distribution of muscle mass index in the population and the absolute values of the cutoff points. By contrast, variations in cutoff points for gait speed and grip strength appear to have a weak impact on the prevalence rates of sarcopenia[39].

The results of this systematic review revealed a significant relationship between sarcopenia and an increased risk of advanced fibrosis in NAFLD patients despite noticeable heterogeneity across the included studies. Upon examining the various modalities used for assessing fibrosis (such as biopsy, NFS/BARD scores, TE, and FIB-4 scores), a consistent association with sarcopenia was observed for all modalities except for the FIB-4 score. The absence of an association with the FIB-4 score indicates the necessity of selecting the appropriate fibrosis assessment method when exploring the relationship between sarcopenia and advanced fibrosis in NAFLD. Additionally, a recent study examined the effectiveness of noninvasive tests for estimating fibrosis, particularly in Asian populations. A recent multicentric study highlighted that only TE and TE-based combination tests accurately predicted liver fibrosis, whereas the internationally accepted thresholds for other NITs exhibited high false-negative rates[40].

Our systematic review sheds light on the extensive range of outcomes associated with sarcopenia in NAFLD patients. Key findings included an increased risk of NASH and a higher incidence of NASH in those with sarcopenia. Additionally, our meta-analysis revealed the predictive value of sarcopenia for mortality in NAFLD, as demonstrated by two studies with aHR of 1.78 (95%CI: 1.16-2.73) and 1.44 (95%CI: 1.16-1.80[14,23]). Sarcopenia in NAFLD was also associated with cardiovascular risk factors, such as increased intima-media thickness and a higher likelihood of carotid plaque formation. Moreover, sarcopenia was associated with a higher prevalence of insulin resistance, a key player in NAFLD pathogenesis. The relationship between sarcopenia and cardiovascular risks is particularly significant, con-

