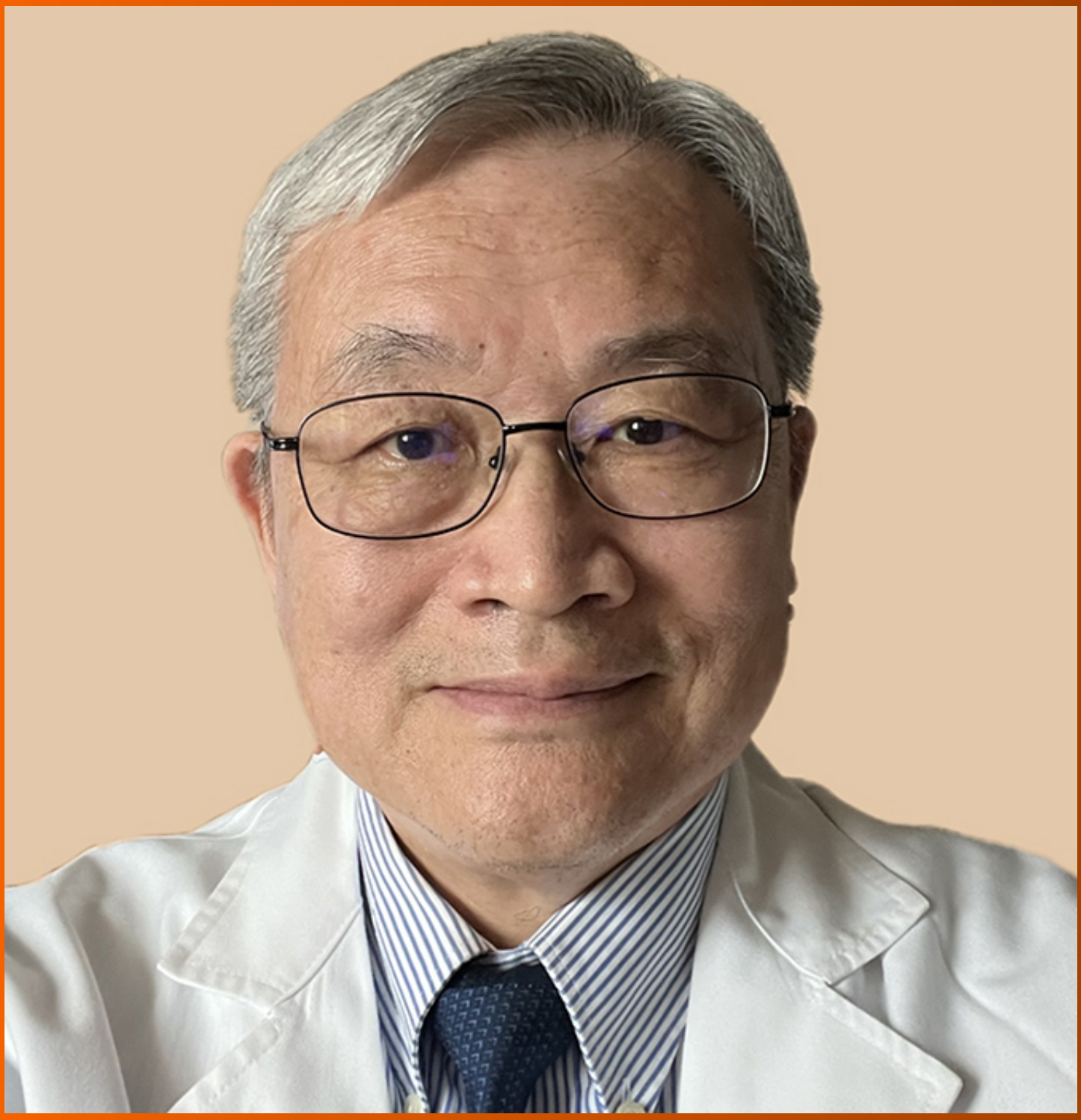
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2024 July 28; 30(28): 3361-3455



Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 30 Number 28 July 28, 2024

EDITORIAL

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Study

Observational Study

Basic Study

LETTER TO THE EDITOR

World Journal of Gastroenterology

Weekly Volume 30 Number 28 July 28, 2024

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, Dar-In Tai, MD, PhD, Senior Attending Doctor, Professor, Department of Gastroenterology and Hepatology, Chang Gung Memorial Hospital, Taipei 105, Taiwan. tai48978@cgmh.org.tw

AIMS AND SCOPE

The primary aim of *World Journal of Gastroenterology* (*WJG*, *World J Gastroenterol*) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJG* mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

INDEXING/ABSTRACTING

The *WJG* is now abstracted and indexed in Science Citation Index Expanded (SCIE), MEDLINE, PubMed, PubMed Central, Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for *WJG* as 4.3; Quartile: Q1. The *WJG*'s CiteScore for 2023 is 7.8.

RESPONSIBLE EDITORS FOR THIS ISSUE

Production Editor: *Yu-Xi Chen*; **Production Department Director:** *Xiang Li*; **Cover Editor:** *Jia-Ru Fan*.

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

PUBLICATION DATE
July 28, 2024

PUBLISHING PARTNER
Shanghai Pancreatic Cancer Institute and Pancreatic Cancer Institute, Fudan University
Biliary Tract Disease Institute, Fudan University

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

POLICY OF CO-AUTHORS
https://www.wjgnet.com/bpg/GerInfo/310

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

PUBLISHING PARTNER's OFFICIAL WEBSITE
https://www.shca.org.cn
https://www.zs-hospital.sh.cn

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3361-3366

DOI: 10.3748/wjg.v30.i28.3361

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Optimization of colorectal cancer screening strategies: New insights

Magie Tamraz, Najib Al Ghossaini, Sally Temraz

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 C

P-Reviewer: Qiu Y

Received: March 16, 2024
Revised: June 7, 2024
Accepted: June 28, 2024
Published online: July 28, 2024
Processing time: 129 Days and 13.1 Hours

Magie Tamraz, Department of Nutrition and Public Health, Holy Spirit University of Kaslik, Jounieh 446, Lebanon

Najib Al Ghossaini, Department of Internal Medicine, Ain Wazein Medical Village, Chouf 1503, Lebanon

Sally Temraz, Department of Internal Medicine, Division of Hematology/Oncology, American University of Beirut Medical Center, Beirut 1107 2020, Lebanon

Corresponding author: Sally Temraz, MD, Associate Professor, Department of Internal Medicine, Division of Hematology/Oncology, American University of Beirut Medical Center, Riad El Solh, Beirut 1107 2020, Lebanon. st29@aub.edu.lb

Abstract

In this editorial, we discuss the article by Agatsuma *et al*. We concentrate specifically on the current routinely used screening tests recommended by society guidelines and delve into the significance of early diagnosis of colorectal cancer (CRC) and its substantial impact on both incidence and mortality rates. Screening is highly recommended, and an early diagnosis stands out as the most crucial predictor of survival for CRC patients. Therefore, it is essential to identify and address the barriers hindering adherence to screening measures, as these barriers can vary among different populations. Furthermore, we focus on screening strategy optimization by selecting high-risk groups. Patients with comorbidities who regularly visit hospitals have been diagnosed at an early stage, showing no significant difference compared to patients undergoing regular screening. This finding highlights the importance of extending screening measures to include patients with comorbidities who do not routinely visit the hospital.

Key Words: Colorectal neoplasms; Early diagnosis; Barriers to adherence; Cancer screening guidelines; Screening tests

Core Tip: Despite the proven mortality benefits, adherence to colorectal cancer (CRC) screening guidelines remains low in many regions worldwide. Because nation-wide screening is not feasible due to limited financial and human resources, it is crucial to identify high-risk groups that ought to participate in screening measures. However, variation in each population is to be considered when implementing screening procedures, and barriers affecting adherence to screening guidelines should be addressed in each specific population. Finally, patients with comorbidities who regularly schedule visits to the hospital are diagnosed at an early stage similar to those who undergo periodic screening. This underscores the importance of encouraging patients with comorbidities who do not attend routine visits to undergo screening to reduce the burden of late-stage CRC diagnosis.

Citation: Tamraz M, Al Ghossaini N, Temraz S. Optimization of colorectal cancer screening strategies: New insights. *World J Gastroenterol* 2024; 30(28): 3361-3366
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3361.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3361

INTRODUCTION

Globally, colorectal cancer (CRC) ranks third following breast and lung cancer. It is expected that the incidence of CRC will increase 1.6-fold by the year 2030[1], and in 2040, it is estimated that there will be approximately 3.2 million cases diagnosed worldwide[2]. The 5-year relative survival rate of CRC has shown a notable increase of 15% from the mid-1970s to the years 2012-2018[3,4]. This trend is primarily attributed to advancements in earlier detection facilitated by routine clinical examinations and screening strategies. Early-stage diagnosis is the most significant predictor of survival among CRC patients mounting to a 5-year survival rate of 91% in early-stage cases compared to a 5-year survival rate of 14% in metastatic cases[5].

Various screening tests are provided for the early detection of CRC and adenomatous polyps. These tests vary in terms of sensitivity and specificity, effectiveness, convenience, safety, accessibility, and cost. Screening tests could be categorized into four groups: Stool-based tests, endoscopic visualization, radiologic visualization, and blood-based markers. Stool-based tests include fecal immunochemical test (FIT) for blood, which directly detects hemoglobin in stool samples, and Guaiac-based fecal occult blood test (gFOBT), which recognizes hemoglobin by altering guaiac reactant-permeated paper to blue due to a peroxidase reaction, multitarget stool DNA tests with FIT that combines molecular assays to test for DNA (KRAS) mutations in addition to FIT. Endoscopic visualization methods include colonoscopy, sigmoidoscopy, sigmoidoscopy plus FIT or gFOBT[6], and colon capsule endoscopy[7]. Available blood-based markers typically identify CRC at a more developed stage instead of premalignant lesions, and thus, their part in identifying early-stage disease remains uncertain and has not been incorporated in main society guideline recommendations. Presently employed blood-based markers, particularly in the United States, are the septin 9[8], which is a plasma assay that identifies circulating hypermethylated septin 9 DNA in CRC, and the 7-gene biomarker test (*ANXA3*, *CLEC4D*, *LMNB1*, *PRRG4*, *TNFAIP6*, *VNN1*, and *IL2RB*)[9]. Table 1 summarizes the specificity and sensitivity of the abovementioned tests as well as the recommended frequency of each screening test.

Another technique of defining screening tests for CRC is by distinguishing between 1-step or 2-step approaches. The 1-step approach such as colonoscopy simultaneously serves both diagnostic and therapeutic purposes. Colonoscopy is effective in diagnosing early lesions or polyps and enables their removal during the same procedure. Polypectomy can reduce the risk of mortality from CRC by 53%[10]. Consequently, the European Society of Gastrointestinal Endoscopy recommendation involves the removal of all polyps, with the exception of miniscule rectal and rectosigmoid polyps that are known to be hyperplastic[11]. In contrast, the 2-step approaches (FIT, gFOBT, CTC, and colon capsule) will require a follow-up colonoscopy if the initial test results are positive. This requirement is a drawback of the 2-step tests, contributing to why colonoscopy remains the gold standard approach for screening[12].

Screening tests are efficient in the context of increased adherence of the population to screening guidelines. Candidates for screening include adults with no signs or symptoms of CRC who are at an average risk of developing CRC and have no personal or family history of genetic disorders such as Lynch syndrome or familial adenomatous polyposis. However, patients with risk factors for CRC are included in high-screening programs. Furthermore, individuals with abnormal findings such as cancer or polyps should undergo surveillance colonoscopy regardless of their age[13]. Figure 1 summarizes the most updated societal recommendations for the screening of CRC.

Screening has significantly impacted the incidence and mortality rates of CRC. The use of gFOBT screening resulted in a reduced incidence of CRC by 20% in the United States and 60% in Japan. Flexible sigmoidoscopy reduced the incidence of CRC by 26% in the United Kingdom and 10% in Italy[14]. Regarding mortality rates, a gFOBT screening led to reductions in the mortality rate of 31.7% in China, 30% in Japan, 18% in Denmark, 16% in France, 16% in Sweden, 15% in the United Kingdom, and 13% in Italy. However, 1-year screening with gFOBT resulted in a higher reduction of mortality rates compared to 2-year screening (32% *vs* 18%)[3]. It is without a doubt that screening is crucial in addressing the high incidence and mortality rates of CRC.

Table 1 Comparison between different screening tests for colorectal cancer

Test type	Specificity, %	Sensitivity, %	Frequency, years	Ref.
Stool-based tests				
FIT	90-95	71-91	1-2	[23]
gFOBT	88-98	50-75	1-2	[24-26]
MT-sDNA	92.70	93.90	1-3	[27]
Endoscopic visualization				
Colonoscopy	86	95	10	[28]
Sigmoidoscopy	87	95[1]	5	[28]
Colon capsule	76-98.2	81-87	5	[29,30]
Radiologic visualization				
CTC	88	84	5	[28]

[1]Examining the distal part of the colon that is within reach of the sigmoidoscope.
CTC: Computed tomography colonography; FIT: Fecal immunochemical test; gFOBT: Guaiac-based fecal occult blood test; MT-sDNA: Multitarget stool DNA.

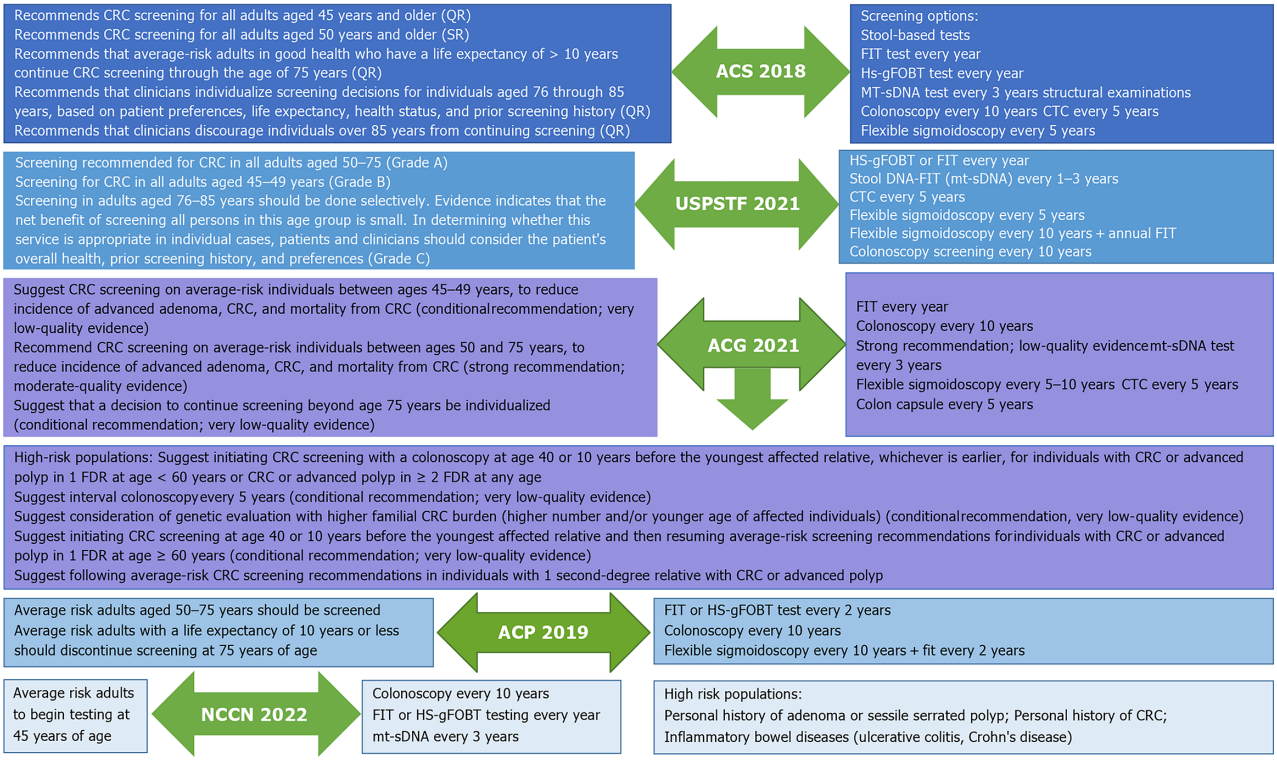


Figure 1 Colorectal cancer screening guideline recommendations from several societies. ACG: American College of Gastroenterology; ACP: American College of Physicians; ACS: American Cancer Society; CRC: Colorectal cancer; CTC: Computed tomography colonography; FDR: First-degree relative; FIT: Fecal immunochemical test; gFOBT: Guaiac-based fecal occult blood test; MT-sDNA: Multitarget stool DNA; NCCN: National Comprehensive Cancer Network; QR: Qualified recommendation; SR: Strong recommendation; USPSTF: United States Preventative Service Task Force.

BARRIERS TO APPROPRIATE SCREENING STRATEGIES

Despite the proven mortality benefits, adherence to screening guidelines varies significantly between countries. In Europe, the Netherlands has the highest adherence to screening guidelines, with a rate of 71.3%. Another 12 European countries reported an adherence rate greater than 50% and Italy reported an adherence of 45.7%. However, Poland and Belgium had an adherence rate below 20%[3]. Barriers affecting patient adherence include inadequate information and consciousness about CRC and its screening measures, absence of doctor recommendations, emotional factors such as anxiety, distress, and disgrace, in addition to social, spiritual, and sociodemographic factors including decreased income

and feminine gender[15]. Ideally, total population screening based on clinical recommendations would be the most efficient screening strategy to combat CRC; however, barriers to achieving total population screening are also limited by human and financial resources, in addition to the aforementioned barriers.

Furthermore, establishing clear guidelines regarding who should be screened is still challenging, particularly with an erratic disease such as CRC. For instance, previous recommendations involved screening adults who were older than 50 years. However, recent updates from the American Cancer Society and the United States Preventive Service Task Force have extended screening recommendations to include individuals aged 45 and older due to an increase in the incidence of CRC among younger adults[3]. Furthermore, in a recent systematic review of 24 clinical practice guidelines and five consensus statements, the median overall quality and reporting were 54.0% and 42.0% and the applicability had low quality in 83% of guidelines (24/29) which necessitates a revision and an enhancement of the current guidelines[16].

SCREENING STRATEGY OPTIMIZATION

In their retrospective study recently published in *World J Gastroenterol*, Agatsuma *et al*[17] elucidate the different stages of CRC at the point of diagnosis according to the diagnostic routes by utilizing cancer registries from two Japanese hospitals. They report that CRCs identified during hospital visits for comorbidities were diagnosed earlier, similar to cancer screening, and emphasize that patients with comorbidities without periodic visits to the hospital should be encouraged to undergo screening. With the high rate of CRC cases diagnosed *via* non-screening routes detected during hospital visits for comorbidities, it is practical to also consider the population of patients with comorbidities who are not routinely and periodically visiting the hospital, as this population of patients does not obtain early CRC recognition benefits due to lesser number of hospital visits. However, the challenge of assessing the burden of comorbidities remains unaddressed. Several studies have found that patients with chronic comorbid conditions harboring lower scores are more likely to adhere to screening compared to patients with higher burden comorbidities. In a population-based study conducted in Spain, it was found that patients with multiple minor chronic diseases were more inclined to participate in screening compared to those with three or more major chronic diseases who were likely to participate less in screening programs[12]. Wellbeing systems elaborated in the Population-Based Research Optimizing Screening by Personalized Regimens consortium observed that with increased comorbidity, diagnosis with fecal blood testing only was less common[18]. These patterns indicate a competing emphasis on morbidities and the notion of poor screening benefits for patients with comorbidities[19]. Another factor contributing to the lower adherence of patients with comorbidities to screening guidelines could be attributed to not being provided or suggested a screening measure by their healthcare providers. It was reported that individuals with a chronic disease index score equal to 1 had 8%-9% chance of being given a FIT, whereas those with a score greater than 4 identified as having an elevated disease burden were 13%-23% less likely to be offered a FIT[20]. Thus, tackling adherence to screening in patients with comorbidities not visiting the hospital for their routine check-ups should be addressed in the context of disease burden and screening emphasis.

Screening programs must be tailored to risk groups to provide approaches formulated to their risk of developing CRC [21]. Furthermore, to determine whether screening is suitable in individualized cases, both patients and practitioners ought to take into account the overall health of the patient, previous screening measures, and preferences[22]. In addition to the comorbidity burden, which is highly valuable in identifying patients less likely to adhere to screening, certain risk factors have been linked to tumorigenesis including smoking, lack of activity, excess fatness, and alcohol, which should also be considered when selecting high-risk individuals. However, protective factors such as aspirin and nonsteroidal anti-inflammatory drug use, and dietary interventions such as Mediterranean diet, high intake of fiber from fruits and vegetables, and intake of certain nutraceuticals such as curcumin, resveratrol, and quercetin may play a preventive role. Furthermore, the age at which CRC screening should begin needs to be identified to seize critical CRC cases while at the same time considering the cost-effectiveness of these tests, looking at regional epidemiology, and weighing the anticipated benefits *vs* harms.

CONCLUSION

Screening guidelines with limited adherence will not be effective in reducing CRC mortality and incidence. It is thus crucial to address barriers limiting patient adherence to screening guidelines and target patients with comorbidities who are not receiving routine clinical check-ups. Patients with comorbidities undergoing routine check-ups are diagnosed early similar to patients who undergo regular screening tests. The reason behind this finding is that patients with comorbidities undertake imaging studies and colonoscopies due to an abnormal test result or after experiencing certain symptoms, which might result in an earlier diagnosis. Comorbidities are categorized into two; those with a high burden and those with a low burden. High-burden comorbidities are inversely related to screening measures and should therefore be targeted. As for physician recommendations for screening, this should also be tailored to high-burden comorbidities

FOOTNOTES

Author contributions: Tamraz M contributed to manuscript drafting and writing; Al Ghossaini N contributed to critical revision; Temraz

S contributed to conception and design.

Conflict-of-interest statement: The authors report no relevant conflicts of interest for this article.

Country of origin: Lebanon

ORCID number: Sally Temraz 0000-0002-8995-7946.

Corresponding Author's Membership in Professional Societies: European Society of Medical Oncology; Lebanese Society of Medical Oncology; Lebanese Society of Hematology.

S-Editor: Li L
L-Editor: Filipodia
P-Editor: Zheng XM

REFERENCES

1 **Chung RY**, Tsoi KKF, Kyaw MH, Lui AR, Lai FTT, Sung JJ. A population-based age-period-cohort study of colorectal cancer incidence comparing Asia against the West. *Cancer Epidemiol* 2019; **59**: 29-36 [PMID: 30660075 DOI: 10.1016/j.canep.2019.01.007]

2 **Xi Y**, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. *Transl Oncol* 2021; **14**: 101174 [PMID: 34243011 DOI: 10.1016/j.tranon.2021.101174]

3 **Baidoun F**, Elshiwy K, Elkeraie Y, Merjaneh Z, Khoudari G, Sarmini MT, Gad M, Al-Husseini M, Saad A. Colorectal Cancer Epidemiology: Recent Trends and Impact on Outcomes. *Curr Drug Targets* 2021; **22**: 998-1009 [PMID: 33208072 DOI: 10.2174/1389450121999201117115717]

4 **Sawicki T**, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. *Cancers (Basel)* 2021; **13** [PMID: 33922197 DOI: 10.3390/cancers13092025]

5 **Siegel RL**, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *CA Cancer J Clin* 2023; **73**: 233-254 [PMID: 36856579 DOI: 10.3322/caac.21772]

6 **Diedrich L**, Brinkmann M, Dreier M, Rossol S, Schramm W, Krauth C. Is there a place for sigmoidoscopy in colorectal cancer screening? A systematic review and critical appraisal of cost-effectiveness models. *PLoS One* 2023; **18**: e0290353 [PMID: 37594967 DOI: 10.1371/journal.pone.0290353]

7 **Koffas A**, Papaefthymiou A, Laskaratos FM, Kapsoritakis A, Epstein O. Colon Capsule Endoscopy in the Diagnosis of Colon Polyps: Who Needs a Colonoscopy? *Diagnostics (Basel)* 2022; **12** [PMID: 36140494 DOI: 10.3390/diagnostics12092093]

8 **Leerhoff S**, Raem A, Kolbe EW, Schulz L, Borchers K, Köhler T, Winde G, Kirchner C. Methylated Septin9 identified patients with colorectal carcinoma and showed higher sensitivity than conventional biomarkers in detecting tumor. *Cancer Treat Res Commun* 2023; **36**: 100748 [PMID: 37541105 DOI: 10.1016/j.ctarc.2023.100748]

9 **Alorda-Clara M**, Torrens-Mas M, Morla-Barcelo PM, Martinez-Bernabe T, Sastre-Serra J, Roca P, Pons DG, Oliver J, Reyes J. Use of Omics Technologies for the Detection of Colorectal Cancer Biomarkers. *Cancers (Basel)* 2022; **14** [PMID: 35159084 DOI: 10.3390/cancers14030817]

10 **Das TS**, Rauch J, Shaukat A. Colorectal cancer screening-what does the recent NordICC trial mean for the U.S. population? *Transl Gastroenterol Hepatol* 2023; **8**: 40 [PMID: 38021363 DOI: 10.21037/tgh-23-20]

11 **Ferlitsch M**, Moss A, Hassan C, Bhandari P, Dumonceau JM, Paspatis G, Jover R, Langner C, Bronzwaer M, Nalankilli K, Fockens P, Hazzan R, Gralnek IM, Gschwantler M, Waldmann E, Jeschek P, Penz D, Heresbach D, Moons L, Lemmers A, Paraskeva K, Pohl J, Ponchon T, Regula J, Repici A, Rutter MD, Burgess NG, Bourke MJ. Colorectal polypectomy and endoscopic mucosal resection (EMR): European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2017; **49**: 270-297 [PMID: 28212588 DOI: 10.1055/s-0043-102569]

12 **Jayasinghe M**, Prathiraja O, Caldera D, Jena R, Coffie-Pierre JA, Silva MS, Siddiqui OS. Colon Cancer Screening Methods: 2023 Update. *Cureus* 2023; **15**: e37509 [PMID: 37193451 DOI: 10.7759/cureus.37509]

13 **Williams JR**, Ayscue JM. Colorectal cancer screening: A review of current screening options, timing, and guidelines. *J Surg Oncol* 2023; **127**: 1236-1246 [PMID: 37222695 DOI: 10.1002/jso.27299]

14 **Li N**, Lu B, Luo C, Cai J, Lu M, Zhang Y, Chen H, Dai M. Incidence, mortality, survival, risk factor and screening of colorectal cancer: A comparison among China, Europe, and northern America. *Cancer Lett* 2021; **522**: 255-268 [PMID: 34563640 DOI: 10.1016/j.canlet.2021.09.034]

15 **Lee J**, Ewing B, Holmes D. Barriers and Facilitators to Colorectal Cancer Screening in South Asian Immigrants: A Systematic Review. *Asian Pac J Cancer Prev* 2023; **24**: 1463-1475 [PMID: 37247265 DOI: 10.31557/APJCP.2023.24.5.1463]

16 **Maes-Carballo M**, García-García M, Martín-Díaz M, Estrada-López CR, Iglesias-Álvarez A, Filigrana-Valle CM, Khan KS, Bueno-Cavanillas A. A comprehensive systematic review of colorectal cancer screening clinical practices guidelines and consensus statements. *Br J Cancer* 2023; **128**: 946-957 [PMID: 36476659 DOI: 10.1038/s41416-022-02070-4]

17 **Agatsuma N**, Utsumi T, Nishikawa Y, Horimatsu T, Seta T, Yamashita Y, Tanaka Y, Inoue T, Nakanishi Y, Shimizu T, Ohno M, Fukushima A, Nakayama T, Seno H. Stage at diagnosis of colorectal cancer through diagnostic route: Who should be screened? *World J Gastroenterol* 2024; **30**: 1368-1376 [PMID: 38596494 DOI: 10.3748/wjg.v30.i10.1368]

18 **Klabunde CN**, Zheng Y, Quinn VP, Beaber EF, Rutter CM, Halm EA, Chubak J, Doubeni CA, Haas JS, Kamineni A, Schapira MM, Vacek

PM, Garcia MP, Corley DA; PROSPR consortium. Influence of Age and Comorbidity on Colorectal Cancer Screening in the Elderly. *Am J Prev Med* 2016; **51**: e67-e75 [PMID: 27344108 DOI: 10.1016/j.amepre.2016.04.018]

19 **Gross CP**, Fried TR, Tinetti ME, Ross JS, Genao I, Hossain S, Wolf E, Lewis CL. Decision-making and cancer screening: a qualitative study of older adults with multiple chronic conditions. *J Geriatr Oncol* 2015; **6**: 93-100 [PMID: 25544380 DOI: 10.1016/j.jgo.2014.12.001]

20 **Coronado GD**, Nielson CM, Keast EM, Petrik AF, Suls JM. The influence of multi-morbidities on colorectal cancer screening recommendations and completion. *Cancer Causes Control* 2021; **32**: 555-565 [PMID: 33687606 DOI: 10.1007/s10552-021-01408-2]

21 **Navarro M**, Nicolas A, Ferrandez A, Lanas A. Colorectal cancer population screening programs worldwide in 2016: An update. *World J Gastroenterol* 2017; **23**: 3632-3642 [PMID: 28611516 DOI: 10.3748/wjg.v23.i20.3632]

22 **Davidson KW**, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, Donahue KE, Doubeni CA, Krist AH, Kubik M, Li L, Ogedegbe G, Owens DK, Pbert L, Silverstein M, Stevermer J, Tseng CW, Wong JB; US Preventive Services Task Force. Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. *JAMA* 2021; **325**: 1965-1977 [PMID: 34003218 DOI: 10.1001/jama.2021.6238]

23 **Niedermaier T**, Balavarca Y, Brenner H. Stage-Specific Sensitivity of Fecal Immunochemical Tests for Detecting Colorectal Cancer: Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2020; **115**: 56-69 [PMID: 31850933 DOI: 10.14309/ajg.0000000000000465]

24 **Meklin J**, SyrjÄnen K, Eskelinen M. Fecal Occult Blood Tests in Colorectal Cancer Screening: Systematic Review and Meta-analysis of Traditional and New-generation Fecal Immunochemical Tests. *Anticancer Res* 2020; **40**: 3591-3604 [PMID: 32620599 DOI: 10.21873/anticanres.14349]

25 **Grobbee EJ**, Wisse PHA, Schreuders EH, van Roon A, van Dam L, Zauber AG, Lansdorp-Vogelaar I, Bramer W, Berhane S, Deeks JJ, Steyerberg EW, van Leerdam ME, Spaander MC, Kuipers EJ. Guaiac-based faecal occult blood tests versus faecal immunochemical tests for colorectal cancer screening in average-risk individuals. *Cochrane Database Syst Rev* 2022; **6**: CD009276 [PMID: 35665911 DOI: 10.1002/14651858.CD009276.pub2]

26 **Lin JS**, Perdue LA, Henrikson NB, Bean SI, Blasi PR. Screening for Colorectal Cancer: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2021; **325**: 1978-1998 [PMID: 34003220 DOI: 10.1001/jama.2021.4417]

27 **Imperiale TF**, Porter K, Zella J, Gagrat ZD, Olson MC, Statz S, Garces J, Lavin PT, Aguilar H, Brinberg D, Berkelhammer C, Kisiel JB, Limburg PJ; BLUE-C Study Investigators. Next-Generation Multitarget Stool DNA Test for Colorectal Cancer Screening. *N Engl J Med* 2024; **390**: 984-993 [PMID: 38477986 DOI: 10.1056/NEJMoa2310336]

28 **Knudsen AB**, Zauber AG, Rutter CM, Naber SK, Doria-Rose VP, Pabiniak C, Johanson C, Fischer SE, Lansdorp-Vogelaar I, Kuntz KM. Estimation of Benefits, Burden, and Harms of Colorectal Cancer Screening Strategies: Modeling Study for the US Preventive Services Task Force. *JAMA* 2016; **315**: 2595-2609 [PMID: 27305518 DOI: 10.1001/jama.2016.6828]

29 **Health Quality Ontario**. Colon Capsule Endoscopy for the Detection of Colorectal Polyps: An Evidence-Based Analysis. *Ont Health Technol Assess Ser* 2015; **15**: 1-39 [PMID: 26366239]

30 **Cash BD**, Fleisher MR, Fern S, Rajan E, Haithcock R, Kastenberg DM, Pound D, Papageorgiou NP, Fernández-Urién I, Schmelkin IJ, Rex DK. Multicentre, prospective, randomised study comparing the diagnostic yield of colon capsule endoscopy versus CT colonography in a screening population (the TOPAZ study). *Gut* 2021; **70**: 2115-2122 [PMID: 33443017 DOI: 10.1136/gutjnl-2020-322578]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3367-3372

DOI: 10.3748/wjg.v30.i28.3367

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Tata-box-binding protein-associated factor 15 as a new potential marker in gastrointestinal tumors

Gulsum Ozlem Elpek

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 B
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Shelat VG

Received: May 25, 2024
Revised: June 19, 2024
Accepted: July 2, 2024
Published online: July 28, 2024
Processing time: 60 Days and 6.1 Hours

Gulsum Ozlem Elpek, Department of Pathology, 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

In this editorial, the roles of tata-box-binding protein-associated factor 15 (TAF15) in oncogenesis, tumor behavior, and as a therapeutic target in cancers in the context of gastrointestinal (GI) tumors are discussed concerning the publication by Guo *et al*. TAF15 is a member of the FET protein family with a comprehensive range of cellular processes. Besides, evidence has shown that TAF15 is involved in many diseases, including cancers. TAF15 contributes to carcinogenesis and tumor behavior in many tumors. Besides, its relationship with the mitogen-activated protein kinases (MAPK) signaling pathway makes TAF15 a new target for therapy. Although, the fact that there is few studies investigating the expression of *TAF15* constitutes a potential limitation in GI system, the association of *TAF15* expression with aggressive tumor behavior and, similar to other organ tumors, the influence of TAF15 on the MAPK signaling pathway emphasize that this protein could serve as a new molecular biomarker to predict tumor behavior and target therapeutic intervention in GI cancers. In conclusion, more studies should be performed to better understand the prognostic and therapeutic role of TAF15 in GI tumors, especially in tumors resistant to therapy.

Key Words: Gastrointestinal cancer; Tata-box-binding protein-associated factor 15; Cell proliferation; Cell migration; Prognosis

Core Tip: Recently, the role of tata-binding protein associated factor 15 (TAF15) in many diseases, including cancer, has been suggested. Current results support the hypothesis that *TAF15* expression is related to aggressive behavior by contributing to many pathways that are involved in tumor progression. Although its role in prognosis has not been entirely determined in gastrointestinal cancers, the fact that increased *TAF15* expression is associated with adverse clinicopathological parameters warrants further study with the aim of better understanding its role in predicting prognosis. Moreover, based on its association with the mitogen-activated protein kinases signal pathway, its significance as a therapeutic target, particularly in tumors resistant to treatment awaits investigation.

Citation: Elpek GO. Tata-box-binding protein-associated factor 15 as a new potential marker in gastrointestinal tumors. *World J Gastroenterol* 2024; 30(28): 3367-3372
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3367.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3367

INTRODUCTION

Tata-box-binding protein-associated factor 15 (TAF15) is a type of RNA-binding protein that belongs to FET protein family[1,2]. TAF15 is a crucial regulator of RNA metabolism and plays a significant role in the normal functions of RNA [3-5]. Prior research has shown that chromosomal translocation of *FET* genes, including TAF15 can result in the formation of fusion oncoproteins in several types of tumors, such as sarcomas and leukemias[6-8]. Moreover, the knockdown and inhibition of TAF15 has an impact on the expression of a substantial number of genes, a considerable proportion of which are implicated in the regulation of the cell cycle and programmed cell death[3,9-11]. Multiple studies have demonstrated that TAF15 plays a role in various types of human diseases including malignancies[12-14]. The involvement of TAF15 in the cellular interactions that promotes cell proliferation and migration has been demonstrated, suggesting a role in tumor progression[15-17]. Recent studies have demonstrated that TAF15 has the ability to activate the mitogen-activated protein kinases (MAPK) signaling pathway in malignant tumors[15-17]. Moreover, the contribution of TAF15 in the drug tolerance of cancer cells has been noted[18,19]. Despite the existence of this evidence regarding the relationship of TAF15 with oncogenesis and tumor behavior, the exact role of this protein in these events and whether it can be used as a therapeutic target has not been fully elucidated.

Therefore, in this article, the activity of TAF15, a protein with a multifaceted role, in cancer and its potential as a treatment target are discussed in the context of gastrointestinal (GI) tumors[20-32].

TAF15

This protein is encoded by the *TAF15* gene; this gene is located at chromosome 17q12 and was first reported in 2002 in a case of B-cell anaplastic large cell lymphoma[33]. As noted above, TAF15 is a member of the FET protein family (FUS-EWS), which contributes to cellular processes, including RNA splicing, transcription, mRNA transport, signaling, modification, translation, and preservation of genomic integrity[1,9,11]. Several evidence indicates that the suppression of the *TAF15* gene with siRNA had a significant effect on the expression of a large number of genes, which are predominantly linked to cell proliferation and death[3,4]. On the other hand, the diverse localization of TAF15 at the cell surface and cytoplasm and its primary localization in the cell nucleus suggests that it has a function beyond DNA and RNA binding[34,35]. The contribution of TAF15 to the cellular stress response, cell adhesion, and migration *via* its regulation and interaction with numerous proteins has been demonstrated[36]. Accumulated data pointed out the contribution of TAF15 in different forms of diseases, including malignant tumors.

TAF15 IN CANCERS

Recent experimental studies in lung carcinoma cell cultures have revealed a new role for TAF15 in carcinogenesis in squamous cell carcinomas and its association with long noncoding RNAs in this process[36]. In parallel to these findings, increased expression of *TAF15* is associated with a decrease in survival rates. The contribution of TAF15 in carcinogenesis by stabilizing the MAPK signaling pathway has been also observed, a finding of significant clinical relevance[16]. Additionally, in adenocarcinomas, TAF15 has been shown to participate in interleukin-6-activated epithelial-to-mesenchymal transition and invasion to facilitate metastasis[37]. The experimental inhibition of TAF15 by transcriptional intermediary factor-1γ has been demonstrated to prevent this phenomenon, further highlighting the urgent need to elucidate TAF15-related mechanisms for potential therapeutic interventions[37]. Moreover, blockade of TAF15 with an antibody is a feasible approach for enhancing the cytotoxicity of radiation in lung cancer, and this approach may lead to improved outcomes in non-small cell lung cancer patients with TAF15 overexpression[10].

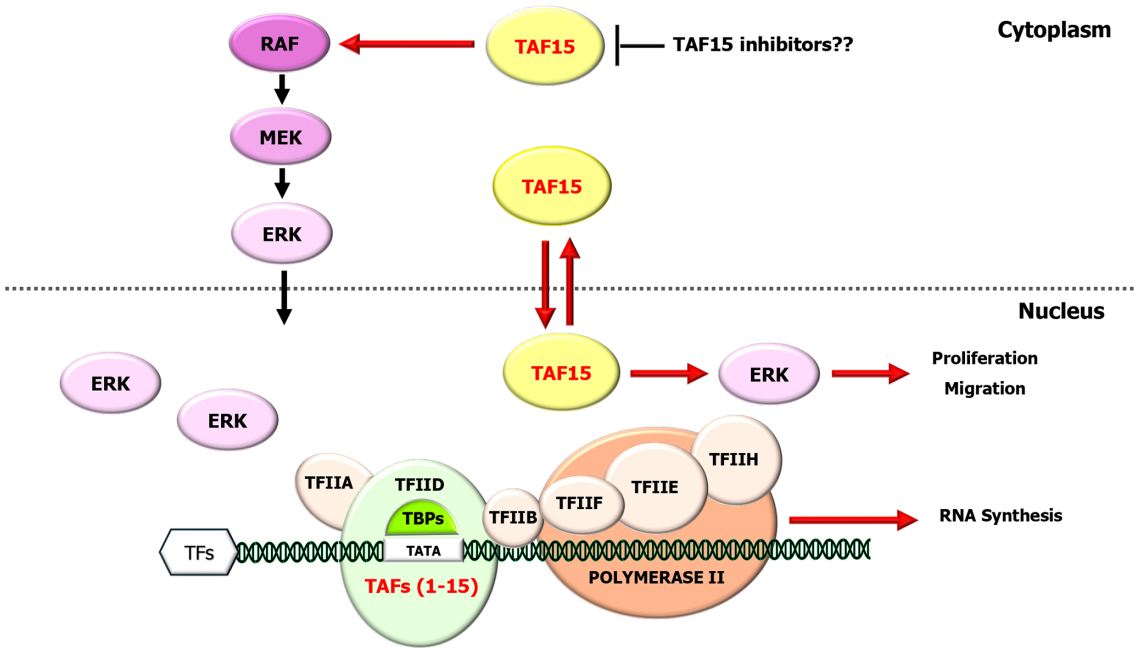


Figure 1 The transcription activation complex involving tata-box-binding protein-associated factor 15, and its relationship with the mitogen-activated protein kinase signaling pathway. For the recruitment of RNA polymerase II to protein coding gene promoters, a sequential addition of specific general transcription factor is required. Transcription factor (TF) IID recognizes a specific sequence, the tata-box, and binds to this motif by tata-box binding protein (TBP) assisted by TBP-associated factors. This process creates a sharp bend in the promoter DNA. TBP recruits TFIIA, then TFIIB, to this promoter. TFIIB recruits RNA polymerase II and TFIIF to the promoter. TFIIE joins to this complex and recruits TFIIH leading to unwind DNA at promoter and form the transcription bubble. The template strand of the transcription bubble engages with the RNA polymerase II active site and RNA synthesis begins. TBP-associated factor 15 shuttles between the nucleus and cytoplasm, and promotes the proliferation, migration and invasion of tumor cells *via* the activation of RAF1/MEK/ERK signaling pathway. MAPK: Mitogen-activated protein kinase; TF: Transcription factor; TBP: Tata-box binding protein; TAF15: Tata-box-binding protein-associated factor 15.

In breast cancer, recruitment of TAF15 by LINC00504 stabilizes CEPB2 mRNA, which is associated with radio resistance and contributes to its overexpression. In addition, LINC00504 silencing increased radiosensitivity by blocking TAF15[17].

In melanomas, the association of TAF15 with oncogenesis and the impact of its suppression on the inhibition of tumor cell proliferation have also been documented[35].

TAF15 IN GI CANCERS

Regarding GI system, in colorectal cancer cell cultures, the interaction of long noncoding RNA blood vessel epicardial substance (BVES) antisense RNA 1 with miR-522-3p and TAF15 has been demonstrated to regulate *BVES* expression, which might offer a perspective for colorectal cancer treatment, but further study is needed[38]. In an elegant study, Tang *et al*[15] revealed significant upregulation of TAF15 in gastric cancer (GC) tumor tissues and cell lines. The overexpression of *TAF15* was found to be correlated with increased tumor size, advanced pathological stage, and invasion. Importantly, the knockdown of TAF15 hindered the proliferation, migration, and invasion of tumor cells in cell culture and restrained tumor growth. Furthermore, this knockdown resulted in substantial decreases in the phosphorylation levels of RAF1, MEK, and ERK1/2, key components of the RAF1/MEK/ERK signaling, indicating the involvement of TAF15 in this pathway and suggesting that it could serve as a promising molecular diagnostic marker or therapeutic target for GC. A human antibody that recognizes a tumor-specific TAF15 antigen that inhibits tumor cell adhesion and spreading in stomach cancer, PAT-BA4, has been described[35].

In GI stromal tumors (GISTs), the influence of *TAF15* expression on oncogenesis and prognosis has been analyzed in a recent report[39]. In this multidisciplinary study, the authors first discovered the significant upregulation of TAF15 in tumor tissues and cell lines. This upregulation was reflected by the overexpression of *TAF15* in 31 patients with GIST and correlated with larger tumor size and high-risk stage, indicating its role in oncogenesis and tumor cell behavior. Although the number of patients was limited, these findings suggest that the TAF15 may be effective in determining the prognosis of GISTs and is worthy of further study. More interestingly, TAF15 knockdown, in addition to suppressing tumor cells and inhibiting tumor growth, also reduced the levels of phosphorylated RAF1, MEK and ERK1/2 in these cells. This observation suggested that TAF15 affects tumor cell behavior by regulating cell proliferation and migration *via* the RAF1/MEK/ERK signaling pathway.

These data support previous findings that TAF15 acts *via* the RAF1/MEK/ERK signaling in tumor progression across different tumor types and that TAF15 may be a potential therapeutic target in GI cancers, particularly in treatment-resistant tumors (Figure 1).

Furthermore, the discovery that α-AMA, an RNAPII inhibitor, is effective in reducing drug tolerance in cancer cells through TAF15 inhibition suggests that this protein may be a promising therapeutic target for preventing posttreatment relapses in solid tumors[40]. These findings indicate that a pharmacological strategy involving the use of novel chemicals that might also prevent aggressive behavior of tumor cells in patients with GI cancers by targeting TAF15.

Therefore, understanding the regulation of TAF15 is highly important considering the significant role of modifications in dysregulation of its expression, which affects malignant transformation and tumor progression in GI tumors. Moreover, new insight into its role as a distinct transcriptional regulator, together with its connection to signaling networks, especially the RAF1/MEK/ERK pathway, will facilitate the advancement of therapies for these tumors.

CONCLUSION

Beyond its involvement in gene fusions, TAF15 is a protein with a broad repertoire of influences on cancers, considering its roles in oncogenesis, tumor progression, and treatment. The expression of *TAF15* in GI malignancies and its relationship with aggressive tumor cell behavior warrant further studies in larger cohorts. The association of TAF15 with progression-related clinicopathological factors and its value as an independent prognostic factor and indicator of aggressive tumor behavior in patients with GI tumors require further study. More importantly, the close relationship between TAF15 and the MAPK signaling pathway in GI cancers suggests that TAF15 may be a potential therapeutic target, especially in treatment-resistant cases.

FOOTNOTES

Author contributions: Elpek GO performed the design of the article, obtained, analyzed and interpreted the data, and wrote the article.

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

Country of origin: Türkiye

ORCID number: Gulsum Ozlem Elpek 0000-0002-1237-5454.

S-Editor: Liu H
L-Editor: A
P-Editor: Zhao S

REFERENCES

1 **Kovar H**. Dr. Jekyll and Mr. Hyde: The Two Faces of the FUS/EWS/TAF15 Protein Family. *Sarcoma* 2011; **2011**: 837474 [PMID: 21197473 DOI: 10.1155/2011/837474]

2 **Law WJ**, Cann KL, Hicks GG. TLS, EWS and TAF15: a model for transcriptional integration of gene expression. *Brief Funct Genomic Proteomic* 2006; **5**: 8-14 [PMID: 16769671 DOI: 10.1093/bfgp/ell015]

3 **Ballarino M**, Jobert L, Dembélé D, de la Grange P, Auboeuf D, Tora L. TAF15 is important for cellular proliferation and regulates the expression of a subset of cell cycle genes through miRNAs. *Oncogene* 2013; **32**: 4646-4655 [PMID: 23128393 DOI: 10.1038/onc.2012.490]

4 **Ibrahim F**, Maragkakis M, Alexiou P, Maronski MA, Dichter MA, Mourelatos Z. Identification of in vivo, conserved, TAF15 RNA binding sites reveals the impact of TAF15 on the neuronal transcriptome. *Cell Rep* 2013; **3**: 301-308 [PMID: 23416048 DOI: 10.1016/j.celrep.2013.01.021]

5 **Luna-Arias JP**, Castro-Muñozledo F. Participation of the TBP-associated factors (TAFs) in cell differentiation. *J Cell Physiol* 2024; **239**: e31167 [PMID: 38126142 DOI: 10.1002/jcp.31167]

6 **Flucke U**, van Noesel MM, Siozopoulou V, Creytens D, Tops BBJ, van Gorp JM, Hiemcke-Jiwa LS. EWSR1-The Most Common Rearranged Gene in Soft Tissue Lesions, Which Also Occurs in Different Bone Lesions: An Updated Review. *Diagnostics (Basel)* 2021; **11** [PMID: 34203801 DOI: 10.3390/diagnostics11061093]

7 **Zhu L**, Bai W, Cheng Q, Fang J. ZNF384-Related Fusion Genes in Acute Lymphoblastic Leukemia. *Cancer Control* 2023; **30**: 10732748231182787 [PMID: 37306722 DOI: 10.1177/10732748231182787]

8 **Georgakopoulos N**, Diamantopoulos P, Micci F, Giannakopoulou N, Zervakis K, Dimitrakopoulou A, Viniou NA. An Adult Patient with Early Pre-B Acute Lymphoblastic Leukemia with t(12;17)(p13;q21)/ZNF384-TAF15. *In Vivo* 2018; **32**: 1241-1245 [PMID: 30150451 DOI: 10.21873/invivo.11371]

9 **Chen L**, Chen Q, Kuang S, Zhao C, Yang L, Zhang Y, Zhu H, Yang R. USF1-induced upregulation of LINC01048 promotes cell proliferation and apoptosis in cutaneous squamous cell carcinoma by binding to TAF15 to transcriptionally activate YAP1. *Cell Death Dis* 2019; **10**: 296 [PMID: 30931936 DOI: 10.1038/s41419-019-1516-2]

10 **Singh AK**, Kapoor V, Thotala D, Hallahan DE. TAF15 contributes to the radiation-inducible stress response in cancer. *Oncotarget* 2020; **11**: 2647-2659 [PMID: 32676166 DOI: 10.18632/oncotarget.27663]

11 **Wang D**, Li Z, Yin H. Long Non-Coding RNA CCAT2 Activates RAB14 and Acts as an Oncogene in Colorectal Cancer. *Front Oncol* 2021; **11**: 751903 [PMID: 34868956 DOI: 10.3389/fonc.2021.751903]

12 **Tetter S**, Arseni D, Murzin AG, Buhidma Y, Peak-Chew SY, Garringer HJ, Newell KL, Vidal R, Apostolova LG, Lashley T, Ghetti B, Ryskeldi-Falcon B. TAF15 amyloid filaments in frontotemporal lobar degeneration. *Nature* 2024; **625**: 345-351 [PMID: 38057661 DOI: 10.1038/s41586-023-06801-2]

13 **Ji J**, Wu S, Bao X, Liu S, Ye Y, Liu J, Guo J, Liu J, Wang X, Xia Z, Wei L, Zhang Y, Hao D, Huang D. Mediating oxidative stress through the Palbociclib/miR-141-3p/STAT4 axis in osteoporosis: a bioinformatics and experimental validation study. *Sci Rep* 2023; **13**: 19560 [PMID: 37949959 DOI: 10.1038/s41598-023-46813-6]

14 **Chen J**, Li X, Liu H, Zhong D, Yin K, Li Y, Zhu L, Xu C, Li M, Wang C. Bone marrow stromal cell-derived exosomal circular RNA improves diabetic foot ulcer wound healing by activating the nuclear factor erythroid 2-related factor 2 pathway and inhibiting ferroptosis. *Diabet Med* 2023; **40**: e15031 [PMID: 36537855 DOI: 10.1111/dme.15031]

15 **Tang L**, Guo C, Li X, Zhang B, Huang L. TAF15 promotes cell proliferation, migration and invasion of gastric cancer *via* activation of the RAF1/MEK/ERK signalling pathway. *Sci Rep* 2023; **13**: 5846 [PMID: 37037864 DOI: 10.1038/s41598-023-31959-0]

16 **Zhu H**, Liu Q, Yang X, Ding C, Wang Q, Xiong Y. LncRNA LINC00649 recruits TAF15 and enhances MAPK6 expression to promote the development of lung squamous cell carcinoma *via* activating MAPK signaling pathway. *Cancer Gene Ther* 2022; **29**: 1285-1295 [PMID: 35228660 DOI: 10.1038/s41417-021-00410-9]

17 **Feng J**, Li Y, Zhu L, Zhao Q, Li D, Li Y, Wu T. STAT1 mediated long non-coding RNA LINC00504 influences radio-sensitivity of breast cancer *via* binding to TAF15 and stabilizing CPEB2 expression. *Cancer Biol Ther* 2021; **22**: 630-639 [PMID: 34908514 DOI: 10.1080/15384047.2021.1964320]

18 **Ruan X**, Zheng J, Liu X, Liu Y, Liu L, Ma J, He Q, Yang C, Wang D, Cai H, Li Z, Liu J, Xue Y. lncRNA LINC00665 Stabilized by TAF15 Impeded the Malignant Biological Behaviors of Glioma Cells *via* STAU1-Mediated mRNA Degradation. *Mol Ther Nucleic Acids* 2020; **20**: 823-840 [PMID: 32464546 DOI: 10.1016/j.omtn.2020.05.003]

19 **Zheng WH**, Long ZQ, Zheng ZQ, Zhang LL, Liang YL, Li ZX, Lv JW, Kou J, Hong XH, He SW, Xu R, Zhou GQ, Liu N, Ma J, Sun Y, Lin L, Wei D. m6A-enriched lncRNA LINC00839 promotes tumor progression by enhancing TAF15-mediated transcription of amine oxidase AOC1 in nasopharyngeal carcinoma. *J Biol Chem* 2023; **299**: 104873 [PMID: 37257820 DOI: 10.1016/j.jbc.2023.104873]

20 **Butler JE**, Kadonaga JT. The RNA polymerase II core promoter: a key component in the regulation of gene expression. *Genes Dev* 2002; **16**: 2583-2592 [PMID: 12381658 DOI: 10.1101/gad.1026202]

21 **Thomas MC**, Chiang CM. The general transcription machinery and general cofactors. *Crit Rev Biochem Mol Biol* 2006; **41**: 105-178 [PMID: 16858867 DOI: 10.1080/10409230600648736]

22 **Akhtar W**, Veenstra GJ. TBP-related factors: a paradigm of diversity in transcription initiation. *Cell Biosci* 2011; **1**: 23 [PMID: 21711503 DOI: 10.1186/2045-3701-1-23]

23 **Feigerle JT**, Weil PA. The C Terminus of the RNA Polymerase II Transcription Factor IID (TFIID) Subunit Taf2 Mediates Stable Association of Subunit Taf14 into the Yeast TFIID Complex. *J Biol Chem* 2016; **291**: 22721-22740 [PMID: 27587401 DOI: 10.1074/jbc.M116.751107]

24 **Bernardini A**, Hollinger C, Willgenss D, Müller F, Devys D, Tora L. Transcription factor IID parks and drives preinitiation complexes at sharp or broad promoters. *Trends Biochem Sci* 2023; **48**: 839-848 [PMID: 37574371 DOI: 10.1016/j.tibs.2023.07.009]

25 **Chen X**, Xu Y. Structural insights into assembly of transcription preinitiation complex. *Curr Opin Struct Biol* 2022; **75**: 102404 [PMID: 35700575 DOI: 10.1016/j.sbi.2022.102404]

26 **Mishal R**, Luna-Arias JP. Role of the TATA-box binding protein (TBP) and associated family members in transcription regulation. *Gene* 2022; **833**: 146581 [PMID: 35597524 DOI: 10.1016/j.gene.2022.146581]

27 **Gura MA**, Mikedis MM, Seymour KA, de Rooij DG, Page DC, Freiman RN. Dynamic and regulated TAF gene expression during mouse embryonic germ cell development. *PLoS Genet* 2020; **16**: e1008515 [PMID: 31914128 DOI: 10.1371/journal.pgen.1008515]

28 **He XD**, Phillips S, Hioki K, Majhi PD, Babbitt C, Tremblay KD, Pobezinsky LA, Mager J. TATA-binding associated factors have distinct roles during early mammalian development. *Dev Biol* 2024; **511**: 53-62 [PMID: 38593904 DOI: 10.1016/j.ydbio.2024.04.002]

29 **Säisä-Borreill S**, Davidson G, Kleiber T, Thevenot A, Martin E, Mondot S, Blottière H, Helleux A, Mengus G, Plateroti M, Duluc I, Davidson I, Freund JN. General transcription factor TAF4 antagonizes epigenetic silencing by Polycomb to maintain intestine stem cell functions. *Cell Death Differ* 2023; **30**: 839-853 [PMID: 36639541 DOI: 10.1038/s41418-022-01109-6]

30 **Kalogeropoulou M**, Voulgari A, Kostourou V, Sandaltzopoulos R, Dikstein R, Davidson I, Tora L, Pintzas A. TAF4b and Jun/activating protein-1 collaborate to regulate the expression of integrin alpha6 and cancer cell migration properties. *Mol Cancer Res* 2010; **8**: 554-568 [PMID: 20353996 DOI: 10.1158/1541-7786.MCR-09-0159]

31 **Li Q**, Wu Q, Li Z, Hu Y, Zhou F, Zhai Z, Yue S, Tian H. LncRNA LINC00319 is associated with tumorigenesis and poor prognosis in glioma. *Eur J Pharmacol* 2019; **861**: 172556 [PMID: 31325436 DOI: 10.1016/j.ejphar.2019.172556]

32 **Xu Y**, Man N, Karl D, Martinez C, Liu F, Sun J, Martinez CJ, Martin GM, Beckedorff F, Lai F, Yue J, Roisman A, Greenblatt S, Duffort S, Wang L, Sun X, Figueroa M, Shiekhattar R, Nimer S. TAF1 plays a critical role in AML1-ETO driven leukemogenesis. *Nat Commun* 2019; **10**: 4925 [PMID: 31664040 DOI: 10.1038/s41467-019-12735-z]

33 **Martini A**, La Starza R, Janssen H, Bilhou-Nabera C, Corveleyn A, Somers R, Aventin A, Foà R, Hagemeijer A, Mecucci C, Marynen P. Recurrent rearrangement of the Ewing's sarcoma gene, EWSR1, or its homologue, TAF15, with the transcription factor CIZ/NMP4 in acute leukemia. *Cancer Res* 2002; **62**: 5408-5412 [PMID: 12359745]

34 **Andersson MK**, Ståhlberg A, Arvidsson Y, Olofsson A, Semb H, Stenman G, Nilsson O, Aman P. The multifunctional FUS, EWS and TAF15 proto-oncoproteins show cell type-specific expression patterns and involvement in cell spreading and stress response. *BMC Cell Biol* 2008; **9**: 37 [PMID: 18620564 DOI: 10.1186/1471-2121-9-37]

35 **Schatz N**, Brändlein S, Rückl K, Hensel F, Vollmers HP. Diagnostic and therapeutic potential of a human antibody cloned from a cancer patient that binds to a tumor-specific variant of transcription factor TAF15. *Cancer Res* 2010; **70**: 398-408 [PMID: 20048082 DOI: 10.1158/0008-5472.CAN-09-2186]

36 **Xiong Y**, Yang C, Yang X, Ding C, Wang Q, Zhu H. LncRNA MIR9-3HG enhances LIMK1 mRNA and protein levels to contribute to the carcinogenesis of lung squamous cell carcinoma *via* sponging miR-138-5p and recruiting TAF15. *Pathol Res Pract* 2022; **237**: 153941 [PMID: 35933883 DOI: 10.1016/j.prp.2022.153941]

37 **Su Z**, Sun Z, Wang Z, Wang S, Wang Y, Jin E, Li C, Zhao J, Liu Z, Zhou Z, Wang Y, Chen X, Liu X, Lei Z, Zhang HT. TIF1γ inhibits lung adenocarcinoma EMT and metastasis by interacting with the TAF15/TBP complex. *Cell Rep* 2022; **41**: 111513 [PMID: 36261009 DOI: 10.1016/j.celrep.2022.111513]

38 **Wen W**, Wang H, Xie S, Wu Z, Zhang C. BVES-AS1 inhibits the malignant behaviors of colon adenocarcinoma cells *via* regulating BVES. *Cell Biol Int* 2021; **45**: 1945-1956 [PMID: 34003551 DOI: 10.1002/cbin.11634]

39 **Guo CM**, Tang L, Li X, Huang LY. TATA-box-binding protein-associated factor 15 is a novel biomarker that promotes cell proliferation and migration in gastrointestinal stromal tumor. *World J Gastroenterol* 2023; **29**: 2932-2949 [PMID: 37274797 DOI: 10.3748/wjg.v29.i19.2932]

40 **Kume K**, Ikeda M, Miura S, Ito K, Sato KA, Ohmori Y, Endo F, Katagiri H, Ishida K, Ito C, Iwaya T, Nishizuka SS. α-Amanitin Restrains Cancer Relapse from Drug-Tolerant Cell Subpopulations *via* TAF15. *Sci Rep* 2016; **6**: 25895 [PMID: 27181033 DOI: 10.1038/srep25895]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3373-3385

DOI: 10.3748/wjg.v30.i28.3373

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Perianal disease in inflammatory bowel disease: Broadening treatment and surveillance strategies for anal cancer

Tatiana Pacheco, Sara Monteiro, Luísa Barros, Jorge Silva

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 A, Grade A, Grade C

Novelty: Grade A, Grade B, Grade B

Creativity or Innovation: Grade A, Grade A, Grade B

Scientific Significance: Grade A, Grade A, Grade A

P-Reviewer: Engida YE; Matsui T

Received: March 26, 2024

Revised: June 17, 2024

Accepted: July 8, 2024

Published online: July 28, 2024

Processing time: 119 Days and 8 Hours

Tatiana Pacheco, Sara Monteiro, Luísa Barros, Jorge Silva, Department of Gastroenterology, Centro Hospitalar do Tâmega e Sousa, Penafiel 4560-136, Portugal

Corresponding author: Tatiana Pacheco, MD, Doctor, Department of Gastroenterology, Centro Hospitalar do Tâmega e Sousa, Avenida do Hospital Padre Américo, 210, Penafiel 4560-136, Portugal. tatianasofiaapacheco@gmail.com

Abstract

The perianal disease affects up to one-third of individuals with Crohn's disease (CD), causing disabling symptoms and significant impairment in quality of life, particularly for those with perianal fistulising CD (PFCD). The collaborative effort between gastroenterologists and surgeons is essential for addressing PFCD to achieve fistula closure and promote luminal healing. Limited fistula healing rates with conventional therapies have prompted the emergence of new biological agents, endoscopic procedures and surgical techniques that show promising results. Among these, mesenchymal stem cells injection is a particularly hopeful therapy. In addition to the burden of fistulas, individuals with perianal CD may face an increased risk of developing anal cancer. This underscores the importance of surveillance programmes and timely interventions to prevent late diagnoses and poor outcomes. Currently, there is no established formal anal screening programme. In this review, we provide an overview of the current state of the art in managing PFCD, including novel medical, endoscopic and surgical approaches. The discussion also focuses on the relevance of establishing an anal cancer screening programme in CD, intending to propose a risk-based surveillance algorithm. The validation of this surveillance programme would be a significant step forward in improving patient care and outcomes.

Key Words: Inflammatory bowel disease; Crohn's disease; Perianal; Fistula; Anus diseases; Management; Anal cancer; Screening

Core Tip: Perianal fistulising Crohn's disease remains one of the most complex phenotypes of inflammatory bowel disease. Effective management involves a multidisciplinary approach. This review seeks to assess the existing evidence and emerging literature to provide clinicians with objective guidance for clinical practice concerning the optimal medical, endoscopic and surgical treatment of perianal fistulas. Future directions in management are also being reviewed. Additionally, the discussion underscores the significance of implementing an anal cancer screening programme, given the heightened risk faced by these patients. An algorithm for anal cancer screening is proposed with the ultimate goal of enhancing patient outcomes.

Citation: Pacheco T, Monteiro S, Barros L, Silva J. Perianal disease in inflammatory bowel disease: Broadening treatment and surveillance strategies for anal cancer. *World J Gastroenterol* 2024; 30(28): 3373-3385
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3373.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3373

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic and progressive inflammatory condition affecting the gastrointestinal tract, categorised into two major subtypes: Crohn's disease (CD) and ulcerative colitis (UC). These conditions are highly disabling and can present with a broad spectrum of clinical manifestations, encompassing both intestinal and extra-intestinal symptoms[1-3]. The perianal CD is one of the many phenotypes of IBD, presenting with skin tags, fissures and anorectal stenosis, as well as fistulas and abscesses, affecting up to one-third of patients[4-6]. Individuals with perianal fistulising CD (PFCD) experience a more challenging condition characterised by a substantial decrease in quality of life due to incapacitating symptoms and limited treatment effectiveness. With the development of novel therapeutics, fistula healing rates seem to be promising[7-9].

Rarely, PFCD can complicate malignancy, as patients might be at increased risk for anal canal-located and fistula-related cancer development[3,10-12]. This risk increases with disease duration[3,11,12]. The patient prognosis is generally poor due to a late diagnosis, and the lack of specificity of symptoms may explain why cancer diagnosis is often delayed [11,13]. For this reason, surveillance programmes aimed at early detection of neoplastic lesions are urgent.

In this review, we discuss the management of PFCD with a focus on emerging therapies. We also propose an anal cancer screening programme for individuals with IBD.

ANATOMY AND CLASSIFICATION

An adequate evaluation, characterisation and classification of perianal lesions are essential for determining the appropriate therapeutic approach.

One commonly used classification system for perianal fistulas is the Parks classification, which categorises fistulas into five anatomical categories based on their relation to the sphincter complex (superficial, intersphincteric, transsphincteric, suprasphincteric and extrasphincteric)[14]. Another major classification system is the American Gastroenterological Association classification, which divides perianal fistulas into two subtypes: Simple and complex. A simple fistula is characterised by a low location, a single external opening, and no association with a perianal abscess, rectovaginal fistula or anorectal stricture. A complex fistula has a high origin, may have multiple external openings, and is often associated with a perianal abscess, a rectovaginal fistula or anorectal stricture[15]. Complex fistulas are more commonly found in CD patients[8].

DIAGNOSIS

Diagnostic modalities for PFCD include examination under anaesthesia (EUA), fistulography, computed tomography [16], magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS).

Imaging with fistulography or pelvic computed tomography has limited accuracy. In contrast, pelvic MRI, rectal EUS and EUA are reasonably accurate methods for classifying PFCD. Combining either pelvic MRI or rectal EUS with surgical evaluation may represent the optimal approach for clinical practice. The choice of imaging modality should depend on local expertise[17].

MEDICAL THERAPY

The management of PFCD constitutes a difficult challenge in treating patients with IBD. In addition to unsatisfactory fistula healing rates, these patients have an increased risk of more severe luminal disease[18]. The treatment goal for fistulas is their closure, which requires addressing and preventing infection and abscesses, as well as promoting luminal

healing[19]. The most recent advances in perianal fistula treatment in CD have shown promising efficacy and underscored the importance of a multidisciplinary approach involving gastroenterologists and surgeons[7,20,21].

Our proposed algorithm for guiding management is presented in Figure 1.

Antibiotics

Antibiotics, especially ciprofloxacin and metronidazole, are commonly used as first-line treatments for PFCD. They are often used as adjuncts to surgery, immunomodulatory or biological treatment to manage perianal sepsis effectively[22].

Several observational studies with small sample sizes have demonstrated the efficacy of metronidazole (at doses of 10-20 mg/kg/day) and ciprofloxacin (at doses of 1000 mg/day) alone or in combination, achieving fistula closure in up to 50% of cases after six to eight weeks of treatment[23,24].

Adding antibiotics to thiopurines, infliximab (IFX) or adalimumab (ADA), has been shown to be significantly more effective than these agents in monotherapy[25,26]. However, side effects such as digestive intolerance with high doses of metronidazole or neuropathy with low but sustained doses, as well as disease recurrence upon discontinuation or dose reduction of antibiotics, limit their long-term use[24].

Thiopurines

Evidence regarding the use of azathioprine or 6-mercaptopurine (6-MP) in monotherapy is indirect, as none of the studies were specifically designed to evaluate the response of perianal disease to the medications. Therefore, the results should be interpreted with caution.

A meta-analysis published in 1995 analysed five studies on azathioprine or 6-MP in PFCD as a secondary outcome. It was found that thiopurines improved fistula symptoms and healing compared with a placebo (odds ratio 4.44, 95%CI: 1.5-13)[27].

A Cochrane review from 2016 reported no differences in fistula response between patients who received azathioprine and those who were given a placebo (3 studies, 18 patients; relative risk: 2.0; 95%CI: 0.67-5.93). The overall quality of the evidence was found to be low[28].

A randomised, double-blind, placebo-controlled trial of azathioprine or 6-MP therapy in patients with PFCD would be of significant interest. Future research should also assess the efficacy and safety of the use of thiopurines with biologics in PFCD. Currently, the most recent guidelines do not recommend the use of thiopurines as monotherapy but rather as an adjuvant to anti-tumour necrosis factor (anti-TNF) agents[19,22].

Tacrolimus

The efficacy of tacrolimus in the treatment of PFCD was evaluated in a multicentre trial where 48 patients were randomised to receive either placebo or tacrolimus orally at a dose of 0.2 mg/kg/day for 10 weeks[29]. Although there was a significantly higher improvement in fistulas (defined as the closure of more than 50% of active fistulous orifices) in the tacrolimus group (43% *vs* 8%), there were no differences in complete healing, defined as the closure of all fistulas for at least four weeks (10% *vs* 8%). Furthermore, significant side effects were observed. However, some studies have shown tacrolimus to be effective in inducing remission and serving as a bridge to treatment with azathioprine or 6-MP[30].

In 2011, a systematic review was published on the effect of tacrolimus on IBD[31]. It also analysed studies related to PFCD and concluded that when administered orally long-term (six months), tacrolimus achieves improvement and remission of perianal fistulas in 57% and 29% of cases, respectively. All studies reported side effects such as tremor, paraesthesia and nephrotoxicity, which usually decrease with a dose reduction or discontinuation of tacrolimus.

A subsequent systematic review and meta-analysis showed similar results, indicating only a modest effect on fistula response and remission rates for systemic administration of tacrolimus in patients with perianal CD, with a tolerable incidence of adverse events[32].

Regarding topical tacrolimus, a recently published systematic review demonstrated no differences in fistula outcomes when compared with placebo[33].

Anti-TNF agents

Anti-TNF drugs revolutionised the treatment of PFCD. Both IFX and ADA have shown their usefulness in the induction and maintenance of remission in PFCD; data on certolizumab pegol (CZP) are scarce and inconclusive. No studies have evaluated the resolution of perianal disease as a primary outcome with ADA or CZP.

In 1999, Present *et al*[34] published the first randomised trial, which included 94 patients with at least one active fistula. Patients were treated with IFX at doses of 5 or 10 mg/kg, compared to a control group treated with a placebo. The 5 mg/kg dose of IFX, administered *via* intravenous infusion and repeated at two and six weeks, was significantly superior to placebo. It resulted in both complete healing of all fistulas (55% *vs* 13%) and a decrease of 50% or more in the number of active fistulas (68% *vs* 26%).

Subsequently, the ACCENT II study demonstrated that IFX (5 mg/kg every eight weeks) was superior to placebo in preventing recurrence at 12 months, with complete fistulous closure rates of 36% and 19%, respectively ($P = 0.009$)[35].

Recent interest has emerged in evaluating and optimising the therapeutic dosing of anti-TNF agents to enhance outcomes. While optimal trough levels have been proposed for treating luminal disease, their adequacy for perianal disease remains incompletely assessed. Several studies have shown a clear exposure-response relationship, with higher serum therapeutic levels of IFX associated with improved outcomes in fistula closure[36-40]. Yarur *et al*[39] demonstrated that achieving IFX levels of ≥ 10.1 mcg/mL during maintenance therapy in PFCD may improve fistula healing rates. Moreover, in the *post hoc* analysis of the ACCENT-II study, it was also observed that higher post-induction IFX concentrations are associated with early fistula response and complete fistula response[40].