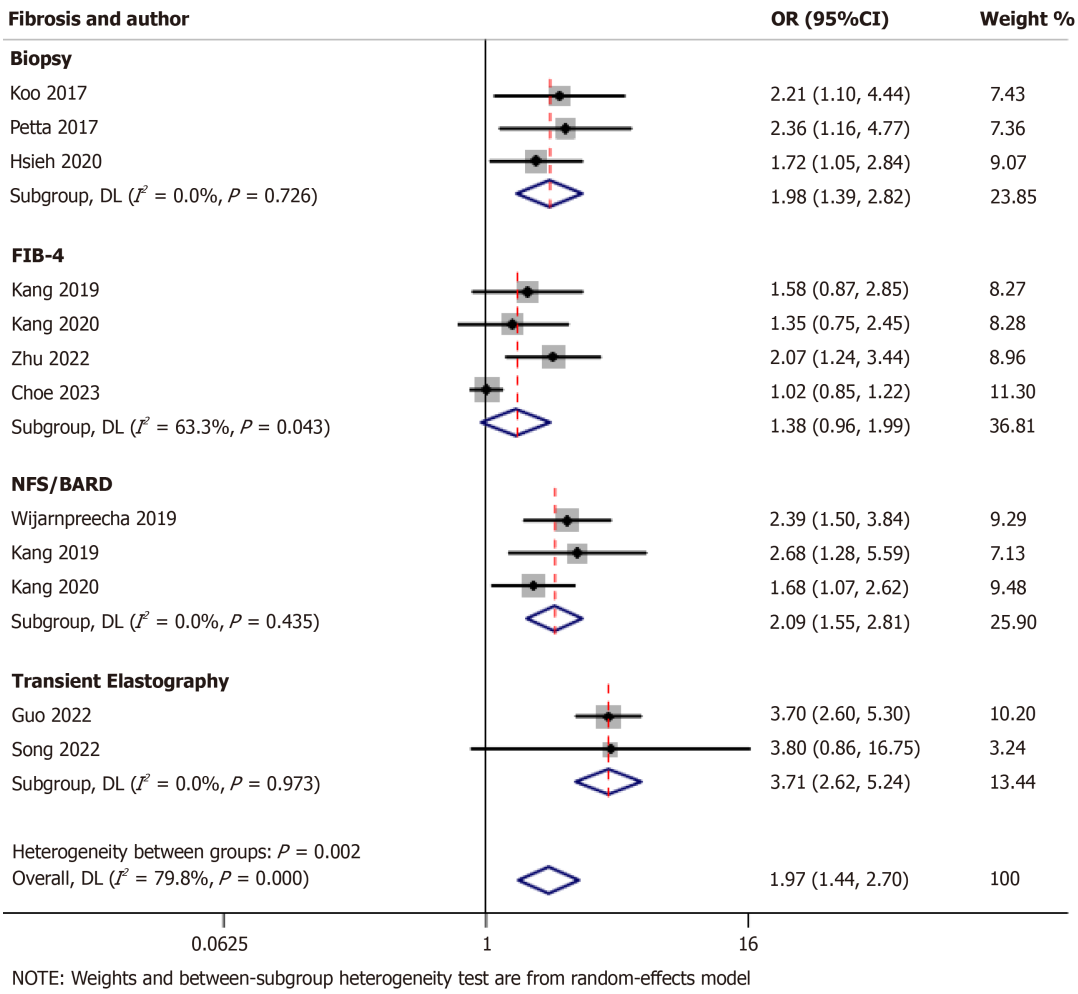

Figure 2 Forest plot showing the odds of advanced fibrosis with sarcopenia in patients with non-alcoholic fatty liver disease with subgroup analysis based on the method of fibrosis assessment. OR: Odds ratio; 95%CI: 95% confidence interval; FIB-4: Fibrosis-4; NFS/BARD: Non-alcoholic fatty liver disease fibrosis scores/body mass index, the ratio of aspartate aminotransferase to alanine aminotransferase, diabetes; DL: DerSimonian and Laird.

sidering the established relationship between NAFLD and adverse cardiovascular events[41]. This association not only highlights the multifaceted impact of sarcopenia in NAFLD but also opens avenues for future research aimed at reducing cardiovascular morbidity and mortality in this patient population.

The strengths of our systematic review include acknowledging the significant heterogeneity in sarcopenia prevalence reports among NAFLD patients and emphasizing the necessity for standardized guidelines in this area. In addition, we examined non-liver-related events in NAFLD patients and their correlation with sarcopenia. Variability in sarcopenia and liver fibrosis assessment methods contributes to the diverse results observed in our review. The inclusion of studies with varied designs and the demographic differences among patient populations could also affect the observed prevalence of sarcopenia and its association with NAFLD outcomes. Although our review explores the association of sarcopenia with mortality and cardiovascular risks, some specific outcomes, such as carotid plaque risk and progression, were only addressed in a limited number of studies, affecting the conclusiveness of these findings. For instance, Zhang *et al*[28] noted a higher sarcopenia prevalence in lean versus non-lean NAFLD patients, a detail we could not further analyze due to data limitations. Moreover, the review's focus on studies primarily from Asian populations, especially South Korea, may limit the generalizability of the findings to Western populations.

CONCLUSION

This systematic review highlights the multifaceted impact of sarcopenia on patients with NAFLD, extending beyond liver-related issues to include cardiovascular risks, mortality, and metabolic complications. The observed variations in prevalence and associations indicate the urgent need for standardized diagnostic criteria and measurement techniques. Our review offers crucial insights into the clinical implications of sarcopenia within the NAFLD context, potentially guiding future research and clinical practice.

FOOTNOTES

Author contributions: Giri S and Lavekar A contributed to the conception and design of the manuscript; Giri S, Angadi S, and Singh A contributed to the literature review, analysis, data collection, and interpretation; Giri S, Anirvan P, and Angadi S drafted the initial manuscript; Giri S and Lavekar A contributed to the critical revision of the initial manuscript; and all the authors approved the final version of the manuscript.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2021 Checklist, and the manuscript was prepared and revised according to the PRISMA 2021 Checklist.

Country/Territory of origin: India

ORCID number: Suprabhat Giri 0000-0002-9626-5243; Prajna Anirvan 0000-0003-4494-0865; Sumaswi Angadi 0000-0002-0066-2616; Ankita Singh 0000-0003-4960-7439; Anurag Lavekar 0000-0002-8192-3581.

S-Editor: Chen YL
L-Editor: A
P-Editor: Zhao YQ

REFERENCES

1 **Loomba R**, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* 2021; **184**: 2537-2564 [PMID: 33989548 DOI: 10.1016/j.cell.2021.04.015]
2 **Cruz-Jentoft AJ**, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, Cooper C, Landi F, Rolland Y, Sayer AA, Schneider SM, Sieber CC, Topinkova E, Vandewoude M, Visser M, Zamboni M; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019; **48**: 16-31 [PMID: 30312372 DOI: 10.1093/ageing/afy169]
3 **Donini LM**, Busetto L, Bischoff SC, Cederholm T, Ballesteros-Pomar MD, Batsis JA, Bauer JM, Boirie Y, Cruz-Jentoft AJ, Dicker D, Frara S, Frühbeck G, Genton L, Gepner Y, Giustina A, Gonzalez MC, Han HS, Heymsfield SB, Higashiguchi T, Laviano A, Lenzi A, Nyulasi I, Parrinello E, Poggiogalle E, Prado CM, Salvador J, Rolland Y, Santini F, Serlie MJ, Shi H, Sieber CC, Siervo M, Vettor R, Villareal DT, Volkert D, Yu J, Zamboni M, Barazzoni R. Definition and Diagnostic Criteria for Sarcopenic Obesity: ESPEN and EASO Consensus Statement. *Obes Facts* 2022; **15**: 321-335 [PMID: 35196654 DOI: 10.1159/000521241]
4 **Dhaliwal A**, Armstrong MJ. Sarcopenia in cirrhosis: A practical overview. *Clin Med (Lond)* 2020; **20**: 489-492 [PMID: 32934043 DOI: 10.7861/clinmed.2020-0089]
5 **Li AA**, Kim D, Ahmed A. Association of Sarcopenia and NAFLD: An Overview. *Clin Liver Dis (Hoboken)* 2020; **16**: 73-76 [PMID: 32922754 DOI: 10.1002/cld.900]
6 **Bhanji RA**, Narayanan P, Allen AM, Malhi H, Watt KD. Sarcopenia in hiding: The risk and consequence of underestimating muscle dysfunction in nonalcoholic steatohepatitis. *Hepatology* 2017; **66**: 2055-2065 [PMID: 28777879 DOI: 10.1002/hep.29420]
7 **Wijarnpreecha K**, Panjawatanan P, Thongprayoon C, Jaruvongvanich V, Ungprasert P. Sarcopenia and risk of nonalcoholic fatty liver disease: A meta-analysis. *Saudi J Gastroenterol* 2018; **24**: 12-17 [PMID: 29451179 DOI: 10.4103/sjg.SJG_237_17]
8 **Page MJ**, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; **372**: n71 [PMID: 33782057 DOI: 10.1136/bmj.n71]
9 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370 DOI: 10.1007/s10654-010-9491-z]
10 **Lee YH**, Kim SU, Song K, Park JY, Kim DY, Ahn SH, Lee BW, Kang ES, Cha BS, Han KH. Sarcopenia is associated with significant liver fibrosis independently of obesity and insulin resistance in nonalcoholic fatty liver disease: Nationwide surveys (KNHANES 2008-2011). *Hepatology* 2016; **63**: 776-786 [PMID: 26638128 DOI: 10.1002/hep.28376]
11 **Koo BK**, Kim D, Joo SK, Kim JH, Chang MS, Kim BG, Lee KL, Kim W. Sarcopenia is an independent risk factor for non-alcoholic steatohepatitis and significant fibrosis. *J Hepatol* 2017; **66**: 123-131 [PMID: 27599824 DOI: 10.1016/j.jhep.2016.08.019]
12 **Petta S**, Ciminnisi S, Di Marco V, Cabibi D, Cammà C, Licata A, Marchesini G, Craxì A. Sarcopenia is associated with severe liver fibrosis in patients with non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2017; **45**: 510-518 [PMID: 28028821 DOI: 10.1111/apt.13889]
13 **Hsieh YC**, Joo SK, Koo BK, Lin HC, Kim W. Muscle alterations are independently associated with significant fibrosis in patients with nonalcoholic fatty liver disease. *Liver Int* 2021; **41**: 494-504 [PMID: 33164304 DOI: 10.1111/liv.14719]
14 **Golabi P**, Gerber L, Paik JM, Deshpande R, de Avila L, Younossi ZM. Contribution of sarcopenia and physical inactivity to mortality in people with non-alcoholic fatty liver disease. *JHEP Rep* 2020; **2**: 100171 [PMID: 32964202 DOI: 10.1016/j.jhepr.2020.100171]
15 **Kang S**, Moon MK, Kim W, Koo BK. Association between muscle strength and advanced fibrosis in non-alcoholic fatty liver disease: a Korean nationwide survey. *J Cachexia Sarcopenia Muscle* 2020; **11**: 1232-1241 [PMID: 32638541 DOI: 10.1002/jcsm.12598]