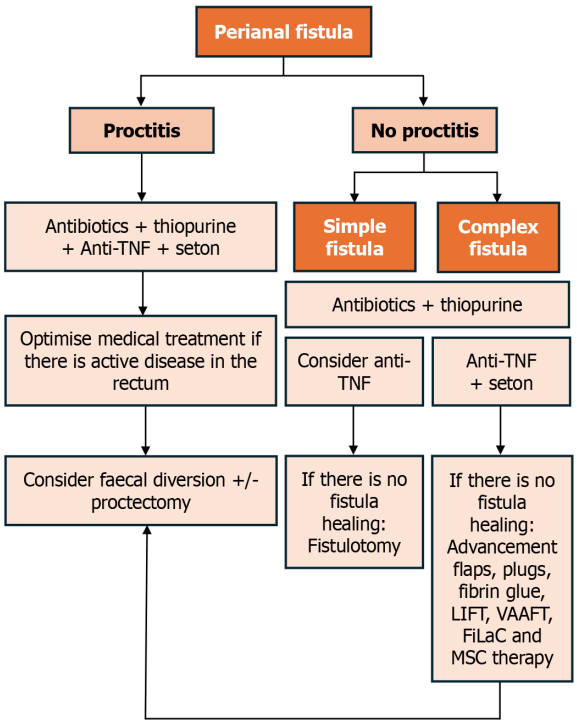


Figure 1 Suggested algorithm for management of perianal fistulising Crohn's disease. TNF: Tumour necrosis factor; LIFT: Ligation of the intersphincteric fistula tract; VAAFT: Video-assisted anal fistula treatment; FiLaC: Fistula laser closure; MSC: Mesenchymal stem cell.

Regarding ADA, evidence of its effectiveness in PFCD comes from several *post hoc* analyses. In the CHARM study, which included 113 patients with PFCD, it was concluded that 30% achieved fistula closure after 26 weeks of treatment, compared to 13% in the placebo group. This effect was maintained throughout the 56 weeks of the study, with 33% achieving fistula closure compared to 13%[41].

The CHOICE study demonstrated the effectiveness of ADA for PFCD following IFX failure, with fistula closure associated with an improvement in quality of life observed in 39% of patients[42].

ADA studies have also found an association between higher serum levels and improved fistula outcomes, with levels above 9.0 mg/mL consistently demonstrating increased rates of fistula closure[36,38,39,43].

The efficacy of CZP in achieving fistula closure has been analysed in the PRECISE 1 and 2 studies, with no significant differences observed compared to placebo in the induction of remission[44,45]. In a subgroup analysis of 58 patients from PRECISE 2 (55 of whom had PFCD) who responded to induction therapy with CZP and were randomised to placebo *vs* anti-TNF, fistula closure was observed at week 26 in 36% of those who received certolizumab *vs* 17% of those who received placebo (P = 0.038). However, this difference disappeared during the follow-up[46]. Based on the available evidence, the recommendation for the use of CZP in the treatment of PFCD is limited.

The local injection of anti-TNF agents into perianal fistulas has been described as an effective therapeutic approach. However, studies on this method have several limitations that should be taken into consideration, including small sample sizes, short follow-up periods, a lack of controls and variability in the technique of injections and outcome measures[7,8].

Vedolizumab

Exploratory analyses from the GEMINI 2 trial suggested that vedolizumab might be effective in PFCD. In this study, data from 57 patients with a draining fistula showed a non-significant trend towards improved fistula healing among patients randomised to vedolizumab compared with those receiving a placebo[47]. A small sample size leads to imprecise estimates of efficacy, which limits the extrapolation of results.

A large multicentre French study on vedolizumab in fistulising CD refractory to anti-TNF agents identified a low success rate of vedolizumab. Less than a quarter of the 102 patients with active perianal CD achieved fistula closure. Furthermore, one-third of patients with inactive perianal lesions at the initiation of vedolizumab treatment experienced perianal CD recurrence[48].

The ENTERPRISE study was the only randomised controlled trial (RCT) that compared two different induction schedules of vedolizumab (300 mg intravenously at weeks 0, 2, 6, 14 and 22 *vs* the same regimen plus an additional dose at week 10). Sustained improvements in fistulising CD were seen with both vedolizumab regimens (42.9% of patients achieved fistula closure at week 30), and an additional dose at week 10 does not appear to affect treatment outcomes[49]. However, the lack of a placebo control group and the small number of participants do not allow the study to provide a definitive answer on the impact of vedolizumab on fistula closure.

Ustekinumab

Limited evidence supports fistula healing with ustekinumab therapy.

No prospective study has specifically evaluated the efficacy of ustekinumab in PFCD.

The Spanish experience in a large multicentre study demonstrated clinical improvement in most of the patients with active PFCD. Specifically, 11 (61%) patients showed clinical improvement in their perianal fistula. However, these promising findings should be considered with caution because of the small number of patients assessed[50].

In the Dutch Initiative on Crohn and Colitis[51] registry, a nationwide prospective observational study, 36% of patients achieved clinical remission of perianal fistulas after 24 weeks of treatment[52].

Results for fistula healing from the pivotal trials of ustekinumab UNITI-1, UNITI-2 and CERTIFI were published as an abstract[53-55]. This *post hoc* analysis of 238 patients with PFCD revealed a non-significant trend towards improved fistula healing in patients randomised to ustekinumab compared with placebo[55].

Further confirmation through RCTs is required to determine the beneficial role of ustekinumab in PFCD.

Hyperbaric oxygen therapy

Hyperbaric oxygen therapy (HBOT) involves the inhalation of 100% oxygen inside a pressurised chamber. While it is a well-established treatment for certain conditions, its role in treating IBD remains somewhat controversial. In the context of PFCD, HBOT has the potential to enhance tissue oxygen levels, creating an inhospitable environment for anaerobic organisms. Additionally, it may diminish active inflammation by suppressing proinflammatory cytokines, enhancing the host antibacterial response, promoting the synthesis of growth factors and stimulating angiogenesis, ultimately aiding in the healing process of fistulas[51]. In a meta-analysis, complete fistula healing in PFCD was reported at 47.64% (22.05%-74.54%), while partial healing was observed at 34.29% (17.33%-56.50%). The majority of adverse events were minor, including intolerance, anxiety, difficulty normalising middle ear pressure, abdominal pain, vomiting, fatigue and visual changes. Severe adverse events primarily involved middle ear barotrauma[56].

HBOT has also been investigated as a potential adjunctive therapy for perianal disease. Feitosa *et al*[57] showed that adjunctive HBOT was associated with significant healing rates (80%) for PFCD.

In the HOT-TOPIC trial, Lansdorp *et al*[58] reported the long-term (week 60) follow-up of 20 patients with therapy-refractory perianal fistulas in CD who underwent 40 sessions of HBOT over a period of eight weeks. Four patients (20%) achieved a fibrotic fistula complex with no other signs of activity on MRI, indicating deep healing. Furthermore, 60% of patients had inactive perianal disease, as measured by the perianal disease activity index at week 60. The absence of a control group is a limitation of this study.

The overall scientific evidence suggests that HBOT is linked with beneficial effects in patients with IBD. However, RCTs are needed to make a definitive recommendation.

ENDOSCOPIC THERAPY

The role of novel endoscopic therapies in PFCD management is now being studied. This growing interest and need are likely related to the lower invasiveness of these procedures compared to surgical ones and the possibility of them being performed on an outpatient basis.

Endoscopic fistulotomy

In a case series involving 29 patients with fistulas and IBD who underwent endoscopic fistulotomy, the technique was found to be both safe and effective. Successful treatment was observed in 26 patients (89.6%)[59]. To date, no single therapy has shown such high success rates. The main limitation of endoscopic fistulotomy is that it cannot be used for long and complex fistulas[59-63].

Endoscopic seton placement

The use of endoscopic seton placement has been described in treating perianal fistula-associated abscesses, irrespective of the presence of underlying IBD. While it proves to be effective in managing simple, single-tract perianal fistulas, it is not feasible when dealing with complex or branched fistulas[62].

Endoscopic clipping with over-the-scope clips

Limited information exists in the literature regarding the use of over-the-scope clips in PFCD.

In a small retrospective study conducted at a single centre on refractory anal fistulas involving 10 patients, including six individuals with perianal CD, the procedure showed technical success in all patients. Permanent fistula closure was achieved in seven out of 10 patients (70%) within a median time of 72 days. Notably, among the six patients with CD, five experienced successful closure of their anal fistulas[64]. The long-term efficacy of it is unknown.

The epithelialisation of the fistula track, along with the presence of inflammation and fibrosis at and around the fistula opening, complicates the implementation of this technique in clinical practice[7,62].

SURGICAL THERAPY

Managing PFCD remains a complex challenge that frequently requires a multidisciplinary approach[65]. Before initiating surgical treatment, it is crucial to evaluate the presence of luminal inflammation, with special attention to proctitis. It is recommended to adopt a conservative approach in such cases because the presence of inflammation is associated with fistula persistence and a higher complication rate, including the need for proctectomy.

Conventional surgical therapies carry the risk of faecal incontinence or permanent stoma, in addition to disappointing fistula closure rates. Consequently, research has been dedicated to finding effective treatments with fewer side effects. Recently, the local injection of mesenchymal stem cells (MSCs) has emerged as the most promising minimally invasive treatment for fistulas, demonstrating higher efficacy in PFCD management[66,67].

Seton placement

There is a consensus that managing sepsis and preventing perianal infections is essential prior to initiating any treatment that impacts the immune system response. Therefore, loose setons should be positioned to prevent abscess formation[9,20,21,68]. It is crucial to avoid treatments that disturb the sphincter, such as a cutting seton[20,69]. The optimal timing of seton removal is uncertain. The removal of setons is necessary to allow for the complete healing of fistula tracks. However, in some cases, patients may necessitate long-term setons to prevent or delay proctectomy[9,21].

Fistulotomy

In cases of simple fistulas without proctitis, fistulotomy is the most commonly employed technique, yielding favourable outcomes. In patients with complex perianal fistulas, the use of fistulotomy may be avoided due to the risks of impairment of continence, recurrence and poor wound healing, making this treatment rarely appropriate in the context of CD[8,9,20].

Ligation of the intersphincteric fistula tract, advancement flaps and infill procedures

Surgical options for PFCD also include ligation of the intersphincteric fistula tract (LIFT), advancement flaps (AF), fistula plugs and fibrin glue injection. A surgical attempt at fistula closure is recommended only after achieving endoscopic remission of the proctitis[20].

For PFCD, no significant differences were observed between AF and LIFT for the overall success rate (61% *vs* 53%, respectively), but continence was better preserved after LIFT[70]. Systematic reviews have demonstrated the effectiveness of anal fistula plugs in approximately 50%-60% of fistulas related to CD[71,72]. This procedure is considered safe, with reasonable success rates, low morbidity and a minimal risk of incontinence. However, the evidence supporting its efficacy is not robust due to factors such as small cohort sizes, heterogeneity and a lack of standardisation. Fibrin glue is a conservative topical technique that preserves sphincter function. In a prospective RCT involving 36 patients with PFCD, fibrin glue therapy demonstrated effective treatment in 38% of cases after eight weeks, compared with 16% of patients who were observed without intervention after seton removal (P = 0.04)[73]. The benefit seemed to be greater for patients with simple fistulas. A meta-analysis revealed no significant difference between fibrin glue and conventional surgery (fistulotomy, seton and AF) concerning recurrence and faecal incontinence rates[74]. Glue and plugs can also be administered *via* endoscopic procedures; however, their efficacy and safety still need to be proven[62].

Prospective studies with a large number of CD patients are further awaited.

Ablative procedures

Newer sphincter-preserving therapies, such as fistula laser closure (FiLaC) and video-assisted anal fistula treatment (VAAFT), have been described for PFCD treatment[75-78]. Both techniques share the fundamental principle of destroying the epithelium of the fistula tract. FiLaC achieves this using laser energy, while VAAFT accomplishes it through cauterisation. Currently, these therapies are only available at a limited number of specialised centres. Despite presenting promising results, the role of these procedures in PFCD needs to be established through comparative studies with other techniques, and additional data are certainly required to make informed decisions.

MSCs

A notable increase in clinical trials has been focused on investigating the safety and effectiveness of MSCs in treating PFCD.

In the first RCT of adipose-derived MSCs (Cx601) for the treatment of 202 patients with complex treatment-refractory PFCD, the results revealed that 50% of patients treated with Cx601 (darvadstrocel) achieved complete remission 24 weeks after treatment, compared to 34% of the placebo group. The administration of stem cell treatment was well tolerated[79].

A subsequent randomised placebo-controlled trial aimed to assess the long-term efficacy and safety of a single local administration of allogeneic expanded adipose-derived stem cells in patients with CD and perianal fistulas. One-year outcome data demonstrated a significantly higher proportion of patients who received Cx601 (darvadstrocel) achieved combined remission (56.3%) compared to controls (38.6%), showing a difference of 17.7% (P = 0.010). Combined remission was defined as the closure of all treated external openings that were draining at baseline and the absence of collections > 2 cm on MRI.

Despite the heterogeneity in protocols using allogeneic or autologous MSCs derived from bone marrow or adipose tissue, different dosages of MSCs, variability in the number of times patients were treated, and use or non-use of scaffolding in delivery, additional studies have demonstrated higher efficacy and a lower incidence of adverse events with MSCs compared to control subjects for the treatment of PFCD[66,67].

The findings from the mentioned studies provide positive indications of MSCs for treating PFCD, demonstrating favourable outcomes in terms of both safety and effectiveness. However, the optimal dosage and the required number of MSC injections for achieving the highest healing rates remain undetermined. Consequently, there is an opportunity to refine treatment protocols in the field of cell-based therapy. The authors believe that this promising area holds the potential to revolutionise the management of PFCD in the foreseeable future.

Faecal diversion and proctectomy

Guidelines recommend that in cases of refractory complex perianal CD, diverting the faecal stream may be necessary to relieve clinical symptoms when medical and local surgical management strategies have failed. Patients should becounselled about the low rates of successful reversal and the likelihood of progression to proctectomy[21,68]. Proctectomy is effective but should be considered a last resort[80].

After faecal diversion, most patients experience initial clinical improvement; however, the probability of restoring bowel continuity is low (around 20%). Furthermore, nearly half of the patients ultimately require proctectomy with a permanent stoma for severe perianal disease[81,82]. Rectal involvement in CD was linked to a reduced likelihood of restoring bowel continuity, and biological therapy did not appear to enhance the outcomes of faecal diversion[81].

ANAL CANCER RISK IN IBD

General considerations and pathogenesis

IBD of the colon is associated with a higher risk of certain complications, including the onset of colorectal cancer (CRC) [83]. CRC significantly contributes to mortality rates among patients with CD[84]. Although the primary focus in IBD is usually on CRC, emerging literature suggests that individuals with IBD, especially those with anal or perianal CD, have an increased risk of anal cancer[10,83,85]. In an analysis of data collected in the French CESAME study from 19486 IBD patients, Beaugerie *et al*[85] found an incidence rate ratio of 9.36 [95%CI: 2.61-33.54] for anorectal cancer in perianal CD patients compared to CD patients without anal and/or perianal involvement. Patients who develop anal cancer are typically those with long-standing anorectal inflammation[11,85]. In fact, early disease onset, disease duration exceeding 10 years, chronic colitis with high inflammatory activity and chronic active fistulas and stenosis appear to be associated with malignant transformation[86]. These risk factors seem to validate the role of chronic inflammation in tumorigenesis, providing yet another reason why we should be increasingly ambitious with our therapeutic targets in perianal CD. In addition to systemic and local inflammatory processes, the pathophysiological mechanisms that lead to anal cancer in IBD are also related to human papilloma virus (HPV) infection, decreased local gut immunity and drug-induced immunosuppression[13,87].

Squamous-cell carcinoma (SCC) is the most frequent histological subtype of anal canal cancer, followed by adenocarcinoma[88]. SCC and adenocarcinoma can also develop from the fistula-lining epithelium in patients with perianal CD. However, in comparison to the overall incidence of anal cancers in CD, the occurrence of cancer in perineal fistulas seems to be relatively low[89].

Symptoms attributed to cancer are non-specific and often similar to those associated with anal and perianal diseases. Bleeding, pain and discharge may be noted[90]. In the series reported by Matsui *et al*[91], among 29 CD patients with rectal and anal cancer, 20 were diagnosed because of cancer-related symptoms (persistent pain in 15 patients, mucus discharge in four patients, and bleeding in four patients). All patients had advanced cancer despite the average duration from symptom onset to diagnosis being only 4.2 months. In case of any changes in anal symptoms, patients with chronic perianal CD should undergo appropriate studies to exclude the development of cancer. This may include a biopsy of any suspicious lesion and a biopsy under anaesthesia or curettage of fistula tracts when needed[83]. Given that the non-specificity of symptoms can lead to a delay in diagnosis and, consequently, a poor prognosis, preventive measures and surveillance programmes aimed at early detection of asymptomatic lesions in high-risk CD patients seem justified.

ANAL CANCER PREVENTION AND SCREENING

HPV vaccination

Anal SCC and high-grade squamous intraepithelial lesion (HSIL), its precursor lesion, are attributable to HPV infection in 80%-85% of cases[90]. Prophylactic vaccination against oncogenic HPV is recommended by the European Crohn's and Colitis Organisation for both young female and young male patients with IBD, similar to what is recommended in most local guidelines for the general population. This recommendation is based on its efficacy in preventing cervical cancer [92]. As an inactivated vaccine, it can be administered to immunocompromised IBD patients.

Screening

Routine screening for anal cancer in the general population is not justified due to its low prevalence. Due to the increased risk of SCC in certain populations, such as HIV-infected patients, men who have sex with men, solid organ transplant recipients or women with genital HPV-related cancer, screening programmes using anal cytology with HPV testing have been recommended for them by several societies, such as the Infectious Diseases Society of America[93]. However, there is no consensus on national and international guidelines regarding screening. Recommendations often vary and may be conflicting, with a low strength of evidence, and largely based on achievements obtained in cervical cytology screening.

Recently, the benefits of diagnosing and treating anal HSIL were demonstrated. In an RCT involving 4459 individuals living with HIV, aged 35 years or older and with a biopsy-proven anal HSIL, it was demonstrated that the risk of anal cancer was significantly lower with the treatment of HSIL than with active monitoring. This finding provides support for the use of screening with cytology and treatment for precursor lesions in this high-risk group[94]. This finding may also be relevant for other groups at increased risk for anal cancer. Perhaps an anal cancer screening programme could also be justified for CD patients with high-risk factors for malignancy, namely those with anal and/or perianal involvement.

The incidence rates for anorectal cancer in the Beaugerie study do not distinguish between the different manifestations of perianal CD, including patients with any anal and/or perianal lesion, and not only fistulising disease. Furthermore, the risk analysis does not specify whether these manifestations occurred in the present or the past. As a result, the algorithm proposed in this article, as illustrated in Figure 1, includes all phenotypes of anal and perianal CD, whether active or not. Modalities of surveillance could be similar to those used in other high-risk groups, which include anal cytology with HPV testing followed by high-resolution anoscopy and biopsy. The significance of randomised transanal biopsy under anaesthesia for cancer surveillance in CD has been previously investigated, and its effectiveness was demonstrated in a Japanese population, with a detection rate of neoplastic lesions reported at 5.8% (6 patients in 103 patients undergoing surveillance)[95]. However, further investigated is warranted.

There is also a lack of evidence and, consequently, recommendations regarding time intervals for screening. We suggest that the evaluation for anorectal cancer should be opportunistically incorporated into monitoring exams or as part of CRC screening programmes. Perhaps screening every 24 to 36 months could be justified, as the risk, although higher than in the general population and in the total IBD population, does not approach that of HIV-positive patients over 35 years of age[93,96]. The optimal frequency of surveillance algorithms should be validated in future studies. Cases with abnormal results should be referred to high-resolution anoscopy and treated accordingly. For patients experiencing pain during a perianal examination or those with anal strictures, alternative surveillance algorithms should be considered.

The diagnostic work-up for anal cancer when malignancy is clinically suspected is not included in our algorithm as it is beyond the scope of the article. However, a heightened suspicion for malignancy should be upheld, and persistent or new symptoms warrant thorough investigation.

The validation of our surveillance programme (Figure 2), which aims to detect asymptomatic lesions early in patients with chronic perianal CD, is urgent to prevent late diagnoses and poor outcomes.

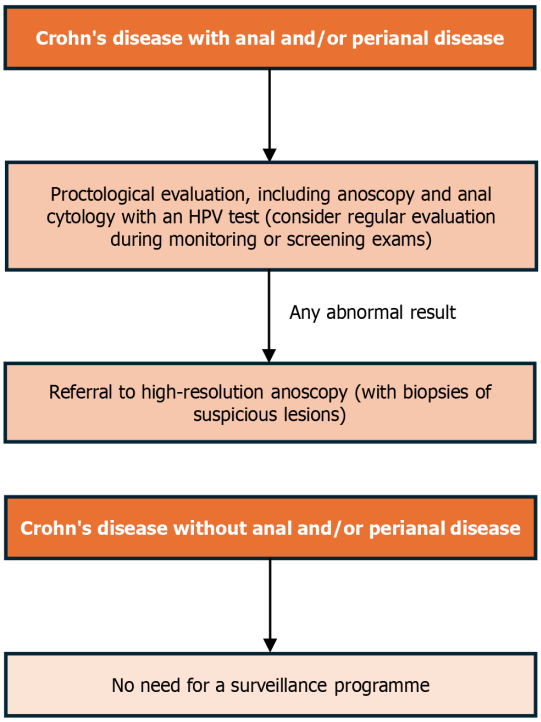


Figure 2 Proposed surveillance algorithm for anal cancer patients with Crohn's disease.

CONCLUSION

PFCD is one of the most feared phenotypes of IBD due to its complexity and the challenges associated with its treatment. A multidisciplinary approach involving gastroenterologists, colorectal surgeons and other healthcare providers is often necessary to optimise outcomes for affected patients. We still need to establish the best management strategies, and future prospective controlled studies can aid in determining the optimal treatment algorithm for IBD. Nevertheless, stem cell injection appears to be the most promising therapy. Patients with PFCD also face an increased risk of anal cancer. To

prevent adverse outcomes, an algorithm for anal cancer screening in IBD is proposed. The optimal management of PFCD should include not only the best medical and surgical therapies but also preventive measures to avoid negative outcomes.

FOOTNOTES

Author contributions: All authors made substantial contributions to the article. Pacheco T performed the literature research and drafted the manuscript; Monteiro S reviewed the manuscript and provided critical input on its intellectual content and structure; Barros L and Silva J critically revised the manuscript; All authors have approved the final version of the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Portugal

ORCID number: Tatiana Pacheco 0009-0006-3041-2729; Sara Monteiro 0000-0002-9966-1348.

S-Editor: Li L
L-Editor: A
P-Editor: Zhang L

REFERENCES

1 **Maaser C**, Sturm A, Vavricka SR, Kucharzik T, Fiorino G, Annese V, Calabrese E, Baumgart DC, Bettenworth D, Borralho Nunes P, Burisch J, Castiglione F, Eliakim R, Ellul P, González-Lama Y, Gordon H, Halligan S, Katsanos K, Kopylov U, Kotze PG, Krustinš E, Laghi A, Limdi JK, Rieder F, Rimola J, Taylor SA, Tolan D, van Rheenen P, Verstockt B, Stoker J; European Crohn's and Colitis Organisation [ECCO] and the European Society of Gastrointestinal and Abdominal Radiology [ESGAR]. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis* 2019; **13**: 144-164 [PMID: 30137275 DOI: 10.1093/ecco-jcc/jjy113]

2 **Roda G**, Chien Ng S, Kotze PG, Argollo M, Panaccione R, Spinelli A, Kaser A, Peyrin-Biroulet L, Danese S. Crohn's disease. *Nat Rev Dis Primers* 2020; **6**: 22 [PMID: 32242028 DOI: 10.1038/s41572-020-0156-2]

3 **Torres J**, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet* 2017; **389**: 1741-1755 [PMID: 27914655 DOI: 10.1016/S0140-6736(16)31711-1]

4 **Eglinton TW**, Barclay ML, Gearry RB, Frizelle FA. The spectrum of perianal Crohn's disease in a population-based cohort. *Dis Colon Rectum* 2012; **55**: 773-777 [PMID: 22706129 DOI: 10.1097/DCR.0b013e31825228b0]

5 **Park SH**, Aniwan S, Scott Harmsen W, Tremaine WJ, Lightner AL, Faubion WA, Loftus EV. Update on the Natural Course of Fistulizing Perianal Crohn's Disease in a Population-Based Cohort. *Inflamm Bowel Dis* 2019; **25**: 1054-1060 [PMID: 30346531 DOI: 10.1093/ibd/izy329]

6 **Wewer MD**, Zhao M, Nordholm-Carstensen A, Weimers P, Seidelin JB, Burisch J. The Incidence and Disease Course of Perianal Crohn's Disease: A Danish Nationwide Cohort Study, 1997-2015. *J Crohns Colitis* 2021; **15**: 5-13 [PMID: 32582937 DOI: 10.1093/ecco-jcc/jjaa118]

7 **Kotze PG**, Shen B, Lightner A, Yamamoto T, Spinelli A, Ghosh S, Panaccione R. Modern management of perianal fistulas in Crohn's disease: future directions. *Gut* 2018; **67**: 1181-1194 [PMID: 29331943 DOI: 10.1136/gutjnl-2017-314918]

8 **Wang X**, Shen B. Advances in Perianal Disease Associated with Crohn's Disease-Evolving Approaches. *Gastrointest Endosc Clin N Am* 2019; **29**: 515-530 [PMID: 31078250 DOI: 10.1016/j.giec.2019.02.011]

9 **Adegbola SO**, Sahnan K, Twum-Barima C, Iqbal N, Reza L, Lung P, Warusavitarne J, Tozer P, Hart A. Current review of the management of fistulising perianal Crohn's disease. *Frontline Gastroenterol* 2021; **12**: 515-523 [PMID: 34712470 DOI: 10.1136/flgastro-2020-101489]

10 **Johansen MP**, Wewer MD, Nordholm-Carstensen A, Burisch J. Perianal Crohn's Disease and the Development of Colorectal and Anal Cancer: A Systematic Review and Meta-analysis. *J Crohns Colitis* 2023; **17**: 361-368 [PMID: 36130090 DOI: 10.1093/ecco-jcc/jjac143]

11 **Kotsafti A**, Scarpa M, Angriman I, Castagliuolo I, Caruso A. Fistula-Related Cancer in Crohn's Disease: A Systematic Review. *Cancers (Basel)* 2021; **13** [PMID: 33809997 DOI: 10.3390/cancers13061445]

12 **Iesalnieks I**, Gaertner WB, Glass H, Strauch U, Hipp M, Agha A, Schlitt HJ. Fistula-associated anal adenocarcinoma in Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 1643-1648 [PMID: 20186945 DOI: 10.1002/ibd.21228]

13 **Wisniewski A**, Fléjou JF, Siproudhis L, Abramowitz L, Svrcek M, Beaugerie L. Anal Neoplasia in Inflammatory Bowel Disease: Classification Proposal, Epidemiology, Carcinogenesis, and Risk Management Perspectives. *J Crohns Colitis* 2017; **11**: 1011-1018 [PMID: 28379306 DOI: 10.1093/ecco-jcc/jjx035]

14 **Parks AG**, Gordon PH, Hardcastle JD. A classification of fistula-in-ano. *Br J Surg* 1976; **63**: 1-12 [PMID: 1267867 DOI: 10.1002/bjs.1800630102]

15 **Sandborn WJ**, Fazio VW, Feagan BG, Hanauer SB; American Gastroenterological Association Clinical Practice Committee. AGA technical review on perianal Crohn's disease. *Gastroenterology* 2003; **125**: 1508-1530 [PMID: 14598268 DOI: 10.1016/j.gastro.2003.08.025]

16 **Singh S**, Proctor D, Scott FI, Falck-Ytter Y, Feuerstein JD. AGA Technical Review on the Medical Management of Moderate to Severe Luminal and Perianal Fistulizing Crohn's Disease. *Gastroenterology* 2021; **160**: 2512-2556.e9 [PMID: 34051985 DOI: 10.1053/j.gastro.2021.04.023]

17 **Schwartz DA**, Wiersema MJ, Dudiak KM, Fletcher JG, Clain JE, Tremaine WJ, Zinsmeister AR, Norton ID; Boardman LA, Devine RM,

Wolff BG, Young-Fadok TM, Diehl NN, Pemberton JH, Sandborn WJ. A comparison of endoscopic ultrasound, magnetic resonance imaging, and exam under anesthesia for evaluation of Crohn's perianal fistulas. *Gastroenterology* 2001; **121**: 1064-1072 [PMID: 11677197 DOI: 10.1053/gast.2001.28676]

18 **Eglinton TW**, Gearry RB. Clinical factors predicting disease course in Crohn's disease. *Expert Rev Clin Immunol* 2010; **6**: 41-45 [PMID: 20383889 DOI: 10.1586/eci.09.76]

19 **Parian AM**, Obi M, Fleshner P, Schwartz DA. Management of Perianal Crohn's Disease. *Am J Gastroenterol* 2023; **118**: 1323-1331 [PMID: 37207318 DOI: 10.14309/ajg.0000000000002326]

20 **Gecse KB**, Bemelman W, Kamm MA, Stoker J, Khanna R, Ng SC, Panés J, van Assche G, Liu Z, Hart A, Levesque BG, D'Haens G; World Gastroenterology Organization, International Organisation for Inflammatory Bowel Diseases IOIBD, European Society of Coloproctology and Robarts Clinical Trials; World Gastroenterology Organization International Organisation for Inflammatory Bowel Diseases IOIBD European Society of Coloproctology and Robarts Clinical Trials. A global consensus on the classification, diagnosis and multidisciplinary treatment of perianal fistulising Crohn's disease. *Gut* 2014; **63**: 1381-1392 [PMID: 24951257 DOI: 10.1136/gutjnl-2013-306709]

21 **Lamb CA**, Kennedy NA, Raine T, Hendy PA, Smith PJ, Limdi JK, Hayee B, Lomer MCE, Parkes GC, Selinger C, Barrett KJ, Davies RJ, Bennett C, Gittens S, Dunlop MG, Faiz O, Fraser A, Garrick V, Johnston PD, Parkes M, Sanderson J, Terry H; IBD guidelines eDelphi consensus group, Gaya DR, Iqbal TH, Taylor SA, Smith M, Brookes M, Hansen R, Hawthorne AB. British Society of Gastroenterology consensus guidelines on the management of inflammatory bowel disease in adults. *Gut* 2019; **68**: s1-s106 [PMID: 31562236 DOI: 10.1136/gutjnl-2019-318484]

22 **Torres J**, Bonovas S, Doherty G, Kucharzik T, Gisbert JP, Raine T, Adamina M, Armuzzi A, Bachmann O, Bager P, Biancone L, Bokemeyer B, Bossuyt P, Burisch J, Collins P, El-Hussuna A, Ellul P, Frei-Lanter C, Furfaro F, Gingert C, Gionchetti P, Gomollon F, González-Lorenzo M, Gordon H, Hlavaty T, Juillerat P, Katsanos K, Kopylov U, Krustins E, Lytras T, Maaser C, Magro F, Marshall JK, Myrelid P, Pellino G, Rosa I, Sabino J, Savarino E, Spinelli A, Stassen L, Uzzan M, Vavricka S, Verstockt B, Warusavitarne J, Zmora O, Fiorino G. ECCO Guidelines on Therapeutics in Crohn's Disease: Medical Treatment. *J Crohns Colitis* 2020; **14**: 4-22 [PMID: 31711158 DOI: 10.1093/ecco-jcc/jjz180]

23 **Bernstein LH**, Frank MS, Brandt LJ, Boley SJ. Healing of perineal Crohn's disease with metronidazole. *Gastroenterology* 1980; **79**: 357-365 [PMID: 7399243 DOI: 10.1016/0016-5085(80)90155-9]

24 **Jakobovits J**, Schuster MM. Metronidazole therapy for Crohn's disease and associated fistulae. *Am J Gastroenterol* 1984; **79**: 533-540 [PMID: 6741906]

25 **Dewint P**, Hansen BE, Verhey E, Oldenburg B, Hommes DW, Pierik M, Ponsioen CI, van Dullemen HM, Russel M, van Bodegraven AA, van der Woude CJ. Adalimumab combined with ciprofloxacin is superior to adalimumab monotherapy in perianal fistula closure in Crohn's disease: a randomised, double-blind, placebo controlled trial (ADAFI). *Gut* 2014; **63**: 292-299 [PMID: 23525574 DOI: 10.1136/gutjnl-2013-304488]

26 **West RL**, van der Woude CJ, Hansen BE, Felt-Bersma RJ, van Tilburg AJ, Drapers JA, Kuipers EJ. Clinical and endosonographic effect of ciprofloxacin on the treatment of perianal fistulae in Crohn's disease with infliximab: a double-blind placebo-controlled study. *Aliment Pharmacol Ther* 2004; **20**: 1329-1336 [PMID: 15606395 DOI: 10.1111/j.1365-2036.2004.02247.x]

27 **Pearson DC**, May GR, Fick GH, Sutherland LR. Azathioprine and 6-mercaptopurine in Crohn disease. A meta-analysis. *Ann Intern Med* 1995; **123**: 132-142 [PMID: 7778826 DOI: 10.7326/0003-4819-123-2-199507150-00009]

28 **Chande N**, Townsend CM, Parker CE, MacDonald JK. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. *Cochrane Database Syst Rev* 2016; **10**: CD000545 [PMID: 27783843 DOI: 10.1002/14651858.CD000545.pub5]

29 **Sandborn WJ**, Present DH, Isaacs KL, Wolf DC, Greenberg E, Hanauer SB, Feagan BG, Mayer L, Johnson T, Galanko J, Martin C, Sandler RS. Tacrolimus for the treatment of fistulas in patients with Crohn's disease: a randomized, placebo-controlled trial. *Gastroenterology* 2003; **125**: 380-388 [PMID: 12891539 DOI: 10.1016/s0016-5085(03)00877-1]

30 **Lowry PW**, Weaver AL, Tremaine WJ, Sandborn WJ. Combination therapy with oral tacrolimus (FK506) and azathioprine or 6-mercaptopurine for treatment-refractory Crohn's disease perianal fistulae. *Inflamm Bowel Dis* 1999; **5**: 239-245 [PMID: 10579116 DOI: 10.1097/00054725-199911000-00001]

31 **McSharry K**, Dalzell AM, Leiper K, El-Matary W. Systematic review: the role of tacrolimus in the management of Crohn's disease. *Aliment Pharmacol Ther* 2011; **34**: 1282-1294 [PMID: 21999607 DOI: 10.1111/j.1365-2036.2011.04873.x]

32 **Iida T**, Nojima M, Nakase H. Therapeutic Efficacy and Adverse Events of Tacrolimus in Patients with Crohn's Disease: Systematic Review and Meta-Analysis. *Dig Dis Sci* 2019; **64**: 2945-2954 [PMID: 30982208 DOI: 10.1007/s10620-019-05619-1]

33 **Salem G**, Ding K, Sakuraba A, Cohen R. Role of topical tacrolimus in the management of proctitis, perianal manifestations in Crohn's disease, and chronic pouchitis: a systematic review. *J Investig Med* 2021 [PMID: 33622709 DOI: 10.1136/jim-2020-001699]

34 **Present DH**, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, Podolsky DK, Sands BE, Braakman T, DeWoody KL, Schaible TF, van Deventer SJ. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999; **340**: 1398-1405 [PMID: 10228190 DOI: 10.1056/NEJM199905063401804]