16 **Choe HJ**, Lee H, Lee D, Kwak SH, Koo BK. Different effects of low muscle mass on the risk of non-alcoholic fatty liver disease and hepatic fibrosis in a prospective cohort. *J Cachexia Sarcopenia Muscle* 2023; **14**: 260-269 [PMID: 36403577 DOI: 10.1002/jcsm.13125]

17 **Kang MK**, Park JG, Lee HJ, Kim MC. Association of low skeletal muscle mass with advanced liver fibrosis in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2019; **34**: 1633-1640 [PMID: 30667551 DOI: 10.1111/jgh.14607]

18 **Wijarnpreecha K**, Kim D, Raymond P, Scribani M, Ahmed A. Associations between sarcopenia and nonalcoholic fatty liver disease and advanced fibrosis in the USA. *Eur J Gastroenterol Hepatol* 2019; **31**: 1121-1128 [PMID: 30888971 DOI: 10.1097/MEG.0000000000001397]

19 **Gan D**, Wang L, Jia M, Ru Y, Ma Y, Zheng W, Zhao X, Yang F, Wang T, Mu Y, Zhu S. Low muscle mass and low muscle strength associate with nonalcoholic fatty liver disease. *Clin Nutr* 2020; **39**: 1124-1130 [PMID: 31053512 DOI: 10.1016/j.clnu.2019.04.023]

20 **Park H**, Jun DW, Park HK, Park KY, Kim M, Hwang HS. A Critical Appraisal of the Definition of Sarcopenia in Patients with Non-Alcoholic Fatty Liver Disease: Pitfall of Adjusted Muscle Mass by Body Weight. *Life (Basel)* 2020; **10** [PMID: 32977654 DOI: 10.3390/life10100218]

21 **Seo DH**, Lee YH, Park SW, Choi YJ, Huh BW, Lee E, Huh KB, Kim SH, Cha BS. Sarcopenia is associated with non-alcoholic fatty liver disease in men with type 2 diabetes. *Diabetes Metab* 2020; **46**: 362-369 [PMID: 31689496 DOI: 10.1016/j.diabet.2019.10.004]

22 **Kang MK**, Park JG. Low Skeletal Muscle Mass Is a Risk Factor for Subclinical Atherosclerosis in Patients with Nonalcoholic Fatty Liver Disease. *Diagnostics (Basel)* 2021; **11** [PMID: 34068776 DOI: 10.3390/diagnostics11050854]