35 **Sands BE**, Anderson FH, Bernstein CN, Chey WY, Feagan BG, Fedorak RN, Kamm MA, Korzenik JR, Lashner BA, Onken JE, Rachmilewitz D, Rutgeerts P, Wild G, Wolf DC, Marsters PA, Travers SB, Blank MA, van Deventer SJ. Infliximab maintenance therapy for fistulizing Crohn's disease. *N Engl J Med* 2004; **350**: 876-885 [PMID: 14985485 DOI: 10.1056/NEJMoa030815]

36 **Gu B**, Venkatesh K, Williams AJ, Ng W, Corte C, Gholamrezaei A, Ghaly S, Xuan W, Paramsothy S, Connor S. Higher infliximab and adalimumab trough levels are associated with fistula healing in patients with fistulising perianal Crohn's disease. *World J Gastroenterol* 2022; **28**: 2597-2608 [PMID: 35949350 DOI: 10.3748/wjg.v28.i23.2597]

37 **Plevris N**, Jenkinson PW, Arnott ID, Jones GR, Lees CW. Higher anti-tumor necrosis factor levels are associated with perianal fistula healing and fistula closure in Crohn's disease. *Eur J Gastroenterol Hepatol* 2020; **32**: 32-37 [PMID: 31567638 DOI: 10.1097/MEG.0000000000001561]

38 **Strik AS**, Löwenberg M, Buskens CJ, B Gecse K, I Ponsioen C, Bemelman WA, D'Haens GR. Higher anti-TNF serum levels are associated with perianal fistula closure in Crohn's disease patients. *Scand J Gastroenterol* 2019; **54**: 453-458 [PMID: 31032686 DOI: 10.1080/00365521.2019.1600014]

39 **Yarur AJ**, Kanagala V, Stein DJ, Czul F, Quintero MA, Agrawal D, Patel A, Best K, Fox C, Idstein K, Abreu MT. Higher infliximab trough levels are associated with perianal fistula healing in patients with Crohn's disease. *Aliment Pharmacol Ther* 2017; **45**: 933-940 [PMID: 28211593 DOI: 10.1111/apt.13970]

40 **Papamichael K**, Vande Casteele N, Jeyarajah J, Jairath V, Osterman MT, Cheifetz AS. Higher Postinduction Infliximab Concentrations Are Associated With Improved Clinical Outcomes in Fistulizing Crohn's Disease: An ACCENT-II Post Hoc Analysis. *Am J Gastroenterol* 2021;

116: 1007-1014 [PMID: 33929379 DOI: 10.14309/ajg.0000000000001111]

41 **Colombel JF**, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, Schreiber S, Byczkowski D, Li J, Kent JD, Pollack PF. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52-65 [PMID: 17241859 DOI: 10.1053/j.gastro.2006.11.041]

42 **Lichtiger S**, Binion DG, Wolf DC, Present DH, Bensimon AG, Wu E, Yu AP, Cardoso AT, Chao J, Mulani PM, Lomax KG, Kent JD. The CHOICE trial: adalimumab demonstrates safety, fistula healing, improved quality of life and increased work productivity in patients with Crohn's disease who failed prior infliximab therapy. *Aliment Pharmacol Ther* 2010; **32**: 1228-1239 [PMID: 20955442 DOI: 10.1111/j.1365-2036.2010.04466.x]

43 **Sirmai L**, Pelletier AL, Gault N, Zallot C, Bouguen G, Bouchard D, Roland Nicaise P, Peyneau M, Sironneau S, Bittencourt MC, Petitcollin A, Fernandez P, Roblin X, Siproudhis L, Abramowitz L. Relationship between clinical remission of perianal fistulas in Crohn's disease and serum adalimumab concentrations: A multi-center cross-sectional study. *World J Gastroenterol* 2022; **28**: 961-972 [PMID: 35317057 DOI: 10.3748/wjg.v28.i9.961]

44 **Sandborn WJ**, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, Bloomfield R, Schreiber S; PRECISE 1 Study Investigators. Certolizumab pegol for the treatment of Crohn's disease. *N Engl J Med* 2007; **357**: 228-238 [PMID: 17634458 DOI: 10.1056/NEJMoa067594]

45 **Schreiber S**, Khaliq-Kareemi M, Lawrance IC, Thomsen OØ, Hanauer SB, McColm J, Bloomfield R, Sandborn WJ; PRECISE 2 Study Investigators. Maintenance therapy with certolizumab pegol for Crohn's disease. *N Engl J Med* 2007; **357**: 239-250 [PMID: 17634459 DOI: 10.1056/NEJMoa062897]

46 **Schreiber S**, Lawrance IC, Thomsen OØ, Hanauer SB, Bloomfield R, Sandborn WJ. Randomised clinical trial: certolizumab pegol for fistulas in Crohn's disease - subgroup results from a placebo-controlled study. *Aliment Pharmacol Ther* 2011; **33**: 185-193 [PMID: 21083671 DOI: 10.1111/j.1365-2036.2010.04509.x]

47 **Feagan BG**, Schwartz D, Danese S, Rubin DT, Lissoos TW, Xu J, Lasch K. Efficacy of Vedolizumab in Fistulising Crohn's Disease: Exploratory Analyses of Data from GEMINI 2. *J Crohns Colitis* 2018; **12**: 621-626 [PMID: 29471381 DOI: 10.1093/ecco-jcc/jjy019]

48 **Chapuis-Biron C**, Bourrier A, Nachury M, Nancey S, Bouhnik Y, Serrero M, Armengol-Debeir L, Buisson A, Tran-Minh ML, Zallot C, Fumery M, Bouguen G, Abitbol V, Viennot S, Chanteloup E, Rajca S, Dib N, Parmentier AL, Peyrin-Biroulet L, Vuitton L; GETAID BioLAP Study Group. Vedolizumab for perianal Crohn's disease: a multicentre cohort study in 151 patients. *Aliment Pharmacol Ther* 2020; **51**: 719-727 [PMID: 32080886 DOI: 10.1111/apt.15665]

49 **Schwartz DA**, Peyrin-Biroulet L, Lasch K, Adsul S, Danese S. Efficacy and Safety of 2 Vedolizumab Intravenous Regimens for Perianal Fistulizing Crohn's Disease: ENTERPRISE Study. *Clin Gastroenterol Hepatol* 2022; **20**: 1059-1067.e9 [PMID: 34597729 DOI: 10.1016/j.cgh.2021.09.028]

50 **Khorrami S**, Ginard D, Marín-Jiménez I, Chaparro M, Sierra M, Aguas M, Sicilia B, García-Sánchez V, Suarez C, Villoria A, Taxonera C, Velasco-Guardado A, Martínez-González J, Gisbert JP. Ustekinumab for the Treatment of Refractory Crohn's Disease: The Spanish Experience in a Large Multicentre Open-label Cohort. *Inflamm Bowel Dis* 2016; **22**: 1662-1669 [PMID: 27306072 DOI: 10.1097/MIB.0000000000000842]

51 **Kirby JP**, Snyder J, Schuerer DJE, Peters JS, Bochicchio GV. Essentials of Hyperbaric Oxygen Therapy: 2019 Review. *Mo Med* 2019; **116**: 176-179 [PMID: 31527935]

52 **Biemans VBC**, van der Meulen-de Jong AE, van der Woude CJ, Löwenberg M, Dijkstra G, Oldenburg B, de Boer NKH, van der Marel S, Bodelier AGL, Jansen JM, Haans JJL, Theeuwen R, de Jong D, Pierik MJ, Hoentjen F. Ustekinumab for Crohn's Disease: Results of the ICC Registry, a Nationwide Prospective Observational Cohort Study. *J Crohns Colitis* 2020; **14**: 33-45 [PMID: 31219157 DOI: 10.1093/ecco-jcc/jjz119]

53 **Sandborn WJ**, Gasink C, Gao LL, Blank MA, Johanns J, Guzzo C, Sands BE, Hanauer SB, Targan S, Rutgeerts P, Ghosh S, de Villiers WJ, Panaccione R, Greenberg G, Schreiber S, Lichtiger S, Feagan BG; CERTIFI Study Group. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med* 2012; **367**: 1519-1528 [PMID: 23075178 DOI: 10.1056/NEJMoa1203572]

54 **Feagan BG**, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR, Blank MA, Johanns J, Gao LL, Miao Y, Adedokun OJ, Sands BE, Hanauer SB, Vermeire S, Targan S, Ghosh S, de Villiers WJ, Colombel JF, Tulassay Z, Seidler U, Salzberg BA, Desreumaux P, Lee SD, Loftus EV Jr, Dieleman LA, Katz S, Rutgeerts P; UNITI-IM-UNITI Study Group. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. *N Engl J Med* 2016; **375**: 1946-1960 [PMID: 27959607 DOI: 10.1056/NEJMoa1602773]

55 **Sands BE**, Gasink C, Jacobstein D, Gao L, Johanns J, Colombel JF, de Villiers WJ, Sandborn WJ. Fistula Healing in Pivotal Studies of Ustekinumab in Crohn's Disease. *Gastroenterology* 2017; **152**: S185 [DOI: 10.1016/s0016-5085(17)30930-7]

56 **Singh AK**, Jha DK, Jena A, Kumar-M P, Sebastian S, Sharma V. Hyperbaric oxygen therapy in inflammatory bowel disease: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2021; **33**: e564-e573 [PMID: 33905214 DOI: 10.1097/MEG.0000000000002164]

57 **Feitosa MR**, Parra RS, Machado VF, Vilar GN, Aquino JC, Rocha JJR, Kotze PG, Féres O. Adjunctive Hyperbaric Oxygen Therapy in Refractory Crohn's Disease: An Observational Study. *Gastroenterol Res Pract* 2021; **2021**: 6628142 [PMID: 33995526 DOI: 10.1155/2021/6628142]

58 **Lansdorp CA**, Buskens CJ, Gecse KB, Löwenberg M, Stoker J, Bemelman WA, D'Haens GRAM, van Hulst RA. Hyperbaric oxygen therapy for the treatment of perianal fistulas in 20 patients with Crohn's disease: Results of the HOT-TOPIC trial after 1-year follow-up. *United European Gastroenterol J* 2022; **10**: 160-168 [PMID: 35146959 DOI: 10.1002/ueg2.12189]

59 **Kochhar G**, Shen B. Endoscopic fistulotomy in inflammatory bowel disease (with video). *Gastrointest Endosc* 2018; **88**: 87-94 [PMID: 29501418 DOI: 10.1016/j.gie.2018.02.034]

60 **Pal P**, Kanaganti S, Banerjee R, Ramchandani M, Nabi Z, Reddy DN, Tandan M. Systematic Review of Endoscopic Management of Stricture, Fistula and Abscess in Inflammatory Bowel Disease. *Gastroenterol Insights* 2023; **14**: 45-63 [DOI: 10.3390/gastroent14010006]

61 **Gold SL**, Cohen-Mekelburg S, Schneider Y, Steinlauf A. Perianal Fistulas in Patients With Crohn's Disease, Part 2: Surgical, Endoscopic, and Future Therapies. *Gastroenterol Hepatol (NY)* 2018; **14**: 521-528 [PMID: 30364296]

62 **Shen B**. Exploring endoscopic therapy for the treatment of Crohn's disease-related fistula and abscess. *Gastrointest Endosc* 2017; **85**: 1133-1143 [PMID: 28153572 DOI: 10.1016/j.gie.2017.01.025]

63 **Shen B**, Kochhar G, Navaneethan U, Liu X, Farraye FA, Gonzalez-Lama Y, Bruining D, Pardi DS, Lukas M, Bortlik M, Wu K, Sood A, Schwartz DA, Sandborn WJ; Global Interventional Inflammatory Bowel Disease Group. Role of interventional inflammatory bowel disease in the era of biologic therapy: a position statement from the Global Interventional IBD Group. *Gastrointest Endosc* 2019; **89**: 215-237 [PMID: 30365985 DOI: 10.1016/j.gie.2018.09.045]

64 **Mennigen R**, Laukötter M, Senninger N, Rijcken E. The OTSC(®) proctology clip system for the closure of refractory anal fistulas. *Tech*

Coloproctol 2015; **19**: 241-246 [PMID: 25715788 DOI: 10.1007/s10151-015-1284-7]

65 **Gecse KB**, Sebastian S, Hertogh Gd, Yassin NA, Kotze PG, Reinisch W, Spinelli A, Koutroubakis IE, Katsanos KH, Hart A, van den Brink GR, Rogler G, Bemelman WA. Results of the Fifth Scientific Workshop of the ECCO [II]: Clinical Aspects of Perianal Fistulising Crohn's Disease-the Unmet Needs. *J Crohns Colitis* 2016; **10**: 758-765 [PMID: 26826183 DOI: 10.1093/ecco-jcc/jjw039]

66 **Cao Y**, Su Q, Zhang B, Shen F, Li S. Efficacy of stem cells therapy for Crohn's fistula: a meta-analysis and systematic review. *Stem Cell Res Ther* 2021; **12**: 32 [PMID: 33413661 DOI: 10.1186/s13287-020-02095-7]

67 **Lightner AL**, Wang Z, Zubair AC, Dozois EJ. A Systematic Review and Meta-analysis of Mesenchymal Stem Cell Injections for the Treatment of Perianal Crohn's Disease: Progress Made and Future Directions. *Dis Colon Rectum* 2018; **61**: 629-640 [PMID: 29578916 DOI: 10.1097/DCR.0000000000001093]

68 **Adamina M**, Bonovas S, Raine T, Spinelli A, Warusavitarne J, Armuzzi A, Bachmann O, Bager P, Biancone L, Bokemeyer B, Bossuyt P, Burisch J, Collins P, Doherty G, El-Hussuna A, Ellul P, Fiorino G, Frei-Lanter C, Furfaro F, Gingert C, Gionchetti P, Gisbert JP, Gomollon F, González Lorenzo M, Gordon H, Hlavaty T, Juillerat P, Katsanos K, Kopylov U, Krustins E, Kucharzik T, Lytras T, Maaser C, Magro F, Marshall JK, Myrelid P, Pellino G, Rosa I, Sabino J, Savarino E, Stassen L, Torres J, Uzzan M, Vavricka S, Verstockt B, Zmora O. ECCO Guidelines on Therapeutics in Crohn's Disease: Surgical Treatment. *J Crohns Colitis* 2020; **14**: 155-168 [PMID: 31742338 DOI: 10.1093/ecco-jcc/jjz187]

69 **Lee MJ**, Heywood N, Sagar PM, Brown SR, Fearnhead NS; pCD Collaborators. Surgical management of fistulating perianal Crohn's disease: a UK survey. *Colorectal Dis* 2017; **19**: 266-273 [PMID: 27423057 DOI: 10.1111/codi.13462]

70 **Stellingwerf ME**, van Praag EM, Tozer PJ, Bemelman WA, Buskens CJ. Systematic review and meta-analysis of endorectal advancement flap and ligation of the intersphincteric fistula tract for cryptoglandular and Crohn's high perianal fistulas. *BJS Open* 2019; **3**: 231-241 [PMID: 31183438 DOI: 10.1002/bjs5.50129]

71 **O'Riordan JM**, Datta I, Johnston C, Baxter NN. A systematic review of the anal fistula plug for patients with Crohn's and non-Crohn's related fistula-in-ano. *Dis Colon Rectum* 2012; **55**: 351-358 [PMID: 22469804 DOI: 10.1097/DCR.0b013e318239d1e4]

72 **Nasseri Y**, Cassella L, Berns M, Zaghiyan K, Cohen J. The anal fistula plug in Crohn's disease patients with fistula-in-ano: a systematic review. *Colorectal Dis* 2016; **18**: 351-356 [PMID: 26749385 DOI: 10.1111/codi.13268]

73 **Grimaud JC**, Munoz-Bongrand N, Siproudhis L, Abramowitz L, Sénéjoux A, Vitton V, Gambiez L, Flourié B, Hébuterne X, Louis E, Coffin B, De Parades V, Savoye G, Soulé JC, Bouhnik Y, Colombel JF, Contou JF, François Y, Mary JY, Lémann M; Groupe d'Etude Thérapeutique des Affections Inflammatoires du Tube Digestif. Fibrin glue is effective healing perianal fistulas in patients with Crohn's disease. *Gastroenterology* 2010; **138**: 2275-2281, 2281.e1 [PMID: 20178792 DOI: 10.1053/j.gastro.2010.02.013]

74 **Cirocchi R**, Santoro A, Trastulli S, Farinella E, Di Rocco G, Vendettuali D, Giannotti D, Redler A, Coccetta M, Gullà N, Boselli C, Avenia N, Sciannameo F, Basoli A. Meta-analysis of fibrin glue versus surgery for treatment of fistula-in-ano. *Ann Ital Chir* 2010; **81**: 349-356 [PMID: 21294388]

75 **Adegbola SO**, Sahnan K, Tozer P, Warusavitarne J. Emerging Data on Fistula Laser Closure (FiLaC) for the Treatment of Perianal Fistulas; Patient Selection and Outcomes. *Clin Exp Gastroenterol* 2021; **14**: 467-475 [PMID: 34908858 DOI: 10.2147/CEG.S269464]

76 **Adegbola SO**, Sahnan K, Tozer PJ, Strouhal R, Hart AL, Lung PFC, Phillips RKS, Faiz O, Warusavitarne J. Symptom Amelioration in Crohn's Perianal Fistulas Using Video-Assisted Anal Fistula Treatment (VAAFT). *J Crohns Colitis* 2018; **12**: 1067-1072 [PMID: 29800373 DOI: 10.1093/ecco-jcc/jjy071]

77 **Cao D**, Li W, Ji Y, Wang X, Cui Z. Efficacy and safety of FiLaC™ for perianal fistulizing Crohn's disease: a systematic review and meta-analysis. *Tech Coloproctol* 2022; **26**: 775-781 [PMID: 35962294 DOI: 10.1007/s10151-022-02682-1]

78 **Schwandner O**. Video-assisted anal fistula treatment (VAAFT) combined with advancement flap repair in Crohn's disease. *Tech Coloproctol* 2013; **17**: 221-225 [PMID: 23179892 DOI: 10.1007/s10151-012-0921-7]

79 **Panés J**, García-Olmo D, Van Assche G, Colombel JF, Reinisch W, Baumgart DC, Dignass A, Nachury M, Ferrante M, Kazemi-Shirazi L, Grimaud JC, de la Portilla F, Goldin E, Richard MP, Leselbaum A, Danese S; ADMIRE CD Study Group Collaborators. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet* 2016; **388**: 1281-1290 [PMID: 27477896 DOI: 10.1016/S0140-6736(16)31203-X]

80 **de Groof EJ**, Cabral VN, Buskens CJ, Morton DG, Hahnloser D, Bemelman WA; research committee of the European Society of Coloproctology. Systematic review of evidence and consensus on perianal fistula: an analysis of national and international guidelines. *Colorectal Dis* 2016; **18**: O119-O134 [PMID: 26847796 DOI: 10.1111/codi.13286]

81 **Yamamoto T**, Shimoyama T. Fecal Diversion in Complex Perianal Fistulizing Crohn's Disease. *Clin Colon Rectal Surg* 2022; **35**: 5-9 [PMID: 35069025 DOI: 10.1055/s-0041-1740028]

82 **Singh S**, Ding NS, Mathis KL, Dulai PS, Farrell AM, Pemberton JH, Hart AL, Sandborn WJ, Loftus EV Jr. Systematic review with meta-analysis: faecal diversion for management of perianal Crohn's disease. *Aliment Pharmacol Ther* 2015; **42**: 783-792 [PMID: 26264359 DOI: 10.1111/apt.13356]

83 **Gordon H**, Biancone L, Fiorino G, Katsanos KH, Kopylov U, Al Sulais E, Axelrad JE, Balendran K, Burisch J, de Ridder L, Derikx L, Ellul P, Greuter T, Iacucci M, Di Jiang C, Kapizioni C, Karmiris K, Kirchgesner J, Laharie D, Lobatón T, Molnár T, Noor NM, Rao R, Saibeni S, Scharl M, Vavricka SR, Raine T. ECCO Guidelines on Inflammatory Bowel Disease and Malignancies. *J Crohns Colitis* 2023; **17**: 827-854 [PMID: 36528797 DOI: 10.1093/ecco-jcc/jjac187]

84 **Yasukawa S**, Matsui T, Yano Y, Sato Y, Takada Y, Kishi M, Ono Y, Takatsu N, Nagahama T, Hisabe T, Hirai F, Yao K, Ueki T, Higashi D, Futami K, Sou S, Sakurai T, Yao T, Tanabe H, Iwashita A, Washio M. Crohn's disease-specific mortality: a 30-year cohort study at a tertiary referral center in Japan. *J Gastroenterol* 2019; **54**: 42-52 [PMID: 29948302 DOI: 10.1007/s00535-018-1482-y]

85 **Beaugerie L**, Carrat F, Nahon S, Zeitoun JD, Sabaté JM, Peyrin-Biroulet L, Colombel JF, Allez M, Fléjou JF, Kirchgesner J, Svrcek M; Cancers et Surrisque Associé aux Maladies Inflammatoires Intestinales En France Study Group. High Risk of Anal and Rectal Cancer in Patients With Anal and/or Perianal Crohn's Disease. *Clin Gastroenterol Hepatol* 2018; **16**: 892-899.e2 [PMID: 29199142 DOI: 10.1016/j.cgh.2017.11.041]

86 **Annese V**, Beaugerie L, Egan L, Biancone L, Bolling C, Brandts C, Dierickx D, Dummer R, Fiorino G, Gornet JM, Higgins P, Katsanos KH, Nissen L, Pellino G, Rogler G, Scaldaferri F, Szymanska E, Eliakim R; ECCO. European Evidence-based Consensus: Inflammatory Bowel Disease and Malignancies. *J Crohns Colitis* 2015; **9**: 945-965 [PMID: 26294789 DOI: 10.1093/ecco-jcc/jjv141]

87 **Selimagic A**, Dozic A, Husic-Selimovic A, Tucakovic N, Cehajic A, Subo A, Spahic A, Vanis N. The Role of Inflammation in Anal Cancer. *Diseases* 2022; **10** [PMID: 35645248 DOI: 10.3390/diseases10020027]

88 **Islami F**, Ferlay J, Lortet-Tieulent J, Bray F, Jemal A. International trends in anal cancer incidence rates. *Int J Epidemiol* 2017; **46**: 924-938

[PMID: 27789668 DOI: 10.1093/ije/dyw276]

89 **Thomas M**, Bienkowski R, Vandermeer TJ, Trostle D, Cagir B. Malignant transformation in perianal fistulas of Crohn's disease: a systematic review of literature. *J Gastrointest Surg* 2010; **14**: 66-73 [PMID: 19826882 DOI: 10.1007/s11605-009-1061-x]

90 **Rao S**, Guren MG, Khan K, Brown G, Renehan AG, Steigen SE, Deutsch E, Martinelli E, Arnold D; ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org. Anal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up(☆). *Ann Oncol* 2021; **32**: 1087-1100 [PMID: 34175386 DOI: 10.1016/j.annonc.2021.06.015]

91 **Matsui T**, Iwashita A, Matsumoto T, Hisabe T, Futami K, Tanabe H. Atlas of Inflammatory Bowel Disease-Associated Intestinal Cancer. Singapore: Springer, 2022 [DOI: 10.1007/978-981-19-3413-1]

92 **Kucharzik T**, Ellul P, Greuter T, Rahier JF, Verstockt B, Abreu C, Albuquerque A, Allocca M, Esteve M, Farraye FA, Gordon H, Karmiris K, Kopylov U, Kirchgesner J, MacMahon E, Magro F, Maaser C, de Ridder L, Taxonera C, Toruner M, Tremblay L, Scharl M, Viget N, Zabana Y, Vavricka S. ECCO Guidelines on the Prevention, Diagnosis, and Management of Infections in Inflammatory Bowel Disease. *J Crohns Colitis* 2021; **15**: 879-913 [PMID: 33730753 DOI: 10.1093/ecco-jcc/jjab052]

93 **Barroso LF**, Stier EA, Hillman R, Palefsky J. Anal Cancer Screening and Prevention: Summary of Evidence Reviewed for the 2021 Centers for Disease Control and Prevention Sexually Transmitted Infection Guidelines. *Clin Infect Dis* 2022; **74**: S179-S192 [PMID: 35416975 DOI: 10.1093/cid/ciac044]

94 **Palefsky JM**, Lee JY, Jay N, Goldstone SE, Darragh TM, Dunlevy HA, Rosa-Cunha I, Arons A, Pugliese JC, Vena D, Sparano JA, Wilkin TJ, Bucher G, Stier EA, Tirado Gomez M, Flowers L, Barroso LF, Mitsuyasu RT, Lensing SY, Logan J, Aboulafia DM, Schouten JT, de la Ossa J, Levine R, Korman JD, Hagensee M, Atkinson TM, Einstein MH, Cracchiolo BM, Wiley D, Ellsworth GB, Brickman C, Berry-Lawhorn JM; ANCHOR Investigators Group. Treatment of Anal High-Grade Squamous Intraepithelial Lesions to Prevent Anal Cancer. *N Engl J Med* 2022; **386**: 2273-2282 [PMID: 35704479 DOI: 10.1056/NEJMoa2201048]

95 **Hirano Y**, Futami K, Higashi D, Mikami K, Maekawa T. Anorectal cancer surveillance in Crohn's disease. *J Anus Rectum Colon* 2018; **2**: 145-154 [PMID: 31559357 DOI: 10.23922/jarc.2018-005]

96 **Albuquerque A**. Cytology in Anal Cancer Screening: Practical Review for Clinicians. *Acta Cytol* 2020; **64**: 281-287 [PMID: 31533094 DOI: 10.1159/000502881]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3386-3392

DOI: 10.3748/wjg.v30.i28.3386

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Is appendoscope a new option for the treatment of acute appendicitis?

Shu-Jiong Feng, Yi-Feng Zhou, Jian-Feng Yang, Hong-Zhang Shen, Guang-Xing Cui, Xiao-Feng Zhang

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

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

P-Reviewer: Elpek GO

Received: April 7, 2024
Revised: June 11, 2024
Accepted: July 1, 2024
Published online: July 28, 2024
Processing time: 107 Days and 15.9 Hours

Shu-Jiong Feng, Yi-Feng Zhou, Jian-Feng Yang, Hong-Zhang Shen, Guang-Xing Cui, Xiao-Feng Zhang, Department of Gastroenterology, Affiliated Hangzhou First People's Hospital, School of Medicine, Westlake University, Hangzhou 310000, Zhejiang Province, China

Shu-Jiong Feng, Yi-Feng Zhou, Jian-Feng Yang, Hong-Zhang Shen, Guang-Xing Cui, Xiao-Feng Zhang, Department of Gastroenterology, Key Laboratory of Integrated Traditional Chinese and Western Medicine for Biliary and Pancreatic Diseases of Zhejiang Province, Hangzhou 310000, Zhejiang Province, China

Shu-Jiong Feng, Yi-Feng Zhou, Jian-Feng Yang, Hong-Zhang Shen, Guang-Xing Cui, Xiao-Feng Zhang, Department of Gastroenterology, Hangzhou Institute of Digestive Diseases, Hangzhou 310000, Zhejiang Province, China

Corresponding author: Xiao-Feng Zhang, MD, Chief Physician, Department of Gastroenterology, Affiliated Hangzhou First People's Hospital, School of Medicine, Westlake University, No. 261 Huansha Road, Hangzhou 310000, Zhejiang Province, China. zxf837@tom.com

Abstract

Acute appendicitis is a common surgical emergency. It is commonly caused by obstruction of the appendiceal lumen due to fecaliths, tumors, or lymphoid hyperplasia. For over a century, appendectomy has been the primary treatment for acute appendicitis. Abraham Groves performed the first open appendectomy in 1883. In 1983, Kurt Semm completed the first laparoscopic appendectomy, heralding a new era in appendectomy. However, appendectomy is associated with certain complications and a rate of negative appendectomies. Studies have suggested controversy over the impact of appendectomy on the development of inflammatory bowel disease and Parkinson's disease, but an increasing number of studies indicate a possible positive correlation between appendectomy and colorectal cancer, gallstones, and cardiovascular disease. With the recognition that the appendix is not a vestigial organ and the advancement of endoscopic technology, Liu proposed the endoscopic retrograde appendicitis therapy. It is an effective minimally invasive alternative for treating uncomplicated acute appendicitis. Our team has developed an appendoscope with a disposable digital imaging system operated through the biopsy channel of a colonoscope and successfully applied it in the treatment of appendicitis. This article provides an overview of the progress in endoscopic treatment for acute appendicitis and offers a new perspective on the future direction of appendiceal disease treatment.

Key Words: Acute appendicitis; Endoscopic technology; Endoscopic retrograde appendicitis therapy; Appendoscope; Appendiceal disease treatment

Core Tip: In this article, our team has developed an appendoscope with a disposable digital imaging system operated through the biopsy channel of a colonoscope and successfully applied it in the treatment of appendicitis. It provides an overview of the progress in endoscopic treatment for acute appendicitis and offers a new perspective on the future direction of appendiceal disease treatment.

Citation: Feng SJ, Zhou YF, Yang JF, Shen HZ, Cui GX, Zhang XF. Is appendoscope a new option for the treatment of acute appendicitis? *World J Gastroenterol* 2024; 30(28): 3386-3392
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3386.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3386

INTRODUCTION

Acute appendicitis is a common surgical emergency, most frequently occurring in the 10-20 age group, with a male-to-female ratio of 1.4:1.0[1]. It is commonly caused by obstruction of the appendiceal lumen due to fecaliths, tumors, or lymphoid hyperplasia[2]. The diagnosis of acute appendicitis still lacks a gold standard and requires a combination of clinical presentation (Alvarado score)[3] and imaging modalities such as ultrasound or computed tomography (CT)/magnetic resonance imaging (MRI) suggestive of appendicitis[4,5]. Liu[6] defines acute uncomplicated appendicitis as an appendiceal diameter greater than 6mm, excluding appendiceal perforation and appendiceal tumors. For pregnant women, children, and patients planning pregnancy who refuse CT scans, colonoscopy can be combined to confirm appendicitis, with endoscopic findings including mucosal edema and the presence of pus or fecaliths at the appendiceal orifice[7]. Acute appendicitis is classified into acute simple appendicitis, acute suppurative appendicitis, acute gangrenous and perforated appendicitis, and inflammatory masses or periappendiceal abscesses formed by omental wrapping[5]. Clinically, acute simple appendicitis and acute suppurative appendicitis are collectively referred to as acute uncomplicated appendicitis; gangrenous and perforated appendicitis or those with periappendiceal abscesses are collectively referred to as acute complicated appendicitis[8].

Traditional treatments for appendicitis include conservative antibiotic therapy and appendectomy, the latter comprising open appendectomy (OA) and laparoscopic appendectomy (LA). Harris[9] performed the first OA in 1883. In 1983, Semm[10] completed the first LA, heralding a new era in appendectomy. For over a century, appendectomy has been the primary treatment for acute appendicitis. However, appendectomy is associated with a series of complications such as wound infection, incisional hernia, intra-abdominal infection, intestinal obstruction, interstitial pneumonia, urinary tract infection, cardiovascular accidents, *etc*[11-13]. Excessive surgical treatment can also lead to negative appendectomies, with recent studies reporting rates as high as 8%-30%[14,15]. Studies have suggested controversy over the impact of appendectomy on the development of inflammatory bowel disease and Parkinson's disease[16,17], but an increasing number of studies indicate a possible positive correlation between appendectomy and colorectal cancer, gallstones, and cardiovascular disease[18-20].

Some scholars have proposed that the appendix is not a vestigial organ but can produce various immunoglobulins. Moreover, due to its unique shape and position, the appendix is considered a reservoir or safe house for intestinal microbiota, playing an important role in regulating the gut flora[21]. With the development of endoscopic minimally invasive technology, Liu *et al*[22] proposed the endoscopic retrograde appendicitis therapy (ERAT). Our team has developed an appendoscope, which has been successfully applied in clinical practice[23]. Both ERAT and the appendoscope can achieve the goal of treating acute appendicitis while preserving the appendix.

ERAT

Inspired by the clinical application of endoscopic retrograde cholangiopancreatography (ERCP), Liu *et al*[22] first proposed ERAT in 2012. The procedure is as follows: A colonoscope with a transparent cap at the tip is inserted into the cecum, using the transparent cap to push aside the Gerlach's valve at the appendiceal orifice, and the Seldinger technique is used for appendiceal cannulation; after successful cannulation, aspiration of the appendiceal lumen is performed to reduce luminal pressure; under X-ray guidance, a contrast agent is injected for appendiceal lumen imaging; appendiceal lumen irrigation and/or lithotripsy are performed; and a stent is placed for drainage. Liu *et al*[24] conducted a multicenter clinical study in 2015, involving 34 patients with a definitive diagnosis of acute uncomplicated appendicitis, one of whom failed cannulation, resulting in a 97% success rate for ERAT. One patient (3%) underwent appendectomy due to perforation within 48 hours postoperatively, and there were no long-term complications at 12-month follow-up,

while two patients (6.2%) underwent appendectomy due to recurrent abdominal pain.

To evaluate the clinical efficacy of ERAT compared to LA in the treatment of acute appendicitis, retrospective studies have been conducted. Yang *et al*[25] included 422 patients (ERAT 79; LA 343) and found that the cure rate within one year and the pain score 6 hours postoperatively in the ERAT group were significantly higher than in the LA group. Moreover, compared to the LA group, the ERAT group had significantly shorter median operative time and median hospital stay. There was no significant difference between the two groups in terms of median time to recurrence and incidence of adverse events at one year. Ding *et al*[26] divided 210 patients with acute appendicitis into ERAT, LA, and OA groups. The results showed that the operative time in the ERAT group was significantly shorter than in the LA and OA groups. The postoperative hospital stay, postoperative bed rest time, surgery-related complications, and hospitalization costs were all significantly lower in the ERAT group compared to the latter two groups.

To compare the efficacy of ERAT with antibiotic treatment for acute uncomplicated appendicitis, Li *et al*[27] conducted a multicenter retrospective study. By comparing treatment success rates, median hospital stays, pain relief rate within 24 hours, and one-year follow-up recurrence rate, the ERAT group outperformed the antibiotic group. This may be because fecaliths, the most common cause of acute appendicitis, can be endoscopically removed during ERAT, and a stent can be placed to relieve luminal pressure. Pata *et al*[28] conducted a meta-analysis comparing ERAT with appendectomy or antibiotic treatment for acute uncomplicated appendicitis. The results showed no significant differences in technical success rates and one-year follow-up treatment efficacy among the three, but ERAT had advantages in postoperative pain relief and hospital stay duration.

ERAT is an effective minimally invasive alternative for treating uncomplicated acute appendicitis. However, current clinical studies are mostly from China. To further evaluate the safety and efficacy of ERAT, a comprehensive, international, multicenter, randomized controlled prospective study is urgently needed.

NON-X-RAY-ASSISTED ERAT FOR SPECIAL POPULATIONS WITH ACUTE UNCOMPLICATED APPENDICITIS

The ERAT procedure requires X-ray assistance, which carries a certain radiation risk. There have been successful cases of ERAT performed without X-ray assistance. Kang *et al*[29] used contrast-enhanced ultrasound instead of endoscopic retrograde appendiceal imaging in a prospective, randomized controlled trial to compare the efficacy of modified ERAT (mERAT) with antibiotic treatment for children with acute uncomplicated appendicitis. The results showed that the overall success rate of mERAT treatment (100.0%) was significantly higher than that of antibiotics (80.9%). The median discharge time in the mERAT group was significantly shorter than that in the antibiotic treatment group (6.00 days ± 1.76 days). mERAT provides a new treatment option for children with acute uncomplicated appendicitis. Liu *et al*[30] reported a case of a pregnant woman at 18 weeks of gestation with acute appendicitis who successfully completed ERAT without anesthesia and X-ray assistance. The patient's abdominal pain was immediately relieved postoperatively, and the pain was completely relieved the next day. The patient was discharged quickly without antibiotic treatment during hospitalization. Thus, non-X-ray-assisted ERAT is an effective treatment method for special populations such as children or pregnant women with acute appendicitis.