23 **Kim D**, Wijarnpreecha K, Sandhu KK, Cholankeril G, Ahmed A. Sarcopenia in nonalcoholic fatty liver disease and all-cause and cause-specific mortality in the United States. *Liver Int* 2021; **41**: 1832-1840 [PMID: 33641244 DOI: 10.1111/liv.14852]

24 **Lee HJ**, Chang JS, Ahn JH, Kim MY, Park KS, Ahn YS, Koh SB. Association Between Low Muscle Mass and Non-alcoholic Fatty Liver Disease Diagnosed Using Ultrasonography, Magnetic Resonance Imaging Derived Proton Density Fat Fraction, and Comprehensive NAFLD Score in Korea. *J Prev Med Public Health* 2021; **54**: 412-421 [PMID: 34875824 DOI: 10.3961/jpmph.21.387]

25 **Lee JH**, Lee HS, Lee BK, Kwon YJ, Lee JW. Relationship between Muscle Mass and Non-Alcoholic Fatty Liver Disease. *Biology (Basel)* 2021; **10** [PMID: 33562473 DOI: 10.3390/biology10020122]

26 **Wang YM**, Zhu KF, Zhou WJ, Zhang Q, Deng DF, Yang YC, Lu WW, Xu J, Yang YM. Sarcopenia is associated with the presence of nonalcoholic fatty liver disease in Zhejiang Province, China: a cross-sectional observational study. *BMC Geriatr* 2021; **21**: 55 [PMID: 33446095 DOI: 10.1186/s12877-020-01910-3]

27 **Almeida NS**, Rocha R, de Souza CA, da Cruz ACS, Ribeiro BDR, Vieira LV, Daltro C, Silva R, Sarno M, Cotrim HP. Prevalence of sarcopenia using different methods in patients with non-alcoholic fatty liver disease. *World J Hepatol* 2022; **14**: 1643-1651 [PMID: 36157861 DOI: 10.4254/wjh.v14.i8.1643]

28 **Zhang X**, He Z, Si Q, Hu X, Yang L, Gu X, Du L, Wang L, Pan L, Li Y, Li J, Yang B. The Association of Sarcopenia and Visceral Obesity with Lean Nonalcoholic Fatty Liver Disease in Chinese Patients with Type 2 Diabetes Mellitus. *J Diabetes Res* 2022; **2022**: 2229139 [PMID: 36387941 DOI: 10.1155/2022/2229139]

29 **Zhu X**, Huang Q, Ma S, Chen L, Wu Q, Wu L, Ma H, Li X, Li Q, Aleteng Q, Hu Y, He W, Gao J, Lin H, Tang H, Gao X, Xia M. Presence of sarcopenia identifies a special group of lean NAFLD in middle-aged and older people. *Hepatol Int* 2023; **17**: 313-325 [PMID: 36327053 DOI: 10.1007/s12072-022-10439-z]

30 **Cho Y**, Park HS, Huh BW, Lee YH, Seo SH, Seo DH, Ahn SH, Hong S, Kim SH. Non-Alcoholic Fatty Liver Disease with Sarcopenia and Carotid Plaque Progression Risk in Patients with Type 2 Diabetes Mellitus. *Diabetes Metab J* 2023; **47**: 232-241 [PMID: 36653888 DOI: 10.4093/dmj.2021.0355]

31 **Chun HS**, Lee M, Lee HA, Lee S, Kim S, Jung YJ, Lee C, Kim H, Kim HY, Yoo K, Kim TH, Ahn SH, Kim SU. Risk Stratification for Sarcopenic Obesity in Subjects With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2023; **21**: 2298-2307.e18 [PMID: 36462755 DOI: 10.1016/j.cgh.2022.11.031]

32 **Lu Y**, Xia Q, Wu L, Xie Z. Gender difference in association between low muscle mass and risk of non-alcoholic fatty liver disease among Chinese adults with visceral obesity. *Front Nutr* 2023; **10**: 1026054 [PMID: 36713086 DOI: 10.3389/fnut.2023.1026054]