ERAT FOR ACUTE COMPLICATED APPENDICITIS

Although ERAT is not routinely used for treating acute complicated appendicitis, there have been successful clinical case reports to date. Song *et al*[31] reported a case of a 73-year-old elderly woman diagnosed with periappendiceal and subhepatic abscesses. Due to poor baseline conditions and no surgical indications after multidisciplinary discussions, the patient underwent ERAT with stent placement. Follow-up CT after 2 months showed no abscess, and there was no recurrence after 6 years of follow-up. Li *et al*[32] reported a case of a 34-year-old woman diagnosed with acute appendicitis complicated by a giant appendiceal abscess and intestinal obstruction. The patient underwent ERAT with stent placement, and the intestinal obstruction was relieved postoperatively. A follow-up CT after 2 months showed complete resolution of the abscess. Cui *et al*[33] from our team performed ERAT on 9 patients diagnosed with appendiceal abscesses, and the patients had good prognoses postoperatively. Therefore, ERAT is an effective treatment method for periappendiceal abscesses, but more extensive clinical studies are needed to confirm this.

FUNNEL-HOOD-ASSISTED ERAT

Luo *et al*[34] performed ERAT using an independently developed funnel-shaped hood with a small-diameter tip. A 33-year-old male patient diagnosed with acute appendicitis experienced immediate relief of abdominal pain symptoms after undergoing Funnel-hood-assisted ERAT, and the patient was discharged three days later. Funnel-hood-assisted ERAT is a technological innovation that can reduce the difficulty of cannulating the appendiceal lumen, and it is expected to improve the success rate of ERAT treatment and promote its clinical application.

SPY-GLASS DS ASSISTED ERAT

The SpyGlass DS is a second-generation cholangioscopy system with an outer sheath diameter of 3.3 mm, equipped with one biopsy channel (diameter 1.2 mm), one fiber optic channel (1.0 mm), and two irrigation channels. It allows direct visualization of the biliary tract and is used for the diagnosis of biliary and pancreatic duct strictures, treatment of difficult stones, and radiofrequency ablation of cholangiocarcinoma. The procedure can be completed without the need for X-ray or ultrasound guidance.

Kong *et al*[35] reported on 14 cases of acute uncomplicated appendicitis treated with SpyGlass DS-assisted ERAT, achieving a 100% success rate. The average operation time was 37.8 minutes ± 22.0 minutes. All patients experienced immediate postoperative relief of abdominal pain. The average postoperative hospital stay was 1.9 days ± 0.7 days. No recurrences were observed during a follow-up period of 2 months to 24 months. Additionally, Kong *et al*[36] successfully performed SpyGlass DS-assisted ERAT on a patient diagnosed with acute appendicitis at 14 weeks + 2 days of pregnancy without anesthesia, and the patient eventually gave birth at full term. Similarly, Wang *et al*[37] performed ERAT using a digital single-operator cholangioscopy system in a pregnant woman. The patient's abdominal pain was significantly relieved postoperatively, and she was discharged without antibiotics. SpyGlass DS-assisted ERAT is a safe and effective alternative for diagnosing and treating acute uncomplicated appendicitis, providing a treatment option for special populations such as pregnant women who need to avoid or refuse X-ray exposure.

APPENDOSCOPIC TREATMENT OF ACUTE APPENDICITIS

Inspired by the SpyGlass DS, our team has successfully developed an appendoscope specifically designed for the diagnosis and treatment of appendiceal diseases, which has passed ethical review and clinical trials and obtained a national patent. The appendoscope is a disposable digital imaging system operated through the biopsy channel of a colonoscope; it is equipped with an LED light source system and has two models with outer sheath diameters of 3.3 mm or 2.6 mm, featuring a biopsy channel (diameter 2.0 mm or 1.2 mm) and two irrigation channels. The distal end of the outer sheath can be adjusted in multiple directions for ease of operation. The appendoscope avoids complications related to X-rays and contrast agents and provides more accurate and intuitive observation under direct endoscopic vision, which is more conducive to the diagnosis and treatment of appendicitis. Compared to the SpyGlass DS, the appendoscope has a more stable imaging system, wider biopsy and irrigation channels, and a price of around 5000 RMB, which is significantly more affordable than the former (approximately 12000 RMB domestically).

Appendoscope Procedure: Patients undergo bowel preparation 6 hours before the procedure with 3.0 L of polyethylene glycol electrolyte solution or 1.5 L of lactulose solution to cleanse the bowel. For patients with clinical symptoms of nausea and vomiting or unable to cooperate with oral laxatives, five 500 mL saline enemas are administered 30 minutes before the procedure. All patients receive intravenous general anesthesia and are positioned supine. A colonoscope (Olympus 290 or Fuji 7000) with a transparent cap is inserted into the cecum, and the appendoscope is advanced through the biopsy channel of the colonoscope into the appendiceal lumen to observe the interior, perform irrigation, stone retrieval, and other treatments as needed. If a fecalith is seen obstructing the appendiceal orifice, stone retrieval treatment can be performed first (Figure 1).

The first clinical application of the appendoscope was reported in June 2022 and published in the top international endoscopy journal, Endoscopy. A 73-year-old female patient was diagnosed with chronic appendicitis. We observed a fecalith obstructing the appendiceal orifice *via* colonoscopy. After removing the fecalith with a retrieval basket, we inserted the appendoscope to observe the appendiceal lumen, where mucosal congestion and edema were visible, with no residual fecalith. The patient experienced significant relief of abdominal pain postoperatively, with no related complications occurring. She was discharged one week later and has had no recurrence to date[23]. The clinical application of the appendoscope for acute uncomplicated appendicitis has been completed in two cases. Both young patients were admitted with abdominal pain and underwent appendoscopic examination after CT imaging suggested appendicitis. One patient had a fecalith removed, and both were discharged within two days postoperatively without complications and had no recurrence during follow-up (Table 1).

The appendoscope has achieved minimally invasive treatment of appendicitis, shortened hospital stay, and saved medical costs, with favorable patient outcomes. Future large-scale clinical studies are still needed to verify the feasibility, safety, and efficacy of appendoscopic treatment for acute appendicitis, providing clinical experience on how to improve the success rate of treatment for acute appendicitis and reduce the incidence of complications.

CONCLUSION

With the booming development of endoscopic minimally invasive technology, the application of ERAT and related new technologies has provided us with a new perspective on the treatment of acute appendicitis. Based on the use of biliary endoscopy systems from ERCP and considering endoscopic costs, we have developed the appendoscope and successfully applied it in the treatment of appendicitis. The appendoscope has the following advantages: (1) Direct insertion into the appendiceal lumen allows for more precise and intuitive operation under direct vision; (2) Avoidance of X-ray and contrast agent hazards; (3) Short operation time, short hospital stay, and fewer postoperative complications; and (4) Low cost, reducing the economic burden on patients. However, due to the small sample size, the study has certain limitations. We will increase the sample size in the later stage for further validation. The application of the appendoscope is not

Table 1 One patient had a fecalith removed, and both were discharged within two days postoperatively without complications and had no recurrence during follow-up

Patient ID	Gender	Age (years)	Chief complaint	Operation time (minutes)	Appendiceal lumen fecalith	Abdominal pain relief time (days)	Hospital stays	Follow-up time (months)	Complications
1	Female	17	Abdominal pain for 3 days	35	Yes	1	2	13	None
2	Male	24	Abdominal pain for 2 days	32	No	1	1	12	None

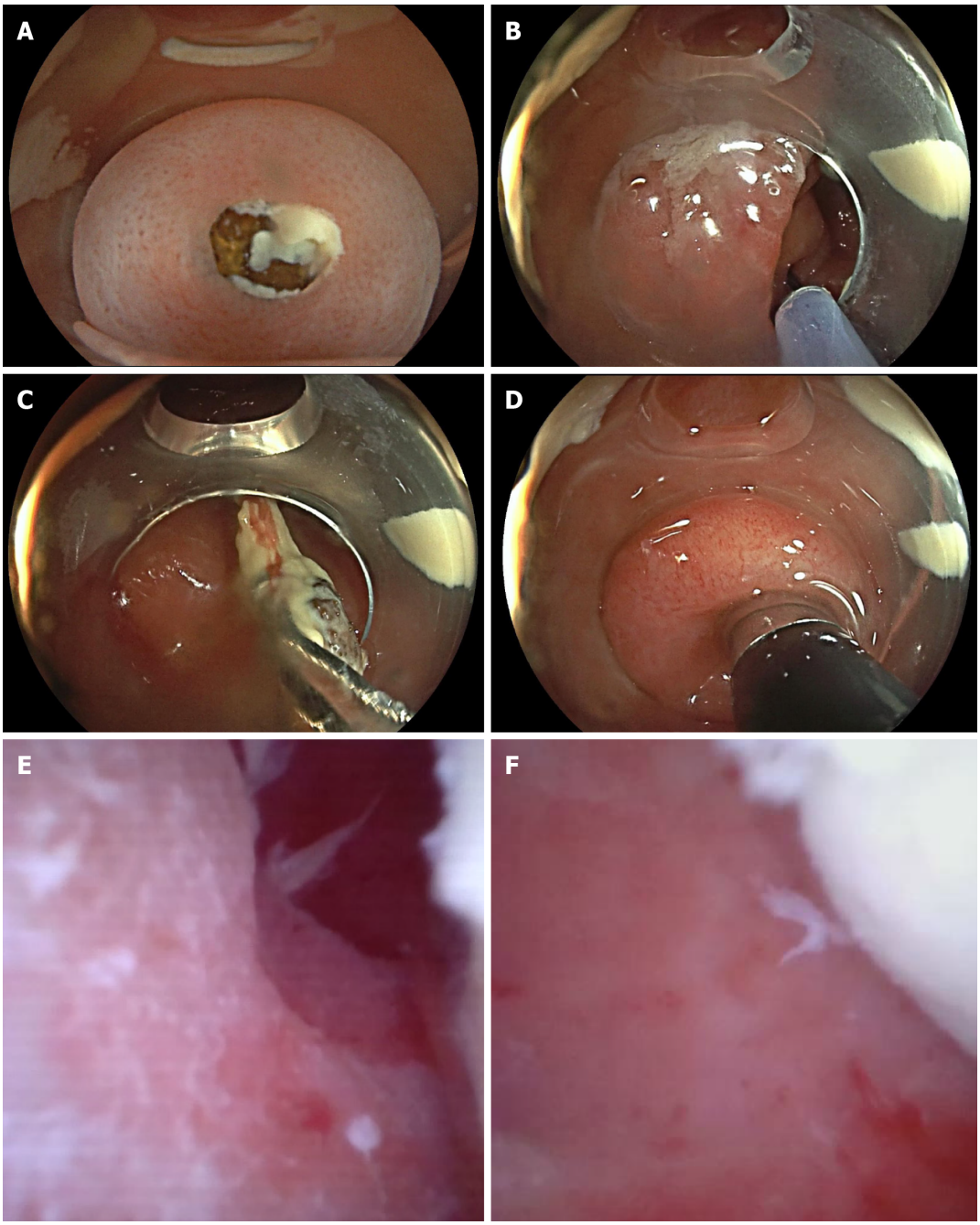


Figure 1 Appendoscope treatment. A: Fecalith obstructing the appendiceal orifice; B: A retrieval basket enters the appendiceal lumen; C: Removal of fecalith with the retrieval basket; D: Insertion of the appendoscope into the appendiceal lumen; E: Images of the appendiceal lumen; F: Pus and localized mucosal congestion and edema.

limited to the diagnosis and treatment of appendicitis. Currently, we are developing specialized biopsy forceps, stents, and other accessories for the appendoscope, which is expected to become an important tool in the diagnosis and treatment of benign and malignant appendiceal diseases.

FOOTNOTES

Author contributions: Feng SJ was responsible for writing the main content of the article; Zhou YF performed appendoscope; Yang JF searched the literature and performed endoscopic retrograde appendicitis therapy; Shen HZ edited figures; Cui GX polished language and performed endoscopic retrograde appendicitis therapy; Zhang XF was mainly responsible for revising the content of the article; and all authors have read and approved the final manuscript.

Supported by the Construction Fund of Key Medical Disciplines of Hangzhou, No. 0020200026; and Key R&D Program of Zhejiang Province, No. 2023C03054.

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

Country of origin: China

ORCID number: Shu-Jiong Feng 0009-0006-7887-8632; Hong-Zhang Shen 0000-0002-1275-2862; Xiao-Feng Zhang 0000-0002-7471-2305.

S-Editor: Chen YL
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Humes DJ**, Simpson J. Acute appendicitis. *BMJ* 2006; **333**: 530-534 [PMID: 16960208 DOI: 10.1136/bmj.38940.664363.AE]
2 **Krzyzak M**, Mulrooney SM. Acute Appendicitis Review: Background, Epidemiology, Diagnosis, and Treatment. *Cureus* 2020; **12**: e8562 [PMID: 32670699 DOI: 10.7759/cureus.8562]
3 **Ohle R**, O'Reilly F, O'Brien KK, Fahey T, Dimitrov BD. The Alvarado score for predicting acute appendicitis: a systematic review. *BMC Med* 2011; **9**: 139 [PMID: 22204638 DOI: 10.1186/1741-7015-9-139]
4 **Eng KA**, Abadeh A, Ligocki C, Lee YK, Moineddin R, Adams-Webber T, Schuh S, Doria AS. Acute Appendicitis: A Meta-Analysis of the Diagnostic Accuracy of US, CT, and MRI as Second-Line Imaging Tests after an Initial US. *Radiology* 2018; **288**: 717-727 [PMID: 29916776 DOI: 10.1148/radiol.2018180318]
5 **Hoffmann JC**, Trimborn CP, Hoffmann M, Schröder R, Förster S, Dirks K, Tannapfel A, Anthuber M, Hollerweger A. Classification of acute appendicitis (CAA): treatment directed new classification based on imaging (ultrasound, computed tomography) and pathology. *Int J Colorectal Dis* 2021; **36**: 2347-2360 [PMID: 34143276 DOI: 10.1007/s00384-021-03940-8]
6 **Liu BR**. Top tips for endoscopic retrograde appendicitis therapy (with video). *Gastrointest Endosc* 2024; **99**: 625-628 [PMID: 38103748 DOI: 10.1016/j.gie.2023.12.019]
7 **Li Y**, Mi C, Li W, She J. Diagnosis of Acute Appendicitis by Endoscopic Retrograde Appendicitis Therapy (ERAT): Combination of Colonoscopy and Endoscopic Retrograde Appendicography. *Dig Dis Sci* 2016; **61**: 3285-3291 [PMID: 27411554 DOI: 10.1007/s10620-016-4245-8]
8 **Bhangu A**, Søreide K, Di Saverio S, Assarsson JH, Drake FT. Acute appendicitis: modern understanding of pathogenesis, diagnosis, and management. *Lancet* 2015; **386**: 1278-1287 [PMID: 26460662 DOI: 10.1016/S0140-6736(15)00275-5]
9 **Harris CW**. Abraham GROVES of Fergus: the first elective appendectomy? *Can J Surg* 1961; **4**: 405-410 [PMID: 13711715]
10 **Semm K**. Endoscopic appendectomy. *Endoscopy* 1983; **15**: 59-64 [PMID: 6221925 DOI: 10.1055/s-2007-1021466]
11 **Kotaluoto S**, Pauniaho SL, Helminen MT, Sand JA, Rantanen TK. Severe Complications of Laparoscopic and Conventional Appendectomy Reported to the Finnish Patient Insurance Centre. *World J Surg* 2016; **40**: 277-283 [PMID: 26482363 DOI: 10.1007/s00268-015-3282-3]
12 **Margenthaler JA**, Longo WE, Virgo KS, Johnson FE, Oprian CA, Henderson WG, Daley J, Khuri SF. Risk factors for adverse outcomes after the surgical treatment of appendicitis in adults. *Ann Surg* 2003; **238**: 59-66 [PMID: 12832966 DOI: 10.1097/01.SLA.0000074961.50020.f8]
13 **Tingstedt B**, Johansson J, Nehez L, Andersson R. Late abdominal complaints after appendectomy--readmissions during long-term follow-up. *Dig Surg* 2004; **21**: 23-27 [PMID: 14665763 DOI: 10.1159/000075378]
14 **Henriksen SR**, Christophersen C, Rosenberg J, Fonnes S. Varying negative appendectomy rates after laparoscopic appendectomy: a systematic review and meta-analysis. *Langenbecks Arch Surg* 2023; **408**: 205 [PMID: 37219616 DOI: 10.1007/s00423-023-02935-z]
15 **Chaochankit W**, Boocha A, Samphao S. Negative appendectomy rate in patients diagnosed with acute appendicitis. *BMC Surg* 2022; **22**: 404 [PMID: 36419019 DOI: 10.1186/s12893-022-01852-0]
16 **Lu HT**, Shen QY, Xie D, Zhao QZ, Xu YM. Lack of association between appendectomy and Parkinson's disease: a systematic review and meta-analysis. *Aging Clin Exp Res* 2020; **32**: 2201-2209 [PMID: 31538320 DOI: 10.1007/s40520-019-01354-9]
17 **Song MY**, Ullah S, Yang HY, Ahmed MR, Saleh AA, Liu BR. Long-term effects of appendectomy in humans: is it the optimal management of

appendicitis? *Expert Rev Gastroenterol Hepatol* 2021; **15**: 657-664 [PMID: 33350352 DOI: 10.1080/17474124.2021.1868298]
18 **Meng W**, Cai SR, Zhou L, Dong Q, Zheng S, Zhang SZ. Performance value of high risk factors in colorectal cancer screening in China. *World J Gastroenterol* 2009; **15**: 6111-6116 [PMID: 20027686 DOI: 10.3748/wjg.15.6111]
19 **Liu Z**, Ma X, Zhu C, Fang JY. Risk of colorectal cancer after appendectomy: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2023; **38**: 350-358 [PMID: 36637673 DOI: 10.1111/jgh.16108]
20 **Wu SC**, Chen WT, Muo CH, Ke TW, Fang CW, Sung FC. Association between appendectomy and subsequent colorectal cancer development: an Asian population study. *PLoS One* 2015; **10**: e0118411 [PMID: 25710790 DOI: 10.1371/journal.pone.0118411]
21 **Kooij IA**, Sahami S, Meijer SL, Buskens CJ, Te Velde AA. The immunology of the vermiform appendix: a review of the literature. *Clin Exp Immunol* 2016; **186**: 1-9 [PMID: 27271818 DOI: 10.1111/cei.12821]
22 **Liu BR**, Song JT, Han FY, Li H, Yin JB. Endoscopic retrograde appendicitis therapy: a pilot minimally invasive technique (with videos). *Gastrointest Endosc* 2012; **76**: 862-866 [PMID: 22840292 DOI: 10.1016/j.gie.2012.05.029]
23 **Feng S**, Ling K, Zhang T, Zhang X, Yan X, Yang J, Zhou Y. Application of an appendoscope in chronic appendicitis. *Endoscopy* 2022; **54**: E296-E297 [PMID: 34215000 DOI: 10.1055/a-1519-6903]
24 **Liu BR**, Ma X, Feng J, Yang Z, Qu B, Feng ZT, Ma SR, Yin JB, Sun R, Guo LL, Liu WG. Endoscopic retrograde appendicitis therapy (ERAT) : a multicenter retrospective study in China. *Surg Endosc* 2015; **29**: 905-909 [PMID: 25106722 DOI: 10.1007/s00464-014-3750-0]
25 **Yang B**, Kong L, Ullah S, Zhao L, Liu D, Li D, Shi X, Jia X, Dalal P, Liu B. Endoscopic retrograde appendicitis therapy versus laparoscopic appendectomy for uncomplicated acute appendicitis. *Endoscopy* 2022; **54**: 747-754 [PMID: 35021234 DOI: 10.1055/a-1737-6381]
26 **Ding W**, Du Z, Zhou X. Endoscopic retrograde appendicitis therapy for management of acute appendicitis. *Surg Endosc* 2022; **36**: 2480-2487 [PMID: 33983458 DOI: 10.1007/s00464-021-08533-8]
27 **Li D**, Yang B, Liao J, Li Y, Liu D, Zhao L, Meng X, Hu H, Kong L, Podda M, Ullah S, Liu B. Endoscopic retrograde appendicitis therapy or antibiotics for uncomplicated appendicitis. *Br J Surg* 2023; **110**: 635-637 [PMID: 36745556 DOI: 10.1093/bjs/znad023]
28 **Pata F**, Nardo B, Ielpo B, Di Martino M, Murzi V, Di Saverio S, Yang B, Ortenzi M, Pisanu A, Pellino G, Podda M. Endoscopic retrograde appendicitis therapy versus appendectomy or antibiotics in the modern approach to uncomplicated acute appendicitis: A systematic review and meta-analysis. *Surgery* 2023; **174**: 1292-1301 [PMID: 37806859 DOI: 10.1016/j.surg.2023.08.029]
29 **Kang J**, Zhang W, Zeng L, Lin Y, Wu J, Zhang N, Xie X, Zhang Y, Liu X, Wang B, Yang R, Jiang X. The modified endoscopic retrograde appendicitis therapy versus antibiotic therapy alone for acute uncomplicated appendicitis in children. *Surg Endosc* 2021; **35**: 6291-6299 [PMID: 33146811 DOI: 10.1007/s00464-020-08129-8]
30 **Liu T**, Jiang K, Bi Y. Endoscopic retrograde appendicitis therapy in a pregnant patient with acute septic appendicitis. *Asian J Surg* 2022; **45**: 2070-2071 [PMID: 35513987 DOI: 10.1016/j.asjsur.2022.04.098]
31 **Song M**, Ullah S, Liu B. Endoscopic Retrograde Appendicitis Therapy for Treating Periappendiceal Abscess: First Human Case Report. *Am J Gastroenterol* 2021; **116**: 1119 [PMID: 34074821 DOI: 10.14309/ajg.0000000000001116]
32 **Li Q**, Liu T, Li A, Liu J, Jiang B, Yang B. Endoscopic retrograde appendicitis therapy for giant periappendiceal abscess with intestinal obstruction. *Endoscopy* 2023; **55**: E1116-E1117 [PMID: 37802112 DOI: 10.1055/a-2173-7756]
33 **Cui G**, Lv W, Wang J, Zhang X. Endoscopic retrograde appendicitis therapy: a novel approach for peri-appendiceal abscess. *Endoscopy* 2022; **54**: E186-E187 [PMID: 33979850 DOI: 10.1055/a-1471-2871]
34 **Luo Q**, Zeng S, Jiang M, Zhang Q, Cheng C. Funnel-hood-assisted endoscopic retrograde appendicitis therapy for acute appendicitis. *Endoscopy* 2024; **56**: E142-E143 [PMID: 38359882 DOI: 10.1055/a-2241-8907]
35 **Kong LJ**, Liu D, Zhang JY, Ullah S, Zhao L, Li D, Yang H, Liu BR. Digital single-operator cholangioscope for endoscopic retrograde appendicitis therapy. *Endoscopy* 2022; **54**: 396-400 [PMID: 33893629 DOI: 10.1055/a-1490-0434]
36 **Kong L**, Zhang H, Liu K, Zhao L, Lu BR. How to manage appendicitis in pregnancy better. *Gastrointest Endosc* 2022; **95**: 1018-1019 [PMID: 35114207 DOI: 10.1016/j.gie.2022.01.011]
37 **Wang F**, Zhou XR, Zhang Y, Wang Y, Du ZQ, Liu WH. Digital single-operator cholangioscopy-guided appendiceal intubation for endoscopic retrograde appendicitis therapy in a pregnant woman (with video). *Gastrointest Endosc* 2023; **98**: 1034-1035 [PMID: 37400039 DOI: 10.1016/j.gie.2023.06.066]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3393-3402

DOI: 10.3748/wjg.v30.i28.3393

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

Three-dimensional visualization technology for guiding one-step percutaneous transhepatic cholangioscopic lithotripsy for the treatment of complex hepatolithiasis

Yong-Qing Ye, Ya-Wen Cao, Rong-Qi Li, En-Ze Li, Lei Yan, Zhao-Wei Ding, Jin-Ming Fan, Ping Wang, Yi-Xiang Wu

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Abdelbasset WK, United Arab Emirates

Received: January 24, 2024
Revised: May 18, 2024
Accepted: June 21, 2024
Published online: July 28, 2024
Processing time: 182 Days and 3.3 Hours

Yong-Qing Ye, Department of Hepatobiliary Surgery, The Second People's Hospital of Foshan, Foshan 528000, Guangdong Province, China

Ya-Wen Cao, Department of Emergency Medicine, Biomedical Innovation Center, The Sixth Affiliated Hospital, Sun Yat-sen University, Guangzhou 510655, Guangdong Province, China

Rong-Qi Li, Department of Hepatobiliary Surgery, Foshan Hospital of Traditional Chinese Medicine, Foshan 528000, Guangdong Province, China

En-Ze Li, Lei Yan, Zhao-Wei Ding, Jin-Ming Fan, Ping Wang, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510282, Guangdong Province, China

Yi-Xiang Wu, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, Guangdong Province, China

Co-first authors: Yong-Qing Ye and Ya-Wen Cao.

Corresponding author: Ping Wang, PhD, Chief Doctor, Deputy Director, Full Professor, Surgeon, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangzhou Medical University, No. 151 Yanjiang West Road, Yuexiu District, Guangzhou 510282, Guangdong Province, China. wangping1219@126.com

Abstract

BACKGROUND

Biliary stone disease is a highly prevalent condition and a leading cause of hospitalization worldwide. Hepatolithiasis with associated strictures has high residual and recurrence rates after traditional multisession percutaneous transhepatic cholangioscopic lithotripsy (PTCSL).

AIM

To study one-step PTCSL using the percutaneous transhepatic one-step biliary fistulation (PTOBF) technique guided by three-dimensional (3D) visualization.

METHODS

This was a retrospective, single-center study analyzing, 140 patients who, between October 2016 and October 2023, underwent one-step PTCSL for hepatolithiasis. The patients were divided into two groups: The 3D-PTOBF group and the PTOBF group. Stone clearance on choledochoscopy, complications, and long-term clearance and recurrence rates were assessed.

RESULTS

Age, total bilirubin, direct bilirubin, Child-Pugh class, and stone location were similar between the 2 groups, but there was a significant difference in bile duct strictures, with biliary strictures more common in the 3D-PTOBF group (P = 0.001). The median follow-up time was 55.0 (55.0, 512.0) days. The immediate stone clearance ratio (88.6% *vs* 27.1%, P = 0.000) and stricture resolution ratio (97.1% *vs* 78.6%, P = 0.001) in the 3D-PTOBF group were significantly greater than those in the PTOBF group. Postoperative complication (8.6% *vs* 41.4%, P = 0.000) and stone recurrence rates (7.1% *vs* 38.6%, P = 0.000) were significantly lower in the 3D-PTOBF group.

CONCLUSION

Three-dimensional visualization helps make one-step PTCSL a safe, effective, and promising treatment for patients with complicated primary hepatolithiasis. The perioperative and long-term outcomes are satisfactory for patients with complicated primary hepatolithiasis. This minimally invasive method has the potential to be used as a substitute for hepatobiliary surgery.

Key Words: Hepatolithiasis; One-step percutaneous transhepatic cholangioscopic lithotripsy; Biliary disease; Three-dimensional visualization; Clinical efficacy

Core Tip: Hepatolithiasis is a clinical benign biliary tract disease with a high incidence and a leading cause of hospitalization, seriously affecting the quality of life of patients. However, current treatment modalities have not achieved good curative effects, with high rates of stone and stenosis retention and recurrence. In the present study we introduce a new technology that one-step percutaneous transhepatic cholangioscopic lithotripsy using the percutaneous transhepatic one-step biliary fistulation (PTOBF) technique guided by three-dimensional (3D) visualization technology. And we performed a randomized trial to assess the efficacy and safety of 3D-PTOBF in the treatment of patients with hepatolithiasis. We found that 3D-PTOBF offered significant improvement of immediate stone clearance ratio and stricture resolution ratio. 3D-PTOBF as a safe, effective, and promising treatment for patients with complicated primary hepatolithiasis.

Citation: Ye YQ, Cao YW, Li RQ, Li EZ, Yan L, Ding ZW, Fan JM, Wang P, Wu YX. Three-dimensional visualization technology for guiding one-step percutaneous transhepatic cholangioscopic lithotripsy for the treatment of complex hepatolithiasis. *World J Gastroenterol* 2024; 30(28): 3393-3402
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3393.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3393

INTRODUCTION

Hepatolithiasis, a common benign biliary tract disease in East Asia, is the presence of gallstones (calculi) in the biliary ducts of the liver. According to statistics, hepatolithiasis accounts for 20% to 45% of patients who undergo surgery for gallstones[1-4]. Women have a lifetime risk of developing gallstones approaching 25%[5]. Gallstones can cause several clinical symptoms, such as biliary colic and jaundice, and some may be lethal. If left untreated, this causes bile flow stagnation, recurrent cholangitis, liver parenchymal destruction, and, eventually, secondary biliary cirrhosis or cholangiocarcinoma[6].

Stone removal is the primary method for treating clinical symptoms. Current treatments for hepatolithiasis include surgical resection of affected liver segments, laparoscopic surgery, endoscopic retrograde cholangiopancreatography, and percutaneous transhepatic cholangioscopy lithotripsy (PTCSL). Surgical intervention is preferred when possible, but many patients are ineligible due to multiple recurring surgeries, comorbidities, or anatomical factors[7]. In traditional PTCSL surgery, an external biliary fistula must be gradually dilated over 2-3 weeks prior to lithotripsy using serial fascial dilators[8]. This method is associated with longer hospitalization, a high recurrence rate, and a high rate of calculus reoperation[9].

Recent technological advances have enabled modifications, such as percutaneous transhepatic one-step biliary fistulation (PTOBF) combined with the rigid choledochoscopy technique, for optimizing the PTCSL procedure[10,11]. One-step PTCSL using the PTOBF technique enables the clearing of intrahepatic stones and the resolution of strictures. Ultrasound (US) images cannot directly reveal the three-dimensional spatial relationships of calculi, bile ducts and blood vessels. Thus, it is difficult to obtain their exact anatomical locations, which will influence the precision of the

operation.Furthermore, the choledochoscope can only acquire the local abdominal information of the patient while the corresponding global abdominal information is a little different from the preoperative two-dimensional (2D) computed tomography (CT) image.

In recent years, 3D visualization technology has been employed to assist in making surgical decisions involving liver resection[12-15]. With the assistance of 3D visualization, the liver anatomy, the morphological structure of the intrahepatic duct system, the location of liver lesions, and the spatial relationship with adjacent liver vessels can be displayed visually and clearly from any angle[14]; this can prevent the excessive resection of liver tissues, maximally maintain functional liver tissues, and aid in the development of individualized surgical plans[16].

The purpose of this study was to introduce a detailed protocol and assess the long-term outcomes of the application of 3D visualization technology in one-step PTCSL for the treatment of complex hepatolithiasis patients with biliary strictures.

MATERIALS AND METHODS

Study design and participants

This retrospective study included 140 patients with complex hepatolithiasis from October 2016 to October 2023 at The First Affiliated Hospital of Guangzhou Medical University. A total of 140 patients underwent one-step PTCSL with or without 3D visualization. All patients provided written informed consent for participation in these procedures. Data were gathered for all patients. For further evaluation, the patients were divided into two groups, the 3D-PTCSL group (n = 70) and the PTCSL group (n = 70), who were subsequently compared. This study was approved by the Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University, No. 09, 2017.

3D model reconstruction

CT data were reconstructed in sections with the IQQA-3D Digital Medicine Central Server. Important anatomical structures on the CT images, such as intrahepatic biliary ducts, calculi, and intrahepatic vessels, were extracted *via* segmentation, and 3D models were generated by the surface rendering algorithm. The spatial distribution of the intrahepatic duct system and the relationship between lesions and surrounding tissues from different perspectives could be observed in the reconstructed model, which can be exported as a standard template library. In the 3D reconstruction model, the anatomical features of the tissue structure, stone distribution, bile duct stricture, malformation, and vascular arrangement were identified by magnifying, deleting, rotating, and transparentizing the patient's organs (Figure 1).

Surgical procedure

All procedures were performed under general anesthesia by an experienced biliary surgeon. The one-step PTOBF technique was utilized as follows (Figures 2 and 3, Video).

Preoperative planning: Important preoperative evaluation data, including the location of intrahepatic stones and strictures, the degree of stricture, the biliary anatomy, and the relationship between the vasculature and biliary system, were collected. The preoperative assessment tools include US, CT, magnetic resonance imaging (MRI) or magnetic resonance cholangiopancreatography, and 3D visualization.

Three-dimensional visualization and US-guided puncture: According to preoperative planning, target biliary tract puncture was performed using an 18G needle and a 0.035-inch hydrophilic guidewire under real-time intraoperative US and 3D-model guidance. The patients were encouraged to hold their breath for 2 minutes during puncture to minimize interference.

Establishing channel: After successful biliary puncture, the sinuses were expanded with serial fascial dilators from 8 Fr to 14 Fr. A 14 Fr sheath was passed over the guidewire to establish the channel for the rigid choledochoscope.

Choledochoscopic stone removal: Small stones were flushed out with a "wash and suction" procedure. Larger stones were removed by using a basket, a clamp, or electrohydraulic shock wave lithotripsy.

Managing anastomotic strictures: Choledochoscopy and cholangiography were used to confirm the degree and location of the anastomotic stricture. A membranous stricture with a thin fibrous layer was designated as a mildly anastomotic stricture and could be expanded with a 16 Fr or 18 Fr dilator. A scar-like stricture of the bile duct was designated a severe anastomotic stricture. These could be dilated by flushing with mannitol solution, inserting a 40-W electric knife to cut the open stricture, and/or employing a 4, 6 or 8 mm balloon dilatation catheter.

Supporting drainage tube insertion: A 14 Fr drainage tube was placed into each hepatolithiasis patient at the end of the procedure. If the patient had strictures, a 16- or 18-Fr supporting drainage tube had to be placed across them. The drainage tubes were exchanged every 2-3 months and removed 6 to 9 months after percutaneous transhepatic cholangioscopy, when the strictures were resolved on the last endoscopic intervention.

Statistical analysis

Statistical analysis was performed using R version 3.4.1. Categorical variables are expressed as frequencies and percentages. Continuous variables are expressed as the mean ± SD or median (range). P values < 0.05 were considered to

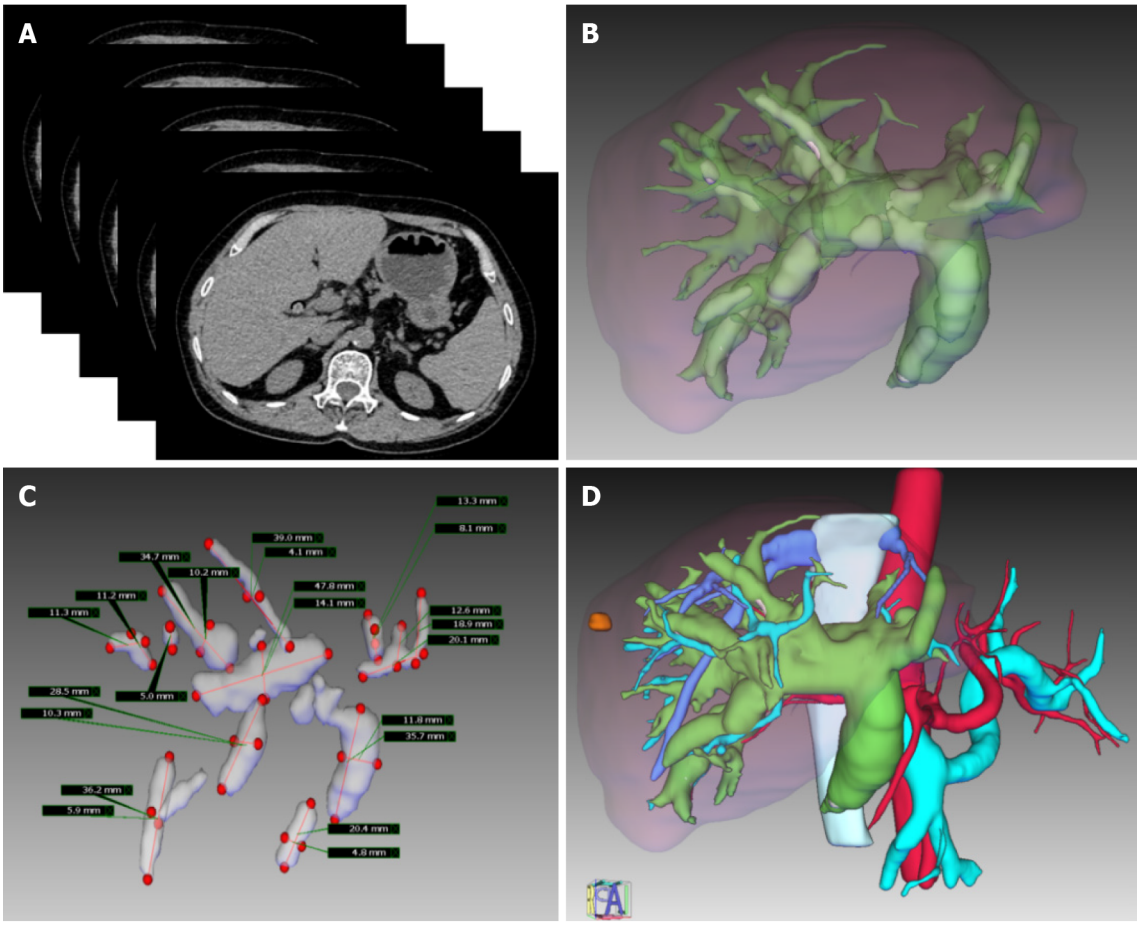


Figure 1 Details of the liver reconstruction model. A: Iterative computed tomography images; B: The bile duct system involves the clearly visible hepatic bile duct segments, and the hepatolithiasis is labeled in yellow; C: Reconstruction of the location and size of the hepatolithiasis; D: A complete liver reconstruction model with intrahepatic bile duct stones.

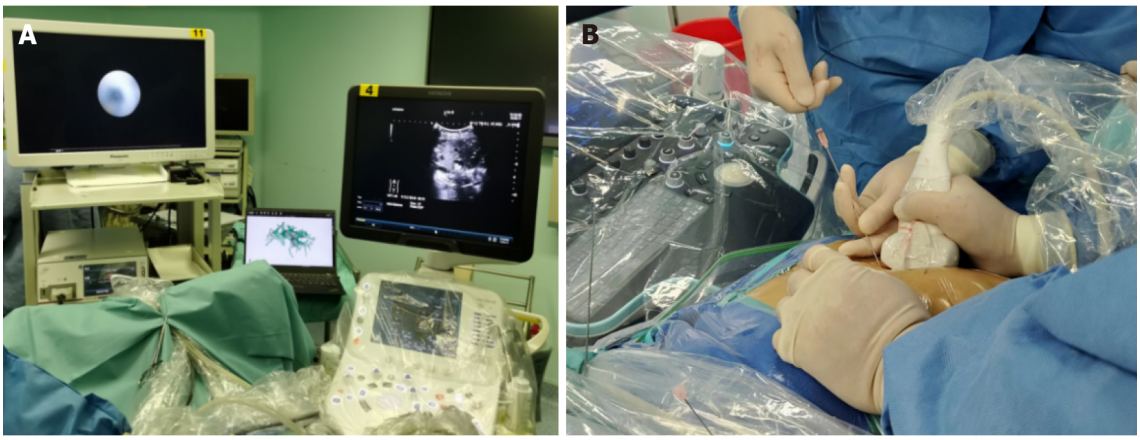


Figure 2 Three-dimensional model combined with real-time B-ultrasonic navigation. A: Combination of three-dimensional reconstruction and B-ultrasound for guiding one-step percutaneous transhepatic cholangioscopic lithotripsy (PTCSL); B: Using the percutaneous transhepatic one-step biliary fistulation technique for one-step PTCSL.

indicate statistical significance.

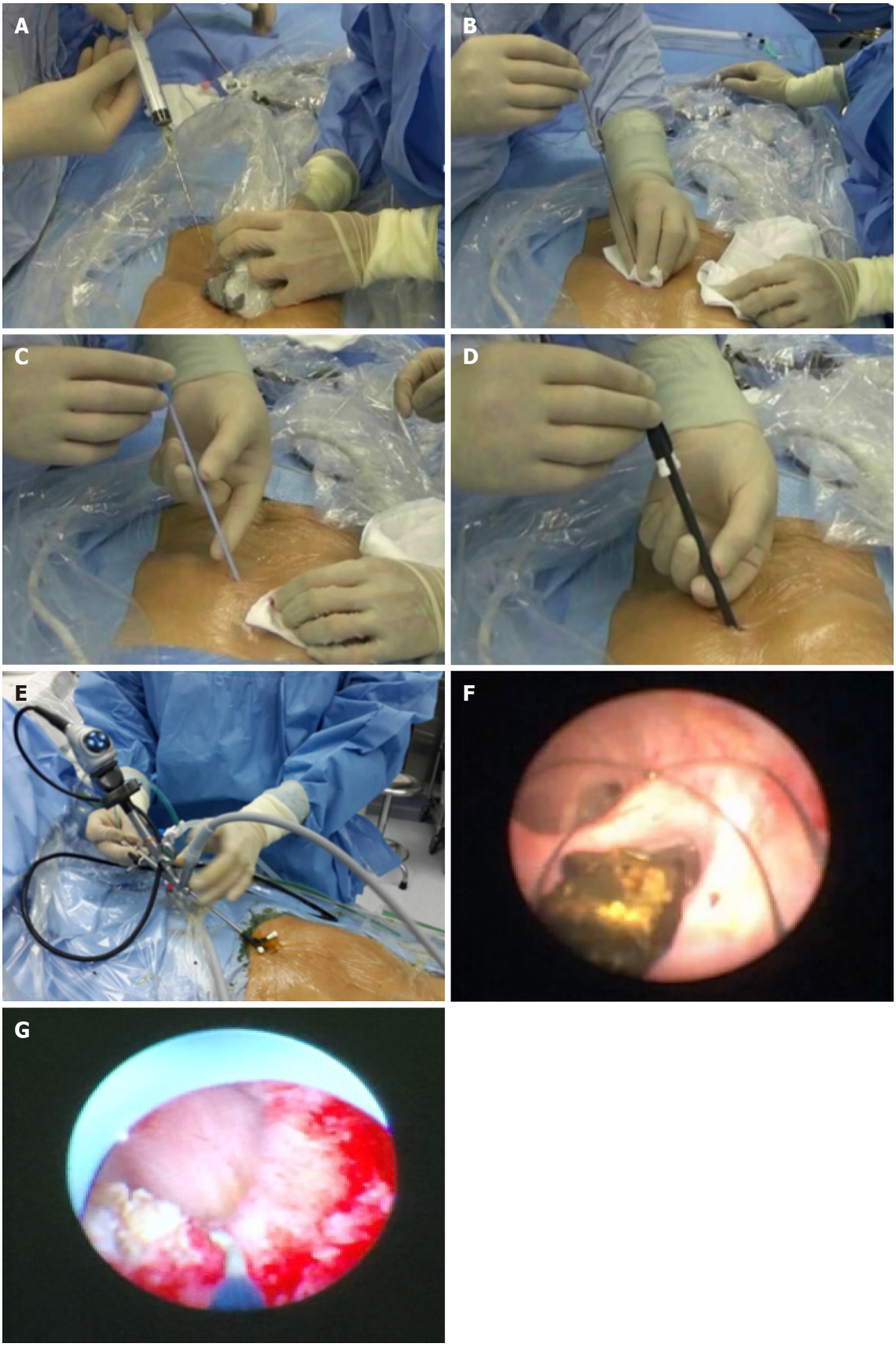


Figure 3 One-step percutaneous transhepatic cholangioscopic lithotripsy using the percutaneous transhepatic one-step biliary fistulation technique. A: Ultrasound-guided puncture; B: Guidewire insertion; C: Fistula dilation D: Sheath insertion; E: Rigid choledochoscope exploration; F: Stone clearance; G: Stricture resolution using an electric knife.

RESULTS

Baseline characteristics of the patients

From October 2016 to October 2022, 140 patients who underwent one-step PTCSL for hepatolithiasis with biliary stricture were eligible and included in the study. There were 54 male and 86 female patients, and the mean age was 19.1 ± 11.4 years. The operation-related data, including age, sex, total bilirubin, direct bilirubin, alanine aminotransferase, Child-Pugh class, stone, and stricture location, were similar between the 2 groups. There was a significant difference in bile duct strictures, with biliary strictures more common in the 3D-PTOBF group (35.7% *vs* 7.1%, $P = 0.001$). A comparison of the specific characteristics of the three groups is listed in Table 1.

Perioperative outcomes

The intraoperative data of the 2 groups are shown in Table 2. The immediate stone clearance ratio in the 3D-PTOBF group was significantly greater than that in the PTOBF group after analysis (88.6% *vs* 27.1%, $P = 0.000$). The postoperative complication rate were significantly lower in the 3D-PTOBF group (8.6% *vs* 41.4%, $P = 0.000$). No significant differences were found between the groups in terms of the operation time and intraoperative blood loss.

Long-term results

The patients were followed up for a period that ranged between 55 and 512 days (mean 55 days). The stricture resolution ratio (97.1% *vs* 78.6%, $P = 0.001$) in the 3D-PTOBF group was significantly better than that in the PTOBF group after analysis. The stone recurrence rate (7.1% *vs* 38.6%, $P = 0.000$) was significantly lower in the 3D-PTOBF group. The final total stone clearance rates were 77.1% (3D-PTOBF) and 78.6% (PTOBF). The reoperation rates were 1.4% (3D-PTOBF) and 12.9% (PTOBF) (Table 3).

DISCUSSION

This study demonstrated that 3D-guided one-step PTCSL using PTOBF is highly effective and safe for the treatment of hepatolithiasis patients with associated biliary strictures. The procedure achieved an immediate stone clearance ratio of 88.6%, a final total stone clearance rate of 77.1%, a stricture resolution rate of 97.1%, a postoperative complication rate of 8.6%, and a stone recurrence rate of 7.1% over a median follow-up period of 55.0 (55.0, 512.0) days.

In one-step PTCSL, 3D visualization of the hepatobiliary tract is carried out beforehand to design an appropriate preoperative plan *via* preoperative CT imaging, in which the calculi and bile duct stricture can be visually located[17]. According to the preoperative plan, percutaneous transhepatic cholangiography is performed intraoperatively under real-time US navigation, and the sinus tract is expanded to an appropriate diameter (16-18 Fr). Then, a protective sheath is used to ensure that biliary endoscopic surgery can be continued immediately without waiting for full recovery of the sinus tissue. This approach significantly shortens the operation and treatment times. Due to the convenience of the operation, multiple punctures can be made in a single operation to create multiple stone-extracting channels, which significantly enhances the success rate of stone extraction[18,19]. This approach is especially suitable when primary intrahepatic bile duct stones are distributed in a diffuse manner. In this case, one stone-extracting channel is not sufficient to clear all the stones.

Previous 2D plane structure generated based on B-US, CT or MRI medical images is very different from the 3D bile duct tree structure. Limited spatial information of 2D structure makes it difficult for determining the spatial locations of the stones, therefore leading to high surgical risk and low efficiency of stone removal. The results of this study are comparable to those of prior studies showing immediate clearance rates of 41%-46% with traditional multisession PTCSL [20]. This reflects the technical complexity of clearing all stones in a single setting from the intrahepatic biliary tree. The limited spatial information available in 2D makes it difficult to determine the spatial locations of the stones, leading to high surgical risk and low stone removal efficiency. However, with 3D visualization technology and adjunctive techniques such as biliary stenting and saline irrigation, the immediate stone clearance ratio in the 3D-PTOBF group was 88.6% (62/70). Three-dimensional visualization can promote one-step PTCSL with a preoperative simulated operation plan, intraoperative digitization of the patient's anatomy, and a predetermined diagnosis.

The long-term recurrence rate of 7.1% (5/70) in our study is also lower than the 15%-40% reported in the literature[21-23]. This may be attributed to the complete initial stone clearance and prolonged biliary stenting performed to prevent stricture recurrence in our patients. Our surgeons employ a rigid choledochoscope to treat stenosed bile ducts and leave an 18 Fr drainage tube in the distal end of the stenosed bile ducts for 6-9 months[11]. Bacterial biofilms and bile sludge cause blockage and restenosis of the bile ducts, so the drainage tubes need to be replaced every 3 months.

The postoperative complication rates were significantly lower in the 3D-PTOBF group than in the PTOBF group (8.6% *vs* 41.4%, $P = 0.000$). The perioperative complication rate of traditional multisession PTCSL is between 15% and 23%. Complex and dangerous situations that may occur in the actual operation can be simulated and estimated by 3D visualization technology prior to the procedure. It is highly important to compare the advantages and disadvantages of different surgical plans through simulation, as this can aid in developing reasonable individual surgical plans and preoperative demonstrations for the patients.

Our study adds to the limited data on the long-term efficacy of one-step PTCSL. Fang *et al*[24] described 3D visualization technology for treating hepatolithiasis patients in 2013. Yang *et al*[20] first described the PTOBF technique in 2013 [20]. However, these studies focused only on immediate outcomes. Our long-term follow-up provides evidence that 3D-guided one-step PTCSL is beneficial and effective for treating complex hepatolithiasis.

Table 1 Comparison of the preoperative data between the two groups, *n* (%)

Variables	3D-PTOBF (*n* = 70)	PTOBF (*n* = 70)	*P* value
Age (year)	50.5 ± 17.3	49.0 ± 17.2	0.608
Sex			
Male	24 (34.3)	30 (42.9)	0.298
Female	46 (65.7)	40 (57.1)	
TBIL (mmol/L)	33.9 ± 32.9	30.8 ± 33.6	0.581
DBIL (mmol/L)	13.3 ± 18.1	13.4 ± 20.3	0.990
ALT (U/L)	59.9 ± 61.7	56.5 ± 57.0	0.791
γ-GGT (U/L)	210.6 ± 262.2	238.0 ± 236.3	0.517
ALB (g/L)	39.3 ± 6.4	37.6 ± 5.8	0.105
PT (second)	14.2 ± 4.5	13.5 ± 1.0	0.174
Child-Pugh score			
Grade A	67 (95.7)	58 (82.9)	0.014
Grade B	3 (4.3)	12 (17.1)	
Stone location			
S1	1 (1.4)	2 (2.9)	0.559
S2	9 (12.9)	12 (17.1)	0.478
S3	11 (15.7)	13 (18.6)	0.654
S4	17 (24.3)	23 (32.9)	0.262
S5	4 (5.7)	5 (7.1)	0.730
S6	12 (17.1)	12 (17.1)	1.0
S7	8 (11.4)	16 (22.9)	0.137
S8	12 (17.1)	17 (24.3)	0.297
Common bile duct	23 (32.9)	13 (18.6)	0.053
Biliary stricture Location			
S1	0	0	1.0
S2	1 (1.4)	8 (11.4)	0.008
S3	8 (11.4)	12 (17.1)	0.334
S4	14 (20.0)	14 (20.0)	1.0
S5	4 (5.7)	6 (8.5)	0.512
S6	11 (15.7)	6 (8.5)	0.196
S7	6 (8.6)	3 (4.2)	0.301
S8	7 (10.0)	8 (11.4)	0.785
Bilio-enteric anastomosis	12 (17.1)	21 (30.0)	0.073
Common bile duct	25 (35.7)	5 (7.1)	0.001

TBIL: Total bilirubin; DBIL: Direct bilirubin; ALT: Alanine aminotransferase; γ-GGT: Gamma-glutamyl transpeptidase; ALB: Albumin; PT: Prothrombin time; 3D: Three-dimensional; PTOBF: Percutaneouas transhepatic one-step biliary fistulation.

Clinically, choledochoscopy can only be performed until maturation of the sinus tract at least 1 week following percutaneous biliary drainage[25]. The one-step approach avoids incremental fistula dilation and reduces overall hospitalization times. We introduced 3D visualization into the one-step PTCSL technique to further show that the reconstructed 3D model can be used to localize lesions with submillimeter accuracy and can therefore contribute to planning an accurate puncture route before surgery and guide the puncture and stone extraction operations during surgery.

Table 2 Patient outcomes, *n* (%)

Variables	3D-PTOBF (*n* = 70)	PTOBF (*n* = 70)	*P* value
Operation time (minute)	55.2 ± 34.0	59.8 ± 37.8	0.448
Intraoperative blood loss (mL)	26.5 ± 52.1	16.4 ± 13.4	0.117
Immediate stone clearance ratio	62 (88.6)	19 (27.1)	0.000[a]
Complications	6 (8.6)	29 (41.4)	0.000[a]
Hemobilia	1 (1.4)	2 (2.9)	0.559
Pulmonary infection	2 (2.9)	4 (5.7)	0.384
Cholangitis	2 (2.9)	14 (20.0)	0.001
Pleural effusion	1 (1.4)	9 (12.9)	0.009

[a]*P* < 0.001.

3D: Three-dimensional; PTOBF: Percutaneous transhepatic one-step biliary fistulation.

Table 3 Long-term results, *n* (%)

Variables	3D-PTOBF (*n* = 70)	PTOBF (*n* = 70)	*P* value
Follow-up time	498.0 ± 373.5	1031.3 ± 573.0	0.000[a]
Final stone clearance ratio	54 (77.1)	55 (78.6)	0.839
Stricture resolution ratio	68 (97.1)	55 (78.6)	0.001
Number of operations	3.0 ± 1.5	2.9 ± 1.8	0.535
Stone recurrence	5 (7.1)	27 (38.6)	0.000[a]
Reoperation rate	1 (1.4)	9 (12.9)	0.009
Late complications	9 (12.9)	9 (12.9)	1.0
Cholangitis	8 (11.4)	7 (10.0)	0.785
Liver failure	1 (1.4)	2 (2.9)	0.559

[a]*P* < 0.001.

3D: Three-dimensional; PTOBF: Percutaneous transhepatic one-step biliary fistulation.

There were still some limitations in this study. This was a single-center study with a limited number of patients; hence, there was some inevitable selection bias between the groups that may have impacted the results.

CONCLUSION

This study confirmed that one-step PTCSL guided by 3D visualization can be used to safely puncture the biliary tract and effectively remove stones, improving the treatment of patients with complicated primary hepatolithiasis. The perioperative and long-term outcomes for these complicated primary hepatolithiasis patients were satisfactory.

FOOTNOTES

Author contributions: Wang P, Li RQ and Ye YQ conceptualized and designed the research; Li EZ, Ding ZW, Fan JM screened patients and acquired clinical data; Cao YW, Wu YX collected blood specimen and performed data analysis; Ye YQ and Cao YW wrote the paper; All the authors have read and approved the final manuscript. Cao YW proposed, designed and conducted data analysis and prepared the first draft of the manuscript. Ye YQ was responsible for patient screening, enrollment, collection of clinical data and blood specimens. Both authors have made crucial and indispensable contributions towards the completion of the project and thus qualified as the co-first authors of the paper.

Supported by The Key Medical Specialty Nurturing Program of Foshan During The 14th Five-Year Plan Period, No. FSPY145205; The Medical Research Project of Foshan Health Bureau, No. 20230814A010024; The Guangzhou Science and Technology Plan Project, No.

202102010251; and the Guangdong Science and Technology Program, No. 2017ZC0222.

Institutional review board statement: This study was approved by the Ethics Committee of The First Hospital of Guangzhou Medical University, No. 09, 2017.

Informed consent statement: Informed consent was obtained from the patients included in this research.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

Country of origin: China

ORCID number: Yong-Qing Ye 0000-0002-3852-0701; Ya-Wen Cao 0000-0003-3036-7594; Rong-Qi Li 0000-0002-0613-8751; Ping Wang 0000-0003-0491-0573; Yi-Xiang Wu 0000-0002-4883-910X.

S-Editor: Li L
L-Editor: A
P-Editor: Yuan YY

REFERENCES

1 **Mori T**, Sugiyama M, Atomi Y. Gallstone disease: Management of intrahepatic stones. *Best Pract Res Clin Gastroenterol* 2006; **20**: 1117-1137 [PMID: 17127192 DOI: 10.1016/j.bpg.2006.05.010]
2 **Otani K**, Shimizu S, Chijiiwa K, Ogawa T, Morisaki T, Sugitani A, Yamaguchi K, Tanaka M. Comparison of treatments for hepatolithiasis: hepatic resection versus cholangioscopic lithotomy. *J Am Coll Surg* 1999; **189**: 177-182 [PMID: 10437840 DOI: 10.1016/S1072-7515(99)00109-X]
3 **Li F**, Zhou Y, Cheng N, Mao H, Jiang L, Li N, Li Q, de Jong MC, Pawlik TM. Epidermal growth factor receptor as a target for anti-proliferative treatment of proliferative cholangitis in hepatolithiasis. *J Surg Res* 2011; **166**: 87-94 [PMID: 20097367 DOI: 10.1016/j.jss.2009.09.058]
4 **Lee JY**, Kim JS, Moon JM, Lim SA, Chung W, Lim EH, Lee BJ, Park JJ, Bak YT. Incidence of Cholangiocarcinoma with or without Previous Resection of Liver for Hepatolithiasis. *Gut Liver* 2013; **7**: 475-479 [PMID: 23898390 DOI: 10.5009/gnl.2013.7.4.475]
5 **Portincasa P**, Moschetta A, Palasciano G. Cholesterol gallstone disease. *Lancet* 2006; **368**: 230-239 [PMID: 16844493 DOI: 10.1016/S0140-6736(06)69044-2]
6 **Lin CC**, Lin PY, Chen YL. Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications. *World J Gastroenterol* 2013; **19**: 375-380 [PMID: 23372360 DOI: 10.3748/wjg.v19.i3.375]
7 **Yoon YI**, Kim KH, Cho HD, Kwon JH, Jung DH, Park GC, Song GW, Ha TY, Lee SG. Long-term perioperative outcomes of pure laparoscopic liver resection versus open liver resection for hepatocellular carcinoma: a retrospective study. *Surg Endosc* 2020; **34**: 796-805 [PMID: 31161292 DOI: 10.1007/s00464-019-06831-w]
8 **Ma S**, Hu S, Gao F, Liang R. Endoscopy Lithotomy for Intrahepatic Gallstones: A Meta-Analysis. *Surg Laparosc Endosc Percutan Tech* 2015; **25**: 269-274 [PMID: 25799257 DOI: 10.1097/SLE.0000000000000138]
9 **Buddingh KT**, Weersma RK, Savenije RA, van Dam GM, Nieuwenhuijs VB. Lower rate of major bile duct injury and increased intraoperative management of common bile duct stones after implementation of routine intraoperative cholangiography. *J Am Coll Surg* 2011; **213**: 267-274 [PMID: 21459631 DOI: 10.1016/j.jamcollsurg.2011.03.004]
10 **Tao H**, Wang P, Sun B, Zhou X, Xie J. One-step Percutaneous Transhepatic Cholangioscopy Combined With High-frequency Needle-knife Electrotomy in Biliary Strictures After Liver Transplantation. *Surg Laparosc Endosc Percutan Tech* 2021; **31**: 787-793 [PMID: 33935263 DOI: 10.1097/SLE.0000000000000946]
11 **Huang MH**, Chen CH, Yang JC, Yang CC, Yeh YH, Chou DA, Mo LR, Yueh SK, Nien CK. Long-term outcome of percutaneous transhepatic cholangioscopic lithotomy for hepatolithiasis. *Am J Gastroenterol* 2003; **98**: 2655-2662 [PMID: 14687812 DOI: 10.1111/j.1572-0241.2003.08770.x]
12 **Liu F**, Cheng Z, Han Z, Yu X, Yu M, Liang P. A three-dimensional visualization preoperative treatment planning system for microwave ablation in liver cancer: a simulated experimental study. *Abdom Radiol (NY)* 2017; **42**: 1788-1793 [PMID: 28161824 DOI: 10.1007/s00261-017-1065-z]
13 **Huang Z**, Zeng S, Zeng X, Wen S, Zhou Y, Cai P, Zhong H, Liu Z, Xiang N, Zhou C, Fang C, Zeng N. Efficacy of hepatectomy for hepatolithiasis using 3D visualization combined with ICG fluorescence imaging: A retrospective cohort study. *World J Surg* 2024; **48**: 1242-1251 [PMID: 38530128 DOI: 10.1002/wjs.12157]
14 **Panwar A**, Das P, Tan LP. 3D Hepatic Organoid-Based Advancements in LIVER Tissue Engineering. *Bioengineering (Basel)* 2021; **8** [PMID: 34821751 DOI: 10.3390/bioengineering8110185]
15 **Gong Y**, Tang Y, Geng Y, Zhou Y, Yu M, Huang B, Sun Z, Tang H, Jian Z, Hou B. Comparative safety and effectiveness of ultrasound-guided radiofrequency ablation combined with preoperative three-dimensional reconstruction versus surgical resection for solitary hepatocellular carcinoma of 3-5 cm. *J Cancer* 2019; **10**: 5568-5574 [PMID: 31632501 DOI: 10.7150/jca.32342]

16 **Yamanaka J**, Saito S, Fujimoto J. Impact of preoperative planning using virtual segmental volumetry on liver resection for hepatocellular carcinoma. *World J Surg* 2007; **31**: 1249-1255 [PMID: 17440774 DOI: 10.1007/s00268-007-9020-8]
17 **Ni ZK**, Lin D, Wang ZQ, Jin HM, Li XW, Li Y, Huang H. Precision Liver Resection: Three-Dimensional Reconstruction Combined with Fluorescence Laparoscopic Imaging. *Surg Innov* 2021; **28**: 71-78 [PMID: 32873180 DOI: 10.1177/1553350620954581]
18 **Ozden I**, Kamiya J, Nagino M, Uesaka K, Sano T, Nimura Y. Clinicoanatomical study on the infraportal bile ducts of segment 3. *World J Surg* 2002; **26**: 1441-1445 [PMID: 12297940 DOI: 10.1007/s00268-002-6544-9]
19 **Kamiya J**, Nagino M, Uesaka K, Sano T, Nimura Y. Clinicoanatomical studies on the dorsal subsegmental bile duct of the right anterior superior segment of the human liver. *Langenbecks Arch Surg* 2003; **388**: 107-111 [PMID: 12684803 DOI: 10.1007/s00423-003-0373-7]
20 **Yang YL**, Zhang C, Zhao G, Wu P, Ma YF, Zhang HW, Shi LJ, Li JY, Lin MJ, Yang SM, Lv Y. Choledochoscopic high-frequency needle-knife electrotomy as an effective treatment for intrahepatic biliary strictures. *J Gastroenterol Hepatol* 2015; **30**: 1438-1443 [PMID: 25765565 DOI: 10.1111/jgh.12951]
21 **Chen C**, Huang M, Yang J, Yang C, Yeh Y, Wu H, Chou D, Yueh S, Nien C. Reappraisal of percutaneous transhepatic cholangioscopic lithotomy for primary hepatolithiasis. *Surg Endosc* 2005; **19**: 505-509 [PMID: 15959714 DOI: 10.1007/s00464-004-8125-5]
22 **Uenishi T**, Hamba H, Takemura S, Oba K, Ogawa M, Yamamoto T, Tanaka S, Kubo S. Outcomes of hepatic resection for hepatolithiasis. *Am J Surg* 2009; **198**: 199-202 [PMID: 19249730 DOI: 10.1016/j.amjsurg.2008.08.020]
23 **Cha SW**. [Management of Intrahepatic Duct Stone]. *Korean J Gastroenterol* 2018; **71**: 247-252 [PMID: 29791982 DOI: 10.4166/kjg.2018.71.5.247]
24 **Fang CH**, Liu J, Fan YF, Yang J, Xiang N, Zeng N. Outcomes of hepatectomy for hepatolithiasis based on 3-dimensional reconstruction technique. *J Am Coll Surg* 2013; **217**: 280-288 [PMID: 23870220 DOI: 10.1016/j.jamcollsurg.2013.03.017]
25 **Cheung MT**, Wai SH, Kwok PC. Percutaneous transhepatic choledochoscopic removal of intrahepatic stones. *Br J Surg* 2003; **90**: 1409-1415 [PMID: 14598423 DOI: 10.1002/bjs.4327]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3403-3417

DOI: 10.3748/wjg.v30.i28.3403

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Retrospective Study

GATIS score for predicting the prognosis of rectal neuroendocrine neoplasms: A Chinese multicenter study of 12-year experience

Xin-Yu Zeng, Ming Zhong, Guo-Le Lin, Cheng-Guo Li, Wei-Zhong Jiang, Wei Zhang, Li-Jian Xia, Mao-Jun Di, Hong-Xue Wu, Xiao-Feng Liao, Yue-Ming Sun, Min-Hao Yu, Kai-Xiong Tao, Yong Li, Rui Zhang, Peng Zhang

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade C, Grade D

Novelty: Grade A, Grade B, Grade B, Grade C

Creativity or Innovation: Grade A, Grade B, Grade B, Grade C

Scientific Significance: Grade A, Grade B, Grade B, Grade C

P-Reviewer: Ono T; Singh A; Sultana N

Received: March 5, 2024

Revised: June 4, 2024

Accepted: July 10, 2024

Published online: July 28, 2024

Processing time: 140 Days and 20.9 Hours

Xin-Yu Zeng, Cheng-Guo Li, Kai-Xiong Tao, Peng Zhang, Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430074, Hubei Province, China

Ming Zhong, Department of Gastrointestinal Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China

Guo-Le Lin, Department of General Surgery, Peking Union Medical College Hospital, Peking Union Medical College, Beijing 100730, China

Wei-Zhong Jiang, Department of Colorectal Surgery, Fujian Medical University, Fuzhou 350401, Fujian Province, China

Wei Zhang, Department of Colorectal Surgery, Changhai Hospital, Shanghai 200433, China

Li-Jian Xia, Department of Gastrointestinal Surgery, The First Affiliated Hospital of Shandong First Medical University, Jinan 250118, Shandong Province, China

Mao-Jun Di, Department of Gastrointestinal Surgery, Taihe Hospital, Hubei University of Medicine, Shiyan 442099, Hubei Province, China

Hong-Xue Wu, Department of Gastrointestinal Surgery I Section, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China

Xiao-Feng Liao, Department of General Surgery, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang 441021, Hubei Province, China

Yue-Ming Sun, Department of Colorectal Surgery, Jiangsu Province Hospital, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Min-Hao Yu, Department of Gastrointestinal Surgery, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China

Yong Li, Department of General Surgery, Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou 510080, Guangdong Province, China

Rui Zhang, Department of Colorectal Surgery, Liaoning Cancer Hospital, Shenyang 110042, Liaoning Province, China

Co-first authors: Xin-Yu Zeng and Ming Zhong.

Co-corresponding authors: Rui Zhang and Peng Zhang.

Corresponding author: Peng Zhang, MD, Surgeon, Department of Gastrointestinal Surgery, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1277 Jiefang Avenue, Wuhan 430074, Hubei Province, China.
zhangpengwh@hust.edu.cn

Abstract

BACKGROUND

There is currently a shortage of accurate, efficient, and precise predictive instruments for rectal neuroendocrine neoplasms (NENs).

AIM

To develop a predictive model for individuals with rectal NENs (R-NENs) using data from a large cohort.

METHODS

Data from patients with primary R-NENs were retrospectively collected from 17 large-scale referral medical centers in China. Random forest and Cox proportional hazard models were used to identify the risk factors for overall survival and progression-free survival, and two nomograms were constructed.

RESULTS

A total of 1408 patients with R-NENs were included. Tumor grade, T stage, tumor size, age, and a prognostic nutritional index were important risk factors for prognosis. The GATIS score was calculated based on these five indicators. For overall survival prediction, the respective C-indexes in the training set were 0.915 (95% confidence interval: 0.866-0.964) for overall survival prediction and 0.908 (95% confidence interval: 0.872-0.944) for progression-free survival prediction. According to decision curve analysis, net benefit of the GATIS score was higher than that of a single factor. The time-dependent area under the receiver operating characteristic curve showed that the predictive power of the GATIS score was higher than that of the TNM stage and pathological grade at all time periods.

CONCLUSION

The GATIS score had a good predictive effect on the prognosis of patients with R-NENs, with efficacy superior to that of the World Health Organization grade and TNM stage.

Key Words: Rectal neuroendocrine neoplasm; Nomogram; Random forest; Prognosis; Overall survival; Progression-free survival

Core Tip: We utilized the data of 1408 patients with rectal neuroendocrine neoplasms from a large multicenter database of 17 large-scale Chinese medical centers. We found that tumor grade, T stage, tumor size, age, and a prognostic nutritional index were independent predictors of prognosis in patients with rectal neuroendocrine neoplasms. In addition, we constructed the GATIS score for overall survival and progression-free survival in these patients, which had a C-index of 0.915 for overall survival and 0.908 for progression-free survival; moreover, it showed a better predictive power than that of the TNM stage and pathological grade.

Citation: Zeng XY, Zhong M, Lin GL, Li CG, Jiang WZ, Zhang W, Xia LJ, Di MJ, Wu HX, Liao XF, Sun YM, Yu MH, Tao KX, Li Y, Zhang R, Zhang P. GATIS score for predicting the prognosis of rectal neuroendocrine neoplasms: A Chinese multicenter study of 12-year experience. *World J Gastroenterol* 2024; 30(28): 3403-3417
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3403.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3403

INTRODUCTION

Neuroendocrine neoplasms (NENs) are rare tumors that originate from embryonic neuroendocrine cells and are characterized by neuroendocrine markers[1,2]. They can occur in various organs throughout the body, with the rectum being a common site of incidence (1.04 per 100000 people)[1,2]. Depending on the presence of hormone-related symptoms, NENs can be divided into functional and non-functional types. Rectal NENs (R-NENs) are mostly non-functional[3]. The overall prognosis of R-NENs is favorable, with a 5-year survival rate as high as 90%[4].

Currently, World Health Organization (WHO) classification and TNM staging are the two most commonly used criteria for the prognosis of NENs[5,6]. According to the morphology and malignant potential of the tumor cells, the WHO 2010 criteria classify R-NENs as G1, G2, or G3. G1 and G2 are neuroendocrine tumors (NETs) with low malignant potential, whereas G3 is usually a neuroendocrine carcinoma (NEC) or mixed adenoneuroendocrine carcinoma (MANEC) with high malignant potential[5]. In recent years, the WHO 2019 classification has also classified some NETs with high malignant potential as G3 to better stratify the prognosis; however, the evaluation criteria remain controversial[7]. Additionally, the WHO classification criteria are not effective in predicting the prognosis of patients with R-NENs[8,9]. TNM staging has a distinct grading system for NET, whereas NEC/MANEC suggests the utilization of colorectal cancer criteria, which are complex in classification and have limited predictive power for patient prognosis. Consequently, developing an effective and precise prognostic assessment tool to guide the clinical diagnosis and treatment of R-NENs is essential.

Nomogram models based on multivariate regression analysis and integrating multiple predictors are useful for the prognostic evaluation of various malignant tumors[10-12]. However, due to the low incidence of R-NENs, a nomogram model with a large sample size is lacking. In this study, we retrospectively collected R-NENs data from 17 large referral hospitals in China and constructed a prognostic model using the Cox proportional hazard model and random forest method. Internal and external validations were conducted to assist in prognosis prediction, diagnosis, and treatment of patients with R-NENs.