33 **Linge J**, Ekstedt M, Dahlqvist Leinhard O. Adverse muscle composition is linked to poor functional performance and metabolic comorbidities in NAFLD. *JHEP Rep* 2021; **3**: 100197 [PMID: 33598647 DOI: 10.1016/j.jhepr.2020.100197]

34 **Zhou T**, Ye J, Lin Y, Wang W, Feng S, Zhuo S, Zhong B. Impact of skeletal muscle mass evaluating methods on severity of metabolic associated fatty liver disease in non-elderly adults. *Br J Nutr* 2023; **130**: 1373-1384 [PMID: 36896599 DOI: 10.1017/S0007114523000399]

35 **Guo W**, Zhao X, Miao M, Liang X, Li X, Qin P, Lu J, Zhu W, Wu J, Zhu C, Xu N, Zhang Q. Association Between Skeletal Muscle Mass and Severity of Steatosis and Fibrosis in Non-alcoholic Fatty Liver Disease. *Front Nutr* 2022; **9**: 883015 [PMID: 35558748 DOI: 10.3389/fnut.2022.883015]

36 **Seo JY**, Cho EJ, Kim MJ, Kwak MS, Yang JI, Chung SJ, Yim JY, Yoon JW, Chung GE. The relationship between metabolic dysfunction-associated fatty liver disease and low muscle mass in an asymptomatic Korean population. *J Cachexia Sarcopenia Muscle* 2022; **13**: 2953-2960 [PMID: 36222309 DOI: 10.1002/jcsm.13099]

37 **Song W**, Yoo SH, Jang J, Baik SJ, Lee BK, Lee HW, Park JS. Association between Sarcopenic Obesity Status and Nonalcoholic Fatty Liver Disease and Fibrosis. *Gut Liver* 2023; **17**: 130-138 [PMID: 36472070 DOI: 10.5009/gnl220041]

38 **Harring M**, Golabi P, Paik JM, Shah D, Racila A, Cable R, Srishord M, Younossi ZM. Sarcopenia Among Patients With Nonalcoholic Fatty Liver Disease (NAFLD) Is Associated With Advanced Fibrosis. *Clin Gastroenterol Hepatol* 2023; **21**: 2876-2888.e5 [PMID: 36848980 DOI: 10.1016/j.cgh.2023.02.013]

39 **Masanés F**, Rojano I Luque X, Salvà A, Serra-Rexach JA, Artaza I, Formiga F, Cuesta F, López Soto A, Ruiz D, Cruz-Jentoft AJ. Cut-off Points for Muscle Mass - Not Grip Strength or Gait Speed - Determine Variations in Sarcopenia Prevalence. *J Nutr Health Aging* 2017; **21**: 825-829 [PMID: 28717813 DOI: 10.1007/s12603-016-0844-5]

40 **Arora U**, Goyal RM, Teh KKJ, Pei Y, Goh GBB, Lin S, Kumar R, Biswas S, Vaishnav M, Swaroop S, Pathak P, Sheikh S, Bharadiya V, Elhence A, Gamanagatti S, Yadav R, Das P, Aggarwal S, Choudhary N, Anirvan P, Singh SP, De A, Duseja A, Shalimar. Poor Performance of Non-invasive Tests for Advanced Fibrosis in Nonalcoholic Fatty Liver Disease: A Multicentric Asian Study. *Dig Dis Sci* 2023; **68**: 4485-4498 [PMID: 37733130 DOI: 10.1007/s10620-023-08085-y]

41 **Wu S**, Wu F, Ding Y, Hou J, Bi J, Zhang Z. Association of non-alcoholic fatty liver disease with major adverse cardiovascular events: A systematic review and meta-analysis. *Sci Rep* 2016; **6**: 33386 [PMID: 27633274 DOI: 10.1038/srep33386]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: office@baishideng.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com