MATERIALS AND METHODS

Patient inclusion

Data of patients with R-NENs admitted to 17 major referral hospitals in China between January 2010 and April 2022 were retrospectively collected. The inclusion criterion was a pathological diagnosis of R-NENs, while the exclusion criterion was simultaneous or metachronous malignancies at other sites (Figure 1). Patient data were collected and analyzed, and the following patients were also excluded during the nomogram construction phase: (1) Patients with metastases at first diagnosis; (2) Patients undergoing neoadjuvant therapy; and (3) Patients with incomplete clinicopathological and follow-up information (Figure 1).

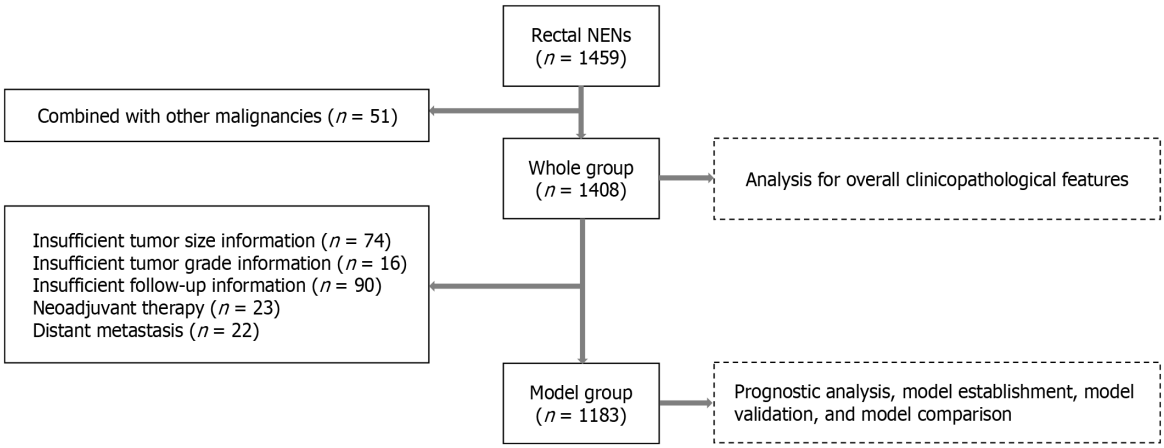


Figure 1 Patient selection flowchart. NENs: Neuroendocrine neoplasms.

Data collection and definition

The clinicopathological characteristics of the patients, including sex, age, preoperative hematologic examination, surgical information, postoperative complications, and pathological conditions were collected. Preoperative hematologic examination indicators were collected from patients at the initial diagnosis or within seven days before surgery. The prognostic nutritional index (PNI) was calculated as serum albumin plus five times the peripheral blood lymphocyte count. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were determined as the ratios of peripheral blood neutrophils and platelets-to-lymphocytes, respectively. The WHO 2010 standard was used to classify R-NENs, while the American Joint Committee on Cancer 8th TNM staging criteria was used for NET, NEC, and MANEC staging[5,6]. Each center conducted individual follow-ups, with the last follow-up in July 2022. The primary outcome measures were overall survival (OS) and progression-free survival (PFS), defined as the time from initial diagnosis to death from any cause or final follow-up, and the time from initial diagnosis to disease progression, patient death (whichever occurred earlier), or final follow-up.

Statistical analysis

SPSS version 25.0 (IBM, Armonk, New York, United States) was used for statistical analysis. Normally distributed data

are expressed as mean ± SD, whereas non-normally distributed data are expressed as median and interquartile range. The *t*-test was used to compare normally distributed data, and the Mann-Whitney *U*-test was used to determine non-normally distributed data. Truncated values, such as PNI, NLR, and PLR, were determined using the X-tile software. Kaplan-Meier and Log-rank tests were employed to map and compare prognostic differences in patients. The Cox proportional hazards model was used to identify independent risk factors for OS and PFS, and the random forest model was used to assess the correlation between each factor and prognosis. Both the random forest and nomogram models were constructed using R 4.0.0. Statistical significance was set at $P < 0.05$ (two-tailed). The statistical methods used in this study were reviewed by Yong Gan from the Huazhong University of Science and Technology.

RESULTS

Patient status

According to the inclusion-exclusion criteria, a total of 1408 patients with R-NENs were included in this study, of whom 591 (42.0%) were female and 817 (58.0%) were male, with a mean age of 51.9 ± 12.1 years. Twenty-three (1.6%) patients received neoadjuvant therapy and 1360 (96.6%) patients underwent complete tumor resection, of which 650 (47.8%) underwent surgery and 710 (52.2%) underwent endoscopic resection. The mean tumor size was 1.3 ± 1.2 cm. The pathological classification was NET in 1307 (92.8%), NEC in 71 (5.0%), MANEC in 14 (1.0%), and unknown in 16 (1.1%) patients. In total, 1149 (81.6%) were classified as G1, 158 (11.2%) as G2, 85 (6.0%) as G3, and 16 (1.1%) as unknown. Of the total group, 1150 (81.7%) had T1, 140 (9.9%) had T2, 82 (5.8%) had T3, and 36 (2.6%) had T4 disease. A total of 103 patients (7.3%) had lymph node metastases, and 61 (4.3%) had lymphovascular invasion. Ninety patients (6.4%) received postoperative adjuvant therapy. Table 1 summarizes the demographic and clinicopathological characteristics of the patients.

Nomogram construction and validation for OS prediction

Patients who did not receive neoadjuvant therapy, did not have distant metastases at diagnosis, or had complete data were screened for nomogram construction (Figure 1). A total of 1183 patients with R-NENs were included in this study. The median follow-up time for the entire group was 34 months, and 44 patients (3.7%) died during the follow-up period. The 1-year, 3-year, and 5-year OS rates were 98.9%, 96.2%, and 94.7%, respectively. The patients were randomly divided into a training dataset (819 cases, 69.2%) and a validation set (364 cases, 30.8%) in a 7:3 ratio. The clinicopathological characteristics of the two groups of patients are shown in Table 2. There was no significant difference at baseline.

The median follow-up time of patients in the training dataset was 34 months, and the 1-year, 3-year, and 5-year OS rates were 98.5%, 95.4%, and 94.2%, respectively. The optimal cut-off values of the PLR, NLR, and PNI hematologic indicators for prognosis according to X-tile were 128.00, 1.45, and 46.57, respectively. A univariate analysis suggested that age, PNI, tumor size, pathological type and grade, T stage, lymph node metastasis, TNM stage, lymphovascular invasion, CgA staining, and adjuvant therapy were associated with prognosis. In the multivariate analysis, considering the collinearity of the TNM stage with the T stage and LNM, TNM stage was not included. The multivariate analysis showed that PNI [hazard ratio (HR) = 0.233, 95% confidence interval (CI): 0.109-0.499, $P < 0.001$], tumor size (1-2 cm, HR = 1.706, 95%CI: 0.538-5.413, $P = 0.364$; > 2 cm, HR = 3.349, 95%CI: 1.314-8.533, $P = 0.011$) and pathological grade (G2, HR = 4.211, 95%CI: 1.603-11.059, $P = 0.004$; G3, HR = 30.681, 95%CI: 10.821-86.988, $P < 0.001$) were independent factors for OS. The results of the log-rank test and Cox proportional hazards regression analysis for OS are shown in Table 3.

The random forest model was used for further screening and 1000 random trees were used. The model tended to stabilize when the size of the training tree reached approximately 100. The importance of each clinicopathological factor was assessed and ranked. The five indicators of minimal depth were tumor grade, T stage, tumor size, patient age, and PNI. After considering the screening results of the two models, the five indicators, namely, tumor grade, T stage, tumor size, age, and PNI, were included in the construction of the prognostic nomogram for OS in patients (Figure 2A).

Bootstrap resampling (1000 iterations) was used to verify the accuracy of the GATIS score; the C-index of OS in the training set was 0.915 (95%CI: 0.866-0.964), while the C-index in the validation set was 0.812 (0.702-0.923). The similarity between the actual and predicted survival rates based on the GATIS scores was verified using a calibration plot. The predicted 3-year and 5-year OS rates were consistent with the actual survival rates within a 10% error range indicated by the dotted line. Similar results were obtained for the validation set (Figure 3A-D). The decision curve analysis of the training dataset showed that the net benefit of the GATIS score was greater than that of a single factor (Figure 3E). The time-dependent area under the receiver operating characteristic curve (TD-AUC) showed that the predictive power of the GATIS score for OS was higher than that of the TNM stage and pathological grade at all time points (Figure 3F).

Nomogram construction and validation for PFS prediction

A univariate analysis suggested that age, NLR, PNI, tumor size, pathological type and grade, T stage, lymph node metastasis, TNM stage, lymphovascular invasion, and adjuvant therapy were associated with PFS. In the multivariate analysis, considering the collinearity of the TNM stage with T stage and LNM, TNM stage was not included. A multivariate analysis showed that PNI (HR = 0.365, 95%CI: 0.135-0.583, $P = 0.010$), pathological grade (G2, HR = 2.937, 95%CI: 1.011-8.535, $P = 0.048$; G3, HR = 7.126, 95%CI: 2.685-18.913, $P < 0.001$) and T stage (T2, HR = 2.136, 95%CI: 1.236-5.362, $P = 0.025$; T3, HR = 6.653, 95%CI: 2.368-15.362, $P = 0.001$; T4, HR = 8.365, 95%CI: 3.325-13.325, $P < 0.001$) were independent factors for PFS. The results of the log-rank test and Cox proportional hazards regression analysis for PFS are shown in Table 3.

Table 1 Clinicopathological data of patients with rectal neuroendocrine neoplasms, *n* (%)

	All (*n* = 1408)	*n*
Sex		1408
Female	591 (42.0)	
Male	817 (58.0)	
Age, year	51.9 (12.1)	1408
Size (cm)		1408
≤ 1	872 (61.9)	
1-2	291 (20.7)	
> 2	171 (12.1)	
NA	74 (5.3)	
Size (cm)	1.3 (1.2)	1334
Neoadjuvant therapy		1408
No	1385 (98.4)	
Yes	23 (1.6)	
Complete resection		
No	48 (3.4)	
Yes	1360 (96.6)	
Pathological type		1408
NET	1307 (92.8)	
NEC	71 (5.0)	
MANEC	14 (1.0)	
NA	16 (1.1)	
Pathological grade,		1408
G1	1149 (81.6)	
G2	158 (11.2)	
G3	85 (6.0)	
NA	16 (1.1)	
T stage		1408
T1	1150 (81.7)	
T2	140 (9.9)	
T3	82 (5.8)	
T4	36 (2.6)	
Lymphatic metastasis		1408
No	1305 (92.7)	
Yes	103 (7.3)	
TNM stage		1408
I	1133 (80.5)	
II	130 (9.2)	
III	92 (6.5)	
IV	53 (3.8)	
Lymph vascular invasion		1408
No	1347 (95.7)	

Yes	61 (4.3)	
CgA		1408
Negative	1046 (74.3)	
Positive	362 (25.7)	
Syn		1408
Negative	381 (27.1)	
Positive	1027 (72.9)	
Adjuvant therapy		1408
No	1318 (93.6)	
Yes	90 (6.4)	

NET: Neuroendocrine tumor; NEC: Neuroendocrine carcinoma; MANEC: Mixed adenoneuroendocrine carcinoma; CgA: Chromogranin A; Syn: Synaptophysin.

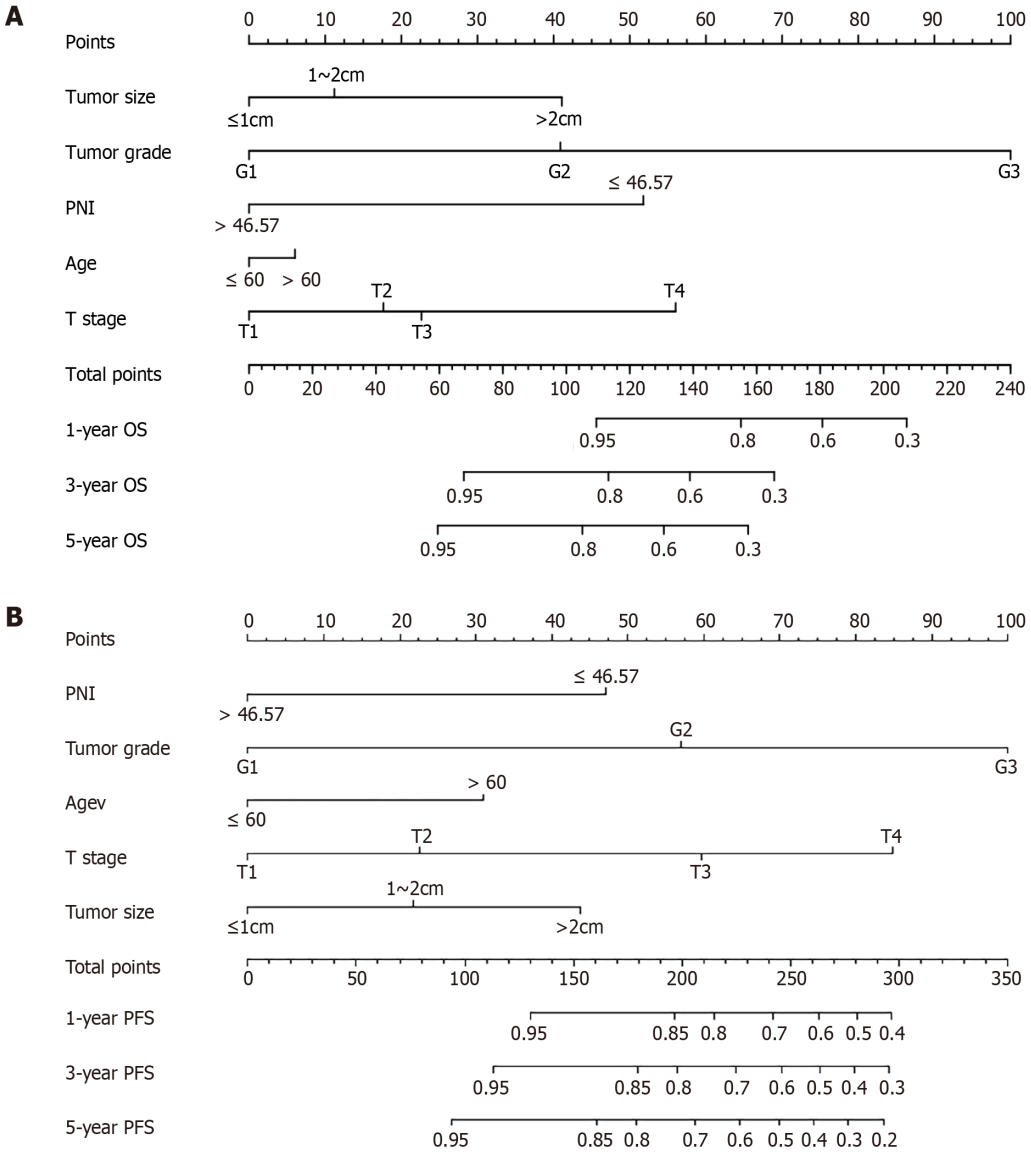


Figure 2 The GATIS score. A: Nomogram for overall survival prediction; B: Nomogram for progression free survival prediction. PNI: Prognostic nutritional index; OS: Overall survival; PFS: Progression free survival.

Table 2 Characteristics of patients with rectal neuroendocrine neoplasms from training database and validation set (2010-2022), *n* (%)

	All	Training set	Validation set	*P* value
	n = 1183	*n* = 819	*n* = 364	
Sex				0.402
Female	504 (42.6)	356 (43.5)	148 (40.7)	
Male	679 (57.4)	463 (56.5)	216 (59.3)	
Age, year	51.7 (12.0)	51.5 (12.0)	52.3 (12.0)	0.291
Size, year	1.1 (1.0)	1.1 (1.0)	1.2 (1.2)	0.187
Size (cm)				0.300
≤ 1	819 (69.2)	568 (69.4)	251 (69.0)	
1-2	246 (20.8)	176 (21.5)	70 (19.2)	
> 2	118 (10.0)	75 (9.2)	43 (11.8)	
PLR	126.1 (52.3)	126.6 (54.3)	124.9 (47.6)	0.588
NLR	2.3 (5.1)	2.2 (2.5)	2.5 (8.4)	0.559
PNI	54.1 (6.8)	54.2 (6.9)	53.9 (6.5)	0.455
Pathological type				0.824
NET	1134 (95.9)	786 (96.0)	348 (95.6)	
NEC	44 (3.7)	29 (3.5)	15 (4.1)	
MANEC	5 (0.4)	4 (0.5)	1 (0.3)	
Pathological grade				0.876
G1	1020 (86.2)	705 (86.1)	315 (86.5)	
G2	114 (9.6)	81 (9.9)	33 (9.1)	
G3	49 (4.1)	33 (4.0)	16 (4.4)	
T stage				0.358
T1	1010 (85.4)	707 (86.3)	303 (83.2)	
T2	111 (9.4)	74 (9.0)	37 (10.2)	
T3	45 (3.8)	29 (3.5)	16 (4.4)	
T4	17 (1.4)	9 (1.1)	8 (2.2)	
Lymphatic metastasis				0.290
No	1107 (93.6)	771 (94.1)	336 (92.3)	
Yes	76 (6.4)	48 (5.9)	28 (7.7)	
TNM stage				0.227
I	996 (84.2)	699 (85.3)	297 (81.6)	
II	108 (9.1)	71 (8.7)	37 (10.2)	
III	79 (6.7)	49 (6.0)	30 (8.2)	
Lymph vascular invasion				0.252
No	1149 (97.1)	799 (97.6)	350 (96.2)	
Yes	34 (2.9)	20 (2.4)	14 (3.8)	
CgA				0.100
Negative	871 (73.6)	615 (75.1)	256 (70.3)	
Positive	312 (26.4)	204 (24.9)	108 (29.7)	
Syn				0.393
Negative	297 (25.1)	212 (25.9)	85 (23.4)	

Positive	886 (74.9)	607 (74.1)	279 (76.6)	
Adjuvant therapy				0.286
No	1125 (95.1)	783 (95.6)	342 (94.0)	
Yes	58 (4.9)	36 (4.4)	22 (6.0)	

PLR: Platelet-to-lymphocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PNI: Prognostic nutritional index; NET: Neuroendocrine tumor; NEC: Neuroendocrine carcinoma; MANEC: Mixed adenoneuroendocrine carcinoma; CgA: Chromogranin A; Syn: Synaptophysin.

Table 3 Univariate and multivariate analysis of clinicopathologic variables in relation to overall survival and progression free survival in patients with rectal neuroendocrine neoplasms

	Univariate analysis[1]		Multivariate analysis[1]		Univariate analysis[2]		Multivariate analysis[2]	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Sex (male)	1.148 (0.588-2.243)	0.686			1.168 (0.648-2.106)	0.605		
Age (> 60)	2.194 (1.115-4.317)	0.023			2.875 (1.505-5.495)	< 0.001		
PLR (> 128.00)	1.592 (0.818-3.100)	0.171			1.313 (0.668-2.577)	0.402		
NLR (> 1.45)	1.629 (0.740-3.585)	0.226			2.322 (1.106-4.874)	0.007		
PNI (> 46.57)	0.144 (0.071-0.295)	0.023	0.233 (0.109-0.499)	< 0.001	0.130 (0.043-0.392)	< 0.001	0.365 (0.135-0.583)	0.010
Size (cm)								
≤ 1	-	Ref.	-	Ref.	-	Ref.		
1-2	6.190 (2.591-14.790)	< 0.001	1.706 (0.538-5.413)	0.364	4.960 (1.956-12.580)	0.001		
> 2	13.439 (5.563-32.468)	< 0.001	3.349 (1.314-8.533)	0.011	10.240 (5.200-25.200)	< 0.001		
Pathological type								
NET	-	Ref.			-	Ref.		
NEC	31.177 (14.961-64.968)	< 0.001			15.121 (5.590-59.102)	< 0.001		
MANEC	27.899 (6.399-121.627)	< 0.001			26.252 (6.203-85.32)	< 0.001		
Pathological grade								
G1	-	Ref.	-	Ref.	-	Ref.	-	Ref.
G2	6.380 (2.582-15.768)	0.001	4.211 (1.603-11.059)	0.004	8.680 (2.830-20.602)	< 0.001	2.937 (1.011-8.535)	0.048
G3	48.175 (21.495-107.969)	< 0.001	30.681 (10.821-86.988)	< 0.001	25.362 (6.362-62.325)	< 0.001	7.126 (2.685-18.913)	< 0.001
T stage								
T1	-	Ref.			-	Ref.	-	Ref.
T2	3.234 (1.169-8.945)	0.024			13.370 (3.794-47.086)	< 0.001	2.136 (1.236-5.362)	0.025
T3	17.473 (7.846-38.915)	< 0.001			12.630 (2.635-18.354)	< 0.001	6.653 (2.368-15.362)	0.001
T4	23.081 (8.328-63.964)	< 0.001			26.368 (5.364-53.258)	< 0.001	8.365 (3.325-13.325)	< 0.001
Lymph node metastasis (yes)	11.387 (5.786-22.409)	< 0.001			12.440 (4.172-37.112)	< 0.001		

TNM stage				
I	-	Ref.	-	Ref.
II	3.280 (1.188-9.056)	0.022	6.839 (1.692-27.650)	0.006
III	14.524 (7.080-29.797)	< 0.001	17.792 (5.982-52.932)	< 0.001
Lymph vascular invasion (yes)	5.753 (2.013-16.447)	0.001	5.899 (1.213-28.703)	< 0.001
CgA (positive)	1.992 (1.012-3.919)	0.046	1.188 (0.608-2.322)	0.512
Syn (positive)	1.161 (0.541-2.492)	0.702	0.960 (0.483-1.908)	0.919
Adjuvant therapy (yes)	10.775 (5.161-22.499)	< 0.001	13.110 (3.737-45.962)	< 0.001

[1]Overall survival.

[2]Progress free survival.

PLR: Platelet-to-lymphocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PNI: Prognostic nutritional index; NET: Neuroendocrine tumor; NEC: Neuroendocrine carcinoma; MANEC: Mixed adenoneuroendocrine carcinoma; CgA: Chromogranin A; Syn: Synaptophysin; HR: Hazard ratio; CI: confidence interval.

The random forest model was used for further screening and 1000 random trees were used. The model tended to stabilize when the size of the training tree reached approximately 100. The importance of each clinicopathological factor was assessed and ranked. The five indicators of minimal depth were tumor grade, T stage, tumor size, patient age, and PNI. After considering the screening results of the two models, the five indicators of tumor grade, T stage, tumor size, age, and PNI were included in the construction of the prognostic nomogram of PFS for R-NENs patients (Figure 2B).

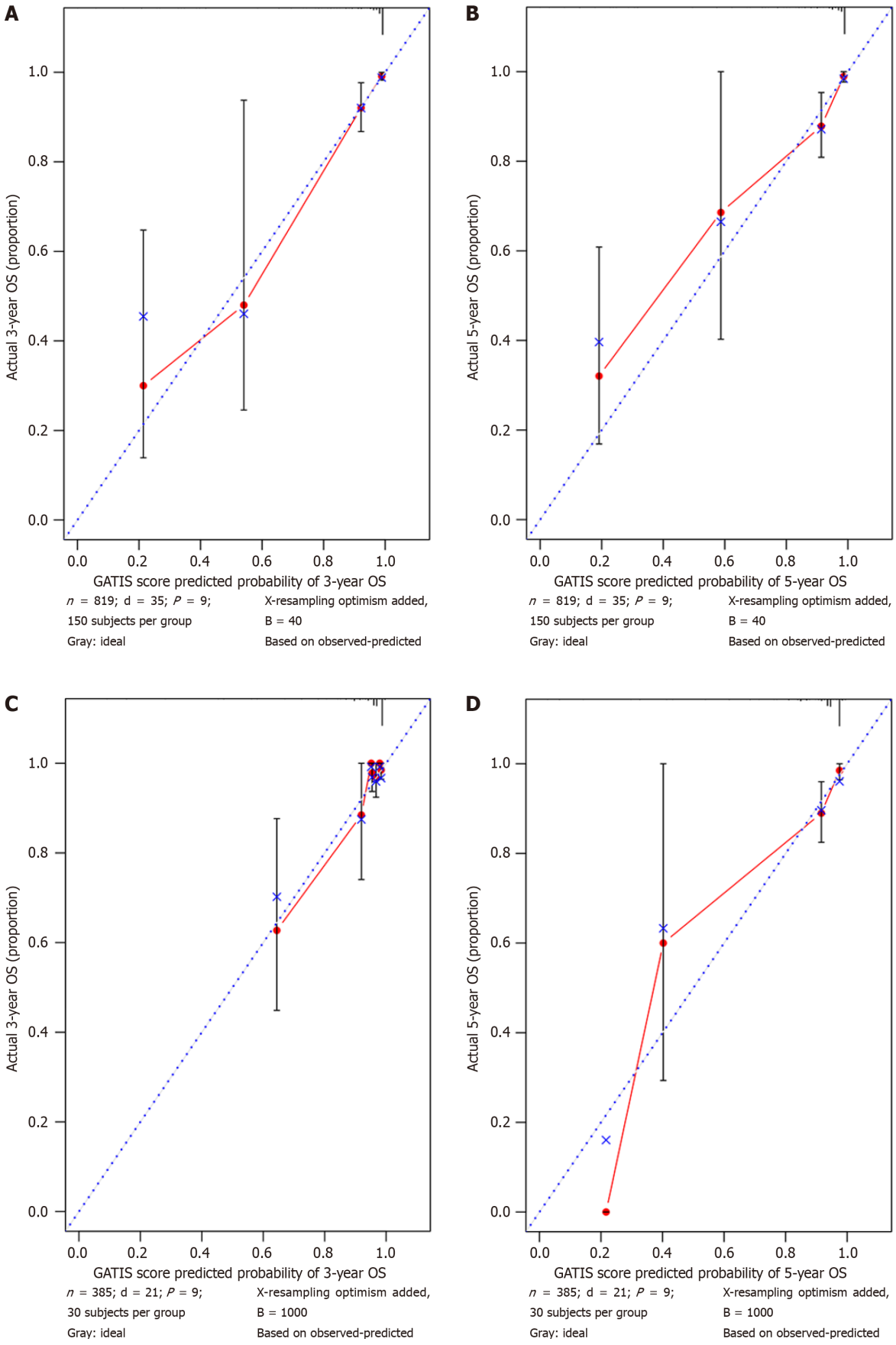
Bootstrap resampling (1000 iterations) was used to verify the accuracy of the GATIS score; the C-index of PFS in the training set was 0.908 (95%CI: 0.872-0.944), while the C-index in the validation set was 0.865 (0.756-0.909). The similarity between the actual and predicted survival rates based on the GATIS scores was verified using a calibration plot. The predicted 3-year and 5-year PFS rates were consistent with the actual survival rates within the 10% error range indicated by the dotted line. Similar results were obtained for the validation set (Figure 4A-D). The decision curve analysis curve of the training dataset showed that the net benefit of the GATIS score was greater than that of a single factor (Figure 4E). The TD-AUC results showed that the predictive power of the GATIS score for PFS was higher than that of the TNM stage and pathological grade at all time points (Figure 4F).

DISCUSSION

Rectal NETs are rare and more common in the elderly, with a poorer prognosis in younger individuals[13,14]. There is no evidence of sex or familial aggregation. Generally, R-NENs have a small diameter, and most patients are classified as G1, with a good prognosis[13,14]. The 5-year survival rate can reach 90%[4]. In this study, the average tumor size was 1.3 cm, and 81.6% of the patients had a pathological grade of G1. The 5-year OS rate was 94.7%, which is consistent with that in previous reports.

Due to their rarity and limited sample size, the prognostic factors of R-NENs, particularly the preoperative hematologic factors, have not been fully elucidated. In this study, we found that the PNI was an independent prognostic factor in patients with R-NENs. PNI, a nutrition-related indicator, has been confirmed to be inversely correlated with patient prognosis in various tumors[15,16]. Our findings suggest that individuals with an elevated preoperative PNI, indicating adequate nutritional status, exhibit a reduced risk of mortality. This finding suggests that preoperative nutritional improvement in patients with R-NENs can help improve their prognosis. Additionally, we found that a large tumor diameter and a high pathological grade were poor prognostic factors, which is consistent with previous studies[17,18].

A random forest is an ensemble algorithm composed of decision trees belonging to the bagging (Bootstrap Aggregation) method of ensemble learning. It is composed of numerous decision trees with no correlation between them. When a new input sample is presented for classification, each decision tree in the forest is judged and classified separately, and the most frequent result is considered the final result. A random forest can assess feature importance, interactions between features, and balance errors; however, it may overfit noisy classification or regression problems. It has been used for the prediction and model construction of various tumors with satisfactory results[19-21]. To the best of our knowledge, this is the first randomized forest study on patients with R-NENs. We analyzed the correlation between clinicopathological factors and patient' prognoses and determined the importance of these factors. Five representative indicators were identified: Tumor grade, T stage, tumor size, age, and PNI.


A
Actual 3-year OS (proportion)
GATIS score predicted probability of 3-year OS
n = 819; d = 35; P = 9;
150 subjects per group
Gray: ideal
X-resampling optimism added,
B = 40
Based on observed-predicted
B
Actual 5-year OS (proportion)
GATIS score predicted probability of 5-year OS
n = 819; d = 35; P = 9;
150 subjects per group
Gray: ideal
X-resampling optimism added,
B = 40
Based on observed-predicted
C
Actual 3-year OS (proportion)
GATIS score predicted probability of 3-year OS
n = 385; d = 21; P = 9;
30 subjects per group
Gray: ideal
X-resampling optimism added,
B = 1000
Based on observed-predicted
D
Actual 5-year OS (proportion)
GATIS score predicted probability of 5-year OS
n = 385; d = 21; P = 9;
30 subjects per group
Gray: ideal
X-resampling optimism added,
B = 1000
Based on observed-predicted

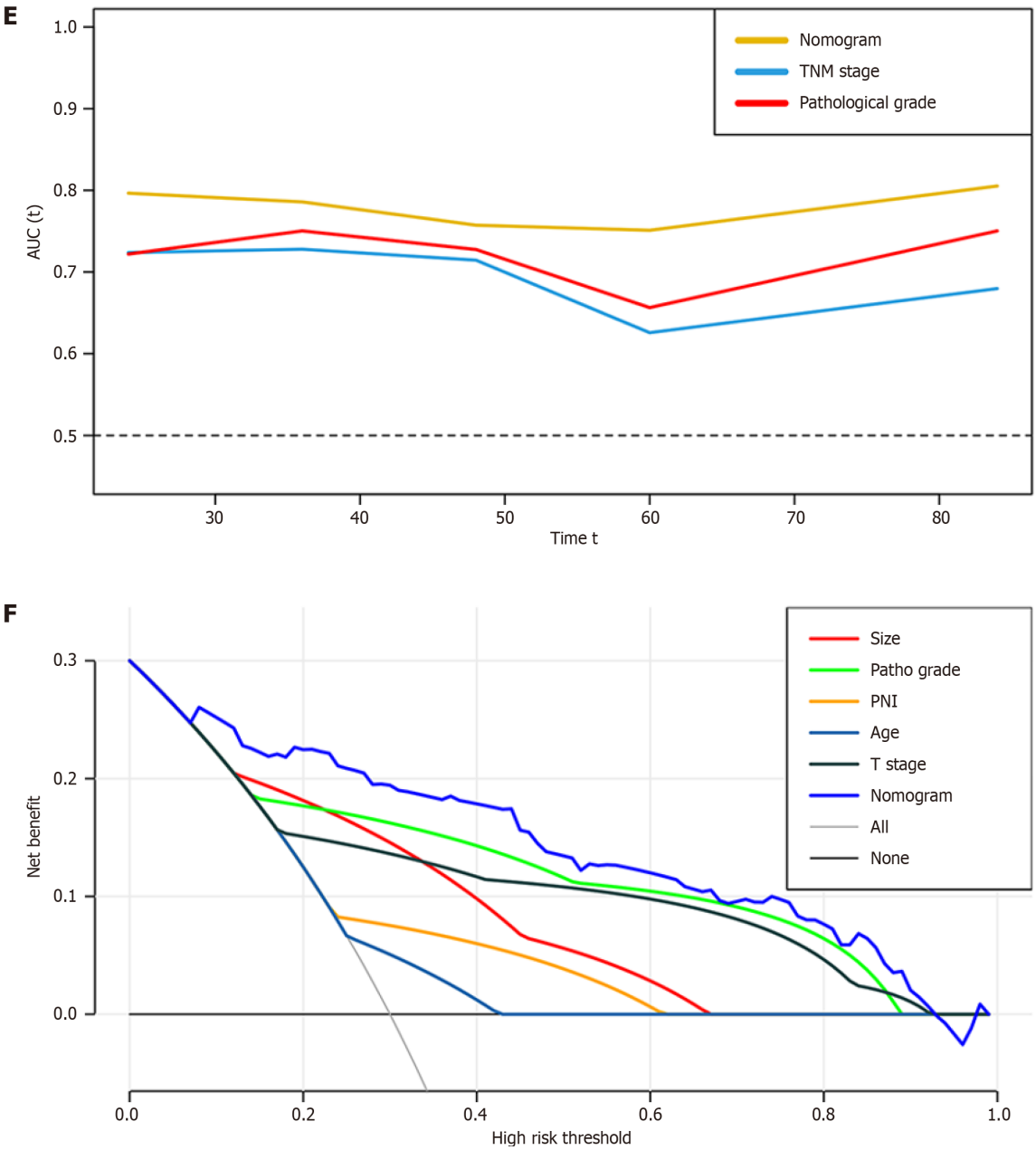
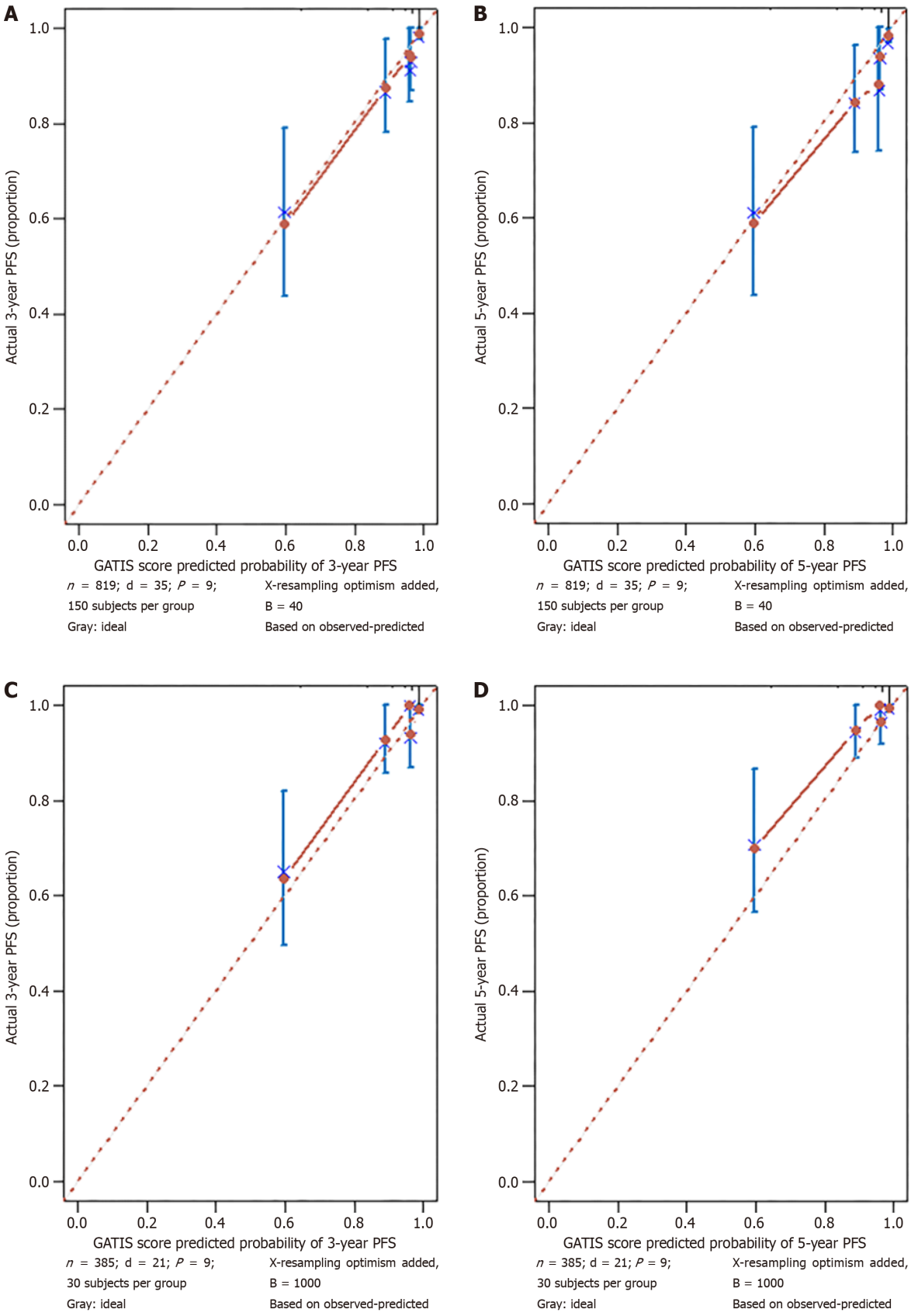


Figure 3 Nomogram validation for overall survival prediction. A: Calibration plot of 3-year overall survival (OS) for the training set; B: Calibration plot of 5-year OS for the training set; C: Calibration plot of 3-year OS for the validation set; D: Calibration plot of 5-year OS for the validation set; E: The decision curve analysis for OS; F: Time-dependent area under the receiver operating characteristic curve analysis for OS. OS: Overall survival; PNI: Prognostic nutritional index; AUC: Area under the receiver operating characteristic curve.

WHO classification and TNM staging are currently the most commonly used prognostic evaluation systems for NENs; however, several studies have shown that their predictive efficacy for R-NENs is limited. Predictive models, including scoring models and nomograms, have been widely studied and applied in the clinical practice for NENs; however, there are few studies on nomogram models for R-NENs, most of which have small sample sizes and do not consider preoperative hematologic factors[8,9,22,23]. In this study, we combined the results of the Cox proportional hazard and random forest models and constructed a prognostic nomogram model for OS and PFS in R-NENs patients according to the five screened variables. Good prediction performance was achieved in both the training and validation sets, and the calibration analysis showed that there was a good fit between the predicted and actual survival rates. In addition, we compared the predictive effect of the GATIS score with that of the WHO and TNM stages. The TD-AUC results showed that the predictive efficacy of the GATIS score was better than that of the WHO and TNM stages at multiple follow-up time points, suggesting that the GATIS scoring tool may provide a more accurate assessment of the prognosis of R-NENs patients.

This study had some limitations. First, the follow-up time of this study was still short, and a longer follow-up is needed to verify the validity of the GATIS score. Second, with the advancement of molecular and gene detection technologies, more prognostic factors (*e.g.*, genes or biological markers) may be identified. These variables were not included in the GATIS score, and further research is required to identify them. Nevertheless, our study provides a useful tool for the


A
Actual 3-year PFS (proportion)
GATIS score predicted probability of 3-year PFS
n = 819; d = 35; P = 9;
150 subjects per group
Gray: ideal
X-resampling optimism added,
B = 40
Based on observed-predicted
B
Actual 5-year PFS (proportion)
GATIS score predicted probability of 5-year PFS
n = 819; d = 35; P = 9;
150 subjects per group
Gray: ideal
X-resampling optimism added,
B = 40
Based on observed-predicted
C
Actual 3-year PFS (proportion)
GATIS score predicted probability of 3-year PFS
n = 385; d = 21; P = 9;
30 subjects per group
Gray: ideal
X-resampling optimism added,
B = 1000
Based on observed-predicted
D
Actual 5-year PFS (proportion)
GATIS score predicted probability of 5-year PFS
n = 385; d = 21; P = 9;
30 subjects per group
Gray: ideal
X-resampling optimism added,
B = 1000
Based on observed-predicted

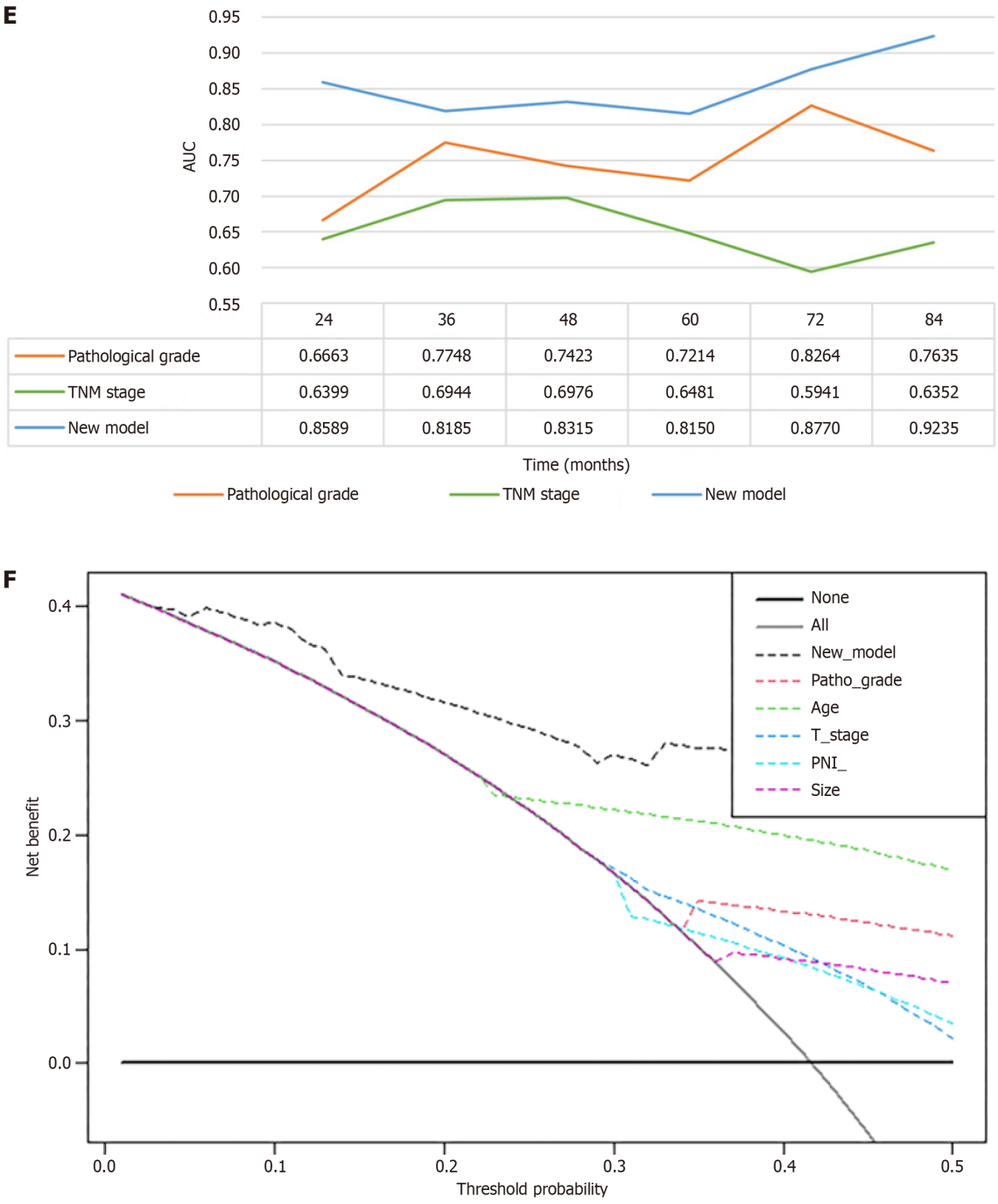


Figure 4 Nomogram validation for progression free survival prediction. A: Calibration plot of 3-year progression free survival (PFS) for the training set; B: Calibration plot of 5-year PFS for the training set; C: Calibration plot of 3-year PFS for the validation set; D: Calibration plot of 5-year PFS for the validation set; E: The decision curve analysis for PFS; F: Time-dependent area under the receiver operating characteristic curve analysis for PFS. PFS: Progression free survival; PNI: Prognostic nutritional index; AUC: Area under the receiver operating characteristic curve.

prognostic assessment of R-NENs after complete resection, offering a reference for diagnosis and treatment based on a large sample size of patients with R-NENs.

CONCLUSION

In conclusion, our study showed that the overall prognosis of patients with R-NENs was favorable, and that tumor size, tumor pathological grade, age, T stage, and preoperative PNI were important factors affecting the prognosis of patients. The GATIS score based on the Cox HR model and random forest had a good predictive effect on the prognosis of patients with R-NENs, and its efficacy was superior than that of the WHO grade and TNM stage. The GATIS score can be used to guide individualized treatment strategies and predict the individualized survival outcomes of patients with R-NENs, to help improve the prognostic evaluation, strengthen patient stratification in clinical trials, and make prognosis-based

decisions for patients with R-NENs.

FOOTNOTES

Author contributions: Zeng XY and Zhong M contributed equally to this article. Li Y, Zhang R, and Zhang P had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; Zeng XY, Li Y, Zhang R, and Zhang P contributed to the concept and design of this study; Zeng XY, Zhong M, Lin GL, Li CG, Jiang WZ, Zhang W, Xia LJ, Di MJ, Wu HX, Liao XF, Sun YM, Yu MH, and Tao KX participated in the acquisition, analysis, or interpretation of data; Zeng XY, Zhong M, and Lin GL drafted the manuscript; Li CG, Jiang WZ, Zhang W, Xia LJ, Di MJ, Wu HX, Liao XF, Sun YM, Yu MH, Tao KX, Li Y, Zhang R, and Zhang P involved in the critical revision of the manuscript for important intellectual content; Li CG, Jiang WZ, Zhang W, Xia LJ, Di MJ, and Wu HX contributed in the statistical analysis; Liao XF, Sun YM, Yu MH, Tao KX, Li Y, Zhang R, and Zhang P provided administrative, technical, or material support; Zhang R and Zhang P contributed to the supervision of this manuscript and should be considered as co-corresponding authors.

Supported by National Natural Science Foundation of China, No. 82072736 and No. 81874184; and the Key Project of Hubei Health Commission, No. WJ2019Q030.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of all 17 participating centers.

Informed consent statement: At the last follow-up, we informed the alive patients about the study in detail and obtained the informed consent signed by them. For patients who were not alive at the time of the study, we contacted their immediate family members, explained the study in detail and obtained the informed consent forms signed by them.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Country of origin: China

ORCID number: Xin-Yu Zeng 0000-0002-9399-5523; Ming Zhong 0000-0002-6933-5760; Guo-Le Lin 0000-0001-6225-3028; Cheng-Guo Li 0000-0003-3715-1171; Wei Zhang 0000-0003-3094-9711; Kai-Xiong Tao 0000-0002-7723-6121; Yong Li 0000-0003-1799-4841; Rui Zhang 0000-0003-0197-1098; Peng Zhang 0009-0005-5192-6537.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Zhang L

REFERENCES

1 **Dasari A**, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, Shih T, Yao JC. Trends in the Incidence, Prevalence, and Survival Outcomes in Patients With Neuroendocrine Tumors in the United States. *JAMA Oncol* 2017; **3**: 1335-1342 [PMID: 28448665 DOI: 10.1001/jamaoncol.2017.0589]
2 **Cives M**, Strosberg JR. Gastroenteropancreatic Neuroendocrine Tumors. *CA Cancer J Clin* 2018; **68**: 471-487 [PMID: 30295930 DOI: 10.3322/caac.21493]
3 **Ambrosini V**, Kunikowska J, Baudin E, Bodei L, Bouvier C, Capdevila J, Cremonesi M, de Herder WW, Dromain C, Falconi M, Fani M, Fanti S, Hicks RJ, Kabasakal L, Kaltsas G, Lewington V, Minozzi S, Cinquini M, Öberg K, Oyen WJG, O'Toole D, Pavel M, Ruszniewski P, Scarpa A, Strosberg J, Sundin A, Taïeb D, Virgolini I, Wild D, Herrmann K, Yao J. Consensus on molecular imaging and theranostics in neuroendocrine neoplasms. *Eur J Cancer* 2021; **146**: 56-73 [PMID: 33588146 DOI: 10.1016/j.ejca.2021.01.008]
4 **Chagpar R**, Chiang YJ, Xing Y, Cormier JN, Feig BW, Rashid A, Chang GJ, You YN. Neuroendocrine tumors of the colon and rectum: prognostic relevance and comparative performance of current staging systems. *Ann Surg Oncol* 2013; **20**: 1170-1178 [PMID: 23212760 DOI: 10.1245/s10434-012-2746-z]
5 **Rindi G**, Arnold R, Bosman F, Al E, WHO classification of tumours of the digestive system: 4th edition. France: International Agency for Research on Cancer, 2010
6 **Amin MB**, Gress DM, Meyer Vega LR, Edge SB, Greene FL, Byrd DR, Brookland RK, Washington MK, Compton CC. AJCC Cancer Staging Manual. Eighth Edition. New York: Springer, 2016: 203-220
7 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]
8 **Chen Q**, Chen J, Deng Y, Zhang Y, Huang Z, Zhao H, Cai J. Nomogram for the prediction of lymph node metastasis and survival outcomes in rectal neuroendocrine tumour patients undergoing resection. *J Gastrointest Oncol* 2022; **13**: 171-184 [PMID: 35284104 DOI:

10.21037/jgo-21-573]
9 **Feng X**, Wei G, Wang W, Zhang Y, Zeng Y, Chen M, Chen Y, Chen J, Zhou Z, Li Y. Nomogram for individually predicting overall survival in rectal neuroendocrine tumours. *BMC Cancer* 2020; **20**: 865 [PMID: 32907602 DOI: 10.1186/s12885-020-07328-9]
10 **Liu B**, Li K, Ma R, Zhang Q. Two web-based dynamic prediction models for the diagnosis and prognosis of gastric cancer with bone metastases: evidence from the SEER database. *Front Endocrinol (Lausanne)* 2023; **14**: 1136089 [PMID: 37293503 DOI: 10.3389/fendo.2023.1136089]
11 **Lin Y**, Wang M, Jia J, Wan W, Wang T, Yang W, Li C, Chen X, Cao H, Zhang P, Tao K. Development and validation of a prognostic nomogram to predict recurrence in high-risk gastrointestinal stromal tumour: A retrospective analysis of two independent cohorts. *EBioMedicine* 2020; **60**: 103016 [PMID: 32980695 DOI: 10.1016/j.ebiom.2020.103016]
12 **Mjaess G**, Peltier A, Roche JB, Lievore E, Lacetera V, Chiacchio G, Beatrici V, Mastroianni R, Simone G, Windisch O, Benamran D, Fourcade A, Nguyen TA, Fournier G, Fiard G, Ploussard G, Roumeguère T, Albisinni S, Diamand R. A Novel Nomogram to Identify Candidates for Focal Therapy Among Patients with Localized Prostate Cancer Diagnosed *via* Magnetic Resonance Imaging-Targeted and Systematic Biopsies: A European Multicenter Study. *Eur Urol Focus* 2023; **9**: 992-999 [PMID: 37147167 DOI: 10.1016/j.euf.2023.04.008]
13 **Maione F**, Chini A, Milone M, Gennarelli N, Manigrasso M, Maione R, Cassese G, Pagano G, Tropeano FP, Luglio G, De Palma GD. Diagnosis and Management of Rectal Neuroendocrine Tumors (NETs). *Diagnostics (Basel)* 2021; **11** [PMID: 33923121 DOI: 10.3390/diagnostics11050771]
14 **Srirajaskanthan R**, Clement D, Brown S, Howard MR, Ramage JK. Optimising Outcomes and Surveillance Strategies of Rectal Neuroendocrine Neoplasms. *Cancers (Basel)* 2023; **15** [PMID: 37345103 DOI: 10.3390/cancers15102766]
15 **Sun H**, Wang H, Pan H, Zuo Y, Zhao R, Huang R, Xue Y, Song H. CD19 (+) B Cell Combined with Prognostic Nutritional Index Predicts the Clinical Outcomes of Patients with Gastric Cancer Who Underwent Surgery. *Cancers (Basel)* 2023; **15** [PMID: 37173997 DOI: 10.3390/cancers15092531]
16 **Jia J**, Zhang L, Wang T, Yang W, Lyu J, Zeng X, Li X, Zeng X, Liu W, Tao K, Zhang P. Association between preoperative skeletal muscle mass depletion and poor relapse-free survival in patients with gastrointestinal stromal tumors after complete resection. *Nutrition* 2022; **98**: 111636 [PMID: 35452975 DOI: 10.1016/j.nut.2022.111636]
17 **Li YW**, He YP, Liu FQ, Peng JJ, Cai SJ, Xu Y, Wang MH. Grade G2 Rectal Neuroendocrine Tumor Is Much More Invasive Compared With G1 Tumor. *Front Oncol* 2021; **11**: 646536 [PMID: 33777809 DOI: 10.3389/fonc.2021.646536]
18 **Duan X**, Zhao M, Zhang S, Xu Z, Mi L, Shi J, Ma X, Liu Y, Li N, Yin X, Han X, Han G, Wang J, Xu J, Yin F. Effects of tumor distance from anal verge on survival outcomes for rectal NENs and lymphatic metastasis risk score for colorectal NENs. *Int J Colorectal Dis* 2020; **35**: 1255-1264 [PMID: 32314191 DOI: 10.1007/s00384-020-03596-w]
19 **Muñoz AJ**, Souto JC, Lecumberri R, Obispo B, Sanchez A, Aparicio J, Aguayo C, Gutierrez D, Palomo AG, Fanjul V, Del Rio-Bermudez C, Viñuela-Benéitez MC, Hernández-Presa MÁ. Development of a predictive model of venous thromboembolism recurrence in anticoagulated cancer patients using machine learning. *Thromb Res* 2023; **228**: 181-188 [PMID: 37348318 DOI: 10.1016/j.thromres.2023.06.015]
20 **Gitto S**, Interlenghi M, Cuocolo R, Salvatore C, Giannetta V, Badalyan J, Gallazzi E, Spinelli MS, Gallazzi M, Serpi F, Messina C, Albano D, Annovazzi A, Anelli V, Baldi J, Aliprandi A, Armiraglio E, Parafioriti A, Daolio PA, Luzzati A, Biagini R, Castiglioni I, Sconfienza LM. MRI radiomics-based machine learning for classification of deep-seated lipoma and atypical lipomatous tumor of the extremities. *Radiol Med* 2023; **128**: 989-998 [PMID: 37335422 DOI: 10.1007/s11547-023-01657-y]
21 **Tian S**, Yu R, Zhou F, Zhan N, Li J, Wang X, Peng X. Prediction of HER2 status *via* random forest in 3257 Chinese patients with gastric cancer. *Clin Exp Med* 2023; **23**: 5015-5024 [PMID: 37318648 DOI: 10.1007/s10238-023-01111-3]
22 **Li J**, Huang L, Liao C, Liu G, Tian Y, Chen S. Two machine learning-based nomogram to predict risk and prognostic factors for liver metastasis from pancreatic neuroendocrine tumors: a multicenter study. *BMC Cancer* 2023; **23**: 529 [PMID: 37296397 DOI: 10.1186/s12885-023-10893-4]
23 **Zheng H**, Zhao Y, He Q, Hao H, Tian Y, Zou B, Jiang L, Qiu X, Zhou Y, Li Z, Xu Y, Zhao G, Xue F, Li S, Fu W, Li Y, Zhou X, Li Y, Zhu Z, Chen J, Xu Z, Cai L, Li E, Li H, Xie J, Zheng C, Lu J, Li P, Huang C. Multi-institutional development and validation of a nomogram to predict recurrence after curative resection of gastric neuroendocrine/mixed adenoneuroendocrine carcinoma. *Gastric Cancer* 2021; **24**: 503-514 [PMID: 32915373 DOI: 10.1007/s10120-020-01119-8]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3418-3427

DOI: 10.3748/wjg.v30.i28.3418

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Positive health: An integrated quantitative approach in patients with chronic gastrointestinal and hepato-pancreatico-biliary disorders

Anja H W M Lemlijn-Slenter, Karolina A P Wijnands, Gijs van der Hamsvoort, Luuk P van Iperen, Nico Wolter, Angelique E de Rijk, Ad A M Masclee

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade B, Grade B

Creativity or Innovation: Grade A, Grade B

Scientific Significance: Grade B, Grade C

P-Reviewer: Lindberg G; Nguyen TVH

Received: March 1, 2024

Revised: June 5, 2024

Accepted: June 24, 2024

Published online: July 28, 2024

Processing time: 144 Days and 14.3 Hours

Anja H W M Lemlijn-Slenter, Karolina A P Wijnands, Luuk P van Iperen, Angelique E de Rijk, Department of Social Medicine, Faculty of Health, Medicine and Life sciences, Care and Public Health Research Institute Maastricht University, Maastricht 6200 MD, Limburg, Netherlands

Anja H W M Lemlijn-Slenter, Nico Wolter, Ad A M Masclee, Division of Gastroenterology and Hepatology, Department of Internal Medicine, School of Nutrition and Translational Research in Metabolism, Maastricht 6200 MD, Limburg, Netherlands

Anja H W M Lemlijn-Slenter, Karolina A P Wijnands, Gijs van der Hamsvoort, Social Medical Affairs, Employee Insurance Agency (UWV), Heerlen 6400 AC, Limburg, Netherlands

Anja H W M Lemlijn-Slenter, Karolina A P Wijnands, Angelique E de Rijk, Department of Research, Academic Knowledge Center Work and Health South-East Netherlands (AKAG-ZON), Heerlen 6400 AC, Limburg, Netherlands

Corresponding author: Anja HWM Lemlijn-Slenter, MD, Insurance Physician, Researcher, Department of Social Medicine, Faculty of Health, Medicine and Life sciences, Care and Public Health Research Institute Maastricht University, PO Box 616, Maastricht 6200 MD, Limburg, Netherlands. a.lemlijn@maastrichtuniversity.nl

Abstract

BACKGROUND

The concept of positive health (PH) supports an integrated approach for patients by taking into account six dimensions of health. This approach is especially relevant for patients with chronic disorders. Chronic gastrointestinal and hepato-pancreatico-biliary (GI-HPB) disorders are among the top-6 of the most prevalent chronically affected organ systems. The impact of chronic GI-HPB disorders on individuals may be disproportionally high because: (1) The affected organ system frequently contributes to a malnourished state; and (2) persons with chronic GI-HPB disorders are often younger than persons with chronic diseases in other organ systems.

AIM

To describe and quantify the dimensions of PH in patients with chronic GI-HPB disorders.

METHODS
Prospective, observational questionnaire study performed between 2019 and 2021 in 235 patients with a chronic GI-HPB disorder attending the Outpatient Department of the Maastricht University Medical Center. Validated questionnaires and data from patient files were used to quantify the six dimensions of PH. Internal consistency was tested with McDonald's Omega. Zero-order Pearson correlations and *t*-tests were used to assess associations and differences. A P value < 0.05 was considered significant.

RESULTS
The GI-HPB patients scored significantly worse in all dimensions of PH compared to control data or norm scores from the general population. Regarding quality of life, participation and daily functioning, GI-HPB patients scored in the same range as patients with chronic disorders in other organ systems, but depressive symptoms (in 35%) and malnutrition (in 45%) were more frequent in patients with chronic GI-HPB disorders. Intercorrelation scores between the six dimensions were only very weak to weak, forcing us to quantify each domain separately.

CONCLUSION
All six dimensions of PH are impaired in the GI-HPB patients. Malnutrition and depressive symptoms are more prevalent compared to patients with chronic disorders in other organ systems.

Key Words: Positive health; Chronic gut disorders; Gastrointestinal disorders; Hepato-pancreatico-biliary disorders; Integrated care

Core Tip: Patients with chronic gastrointestinal and hepato-pancreatico-biliary (GI-HPB) disorders experience complaints in every dimension of positive health (PH). We described and quantified these complaints and compared the outcomes with norm scores and other chronic diseases. We found that GI-HPB patients scored significantly worse in all dimensions of PH compared to control data or norm scores from the general population. Regarding quality of life, participation and daily functioning, GI-HPB patients scored in the same range as patients with chronic disorders in other organ systems, but depressive symptoms (in 35%) and malnutrition (in 45%) were more frequent in patients with chronic GI-HPB disorders.

Citation: Lemlijn-Slenter AHWM, Wijnands KAP, van der Hamsvoort G, van Iperen LP, Wolter N, de Rijk AE, Masclee AAM. Positive health: An integrated quantitative approach in patients with chronic gastrointestinal and hepato-pancreatico-biliary disorders. *World J Gastroenterol* 2024; 30(28): 3418-3427
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3418.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3418

INTRODUCTION

In the past decades, the prevalence of chronic disorders has steadily increased worldwide. In the Netherlands, over 55% of all inhabitants had been diagnosed with at least one chronic disorder in 2021. Increasing age is associated with an even higher prevalence of chronic disorders[1]. These developments have challenged healthcare providers to expand on the conventional definition of "health" beyond the focus on "absence of disease". Chronic disorders significantly impact not only an individual's physical well-being, but also mental well-being and social participation[2,3]. In 2011 Huber *et al*[4] proposed changing the focus of "health" to a more holistic vision, called "positive health" (PH). This approach emphasises a person's ability to cope with life's challenges and maintain self-management in relation to six dimensions: Bodily functions, mental well-being, quality of life (QOL), social participation, daily living and meaningfulness. By assessing a patient's functioning in these six dimensions, a broader insight into their life and well-being is obtained. This integrated approach enables a more structured and potentially more relevant and personalised management of patients with chronic disorders.

There is an ongoing debate of how best to measure and quantify PH. Previous research has focused on the separate dimensions of PH, not on their coherence or through an integrated approach. To map PH during patient consultations, a dialogue tool was developed by the Institute for Positive Health[5]. Using 44 items, the six dimensions of PH are explored and the outcome visualised in a personal spider web. While this tool is helpful for individual clinical consultations, it does not systematically quantify all the PH dimensions to allow individual follow-up, group comparisons or scientific analyses. Recently, we proposed bridging this gap by measuring PH by merging validated, widely available questionnaire instruments that, taken together, cover all the various dimensions of PH[6]. As a next step, PH should be quantified in patients with chronic disorders. Here we focus on a specific group of patients with chronic disorders.

Chronic gastrointestinal and hepato-pancreatico-biliary (GI-HPB) disorders are among the top 6 of most prevalent chronically affected organ systems[7]. The impact of chronic GI-HPB disorders on individuals may be disproportionally

high because the affected organ system frequently contributes to a malnourished state and persons with chronic GI-HPB disorders are often younger than persons with chronic diseases in other organ systems.

The aim of the present study was to describe and quantify the six dimensions of PH in a group of patients with chronic GI-HPB disorders, compare their scores with norm scores (control groups, Dutch population data, disease controls) and test intercorrelations.

MATERIALS AND METHODS

Patient characteristics

In this prospective, single-centre, observational, explorative questionnaire study, chronic GI-HPB patients attending the Gastroenterology-Hepatology Outpatient Clinic of the Maastricht University Medical Center (MUMC) were invited to participate. The MUMC is a university hospital for tertiary care and also provides secondary care for inhabitants of Maastricht and its surrounding area (250000 inhabitants). The Department of Gastroenterology-Hepatology has organised its care for patients with chronic disorders into three clinical pathways for: (1) Inflammatory bowel diseases (IBD); (2) Chronic hepato-pancreatico-biliary (HPB) disorders; and (3) Neurogastroenterology and motility (NGM) disorders, also including functional gastrointestinal disorders, for which it is the national centre of expertise.

This survey is part of a more extensive MUMC study on PH in patients with chronic GI-HPB disorders. In the period from 2019-2021, a group of 1170 GI-HPB patients were contacted, of whom 555 participated (response rate 47.4%). As the questionnaire about meaningfulness was added later, only in a subset of 235 GI-HPB patients were all PH dimensions quantified using the combined, validated questionnaires. The local Medical Ethics Committee confirmed in a written statement that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study (No. METC 2019-1324).

Patients aged 18 years and older who had been diagnosed with a chronic GI-HPB disorder (IBD, HPB or NGM) and were scheduled for an appointment at the Outpatient Clinic were invited by letter to participate in the study. The letter contained detailed information about the study. After signing the informed consent form, the patients received the questionnaires, filled them in and handed them back to the investigator after the clinical consultation. In addition to the questionnaires, clinical data were collected from their electronic patient files (medical diagnoses, duration of complaints, comorbidity, *etc.*).

Dimensions of PH

In Figure 1 the validated questionnaires and scales to quantify each of the six dimensions are presented. Each questionnaire is addressed in more detail below.

Bodily functions

The bodily functions of GI-HPB patients were measured with the Gastrointestinal Symptom Rating Scale (GSRS)[8] that separates abdominal complaints into five symptom clusters on a seven-point Likert scale: Reflux, Abdominal pain, Indigestion, Diarrhoea and Constipation. McDonald's Omega was adequate as a measure of internal consistency for the GSRS (0.857).

In addition, we used the Short Nutritional Assessment Questionnaire (SNAQ), an instrument developed to screen for malnutrition at hospitalisation. It provides insight into weight loss, loss of appetite and additional nutritional support[9]. A score ≥ 2 points to moderate malnutrition, a score ≥ 3 to severe malnutrition.

Mental well-being

To evaluate mental well-being, depressive complaints (nine items) were measured with the Patient Health Questionnaire (PHQ-9)[10] and anxiety complaints (seven items) with the Generalized Anxiety Disorder (GAD-7)[11]. The PHQ-9 (range 0-27) and the GAD-7 (range 0-21) utilise answers on a four-point scale. Range of PHQ-9 scores: 5-9 mild; 10-14 moderate; 15-19 moderately severe; and 20-27 severe. Range of GAD-7 scores: 5-9 mild; 10-14 moderate; and 15-21 severe. A score of ≥ 10 represents the cut-off point to identify moderate to severe depression (PHQ-9) or moderate to severe anxiety disorder (GAD-7), at which point treatment is considered[12]. McDonald's Omega was adequate for PHQ-9 (0.882) and GAD-7 (0.913).

The Short Form-36 (SF-36) is a questionnaire to quantify QOL and consists of nine subscales: *e.g.*, physical functioning, social functioning, and physical or emotional role limitations. We distributed the subscales of the SF-36 over the various PH dimensions to find the best match with a specific dimension. Questions from the Mental Health subscale of the SF-36 were added to the Mental well-being dimension[13]. McDonald's Omega was acceptable (0.864).

Meaningfulness

In 2020 the Disease Experience Questionnaire (DEQ) (Meaningfulness subscale, five items) was introduced and provides insight into the meaningfulness dimension[14]. The DEQ uses a five-point Likert scale. A higher score indicates that the chronic disorder is regarded as a more meaningful experience. Control data at the population level are not yet available. McDonald's Omega was adequate (0.750).

Quality of life

The EuroQol (EQ)-5D-5 L measures QOL in five dimensions using a five-point Likert scale: Mobility, self-care, daily

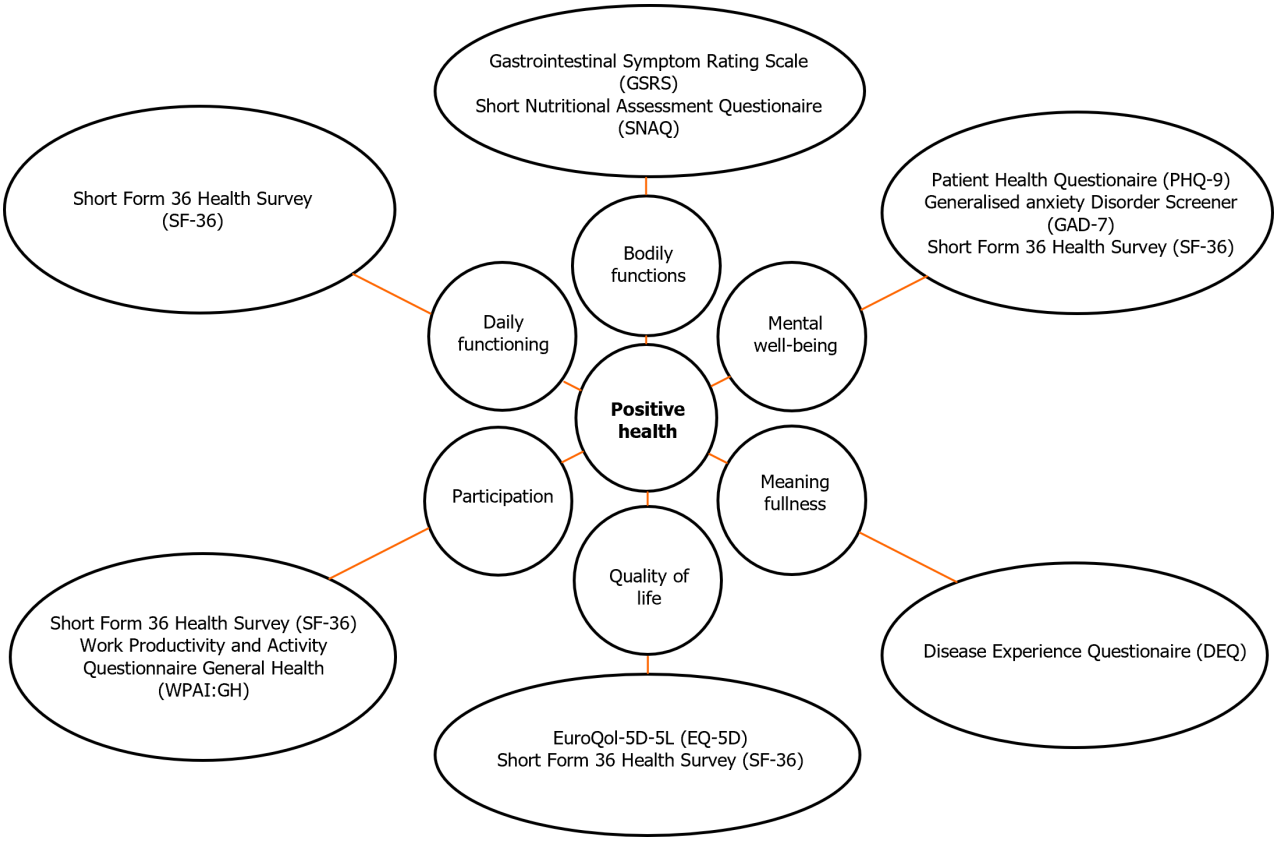


Figure 1 Visual representation of the six dimensions of positive health (boxes of the inner circle) and the validated questionnaires used to quantify each dimension (boxes of the outer circle).

activities, pain/discomfort and anxiety/depression. The patients' health status was measured on a VAS scale (0-100). To obtain the EQ-5D-5L Crosswalk Value, a five-digit code consisting of the answers to the questions in the EQ-5D-5L was mapped to health status scores based on the study of M Versteegh *et al*[15]. Additionally, the SF-36 Vitality and General Health subscales were added to the PH QOL dimension.

Social and work participation

The Social functioning, Emotional role limitations and Physical role limitations subscales of the SF-36 were used to score social and work participation. Participation was also measured with the Work Productivity and Activity Impairment-General Health (WPAI: GH)[16]. Additionally, for the subgroup of working age (18-66 years), a set of questions tailored to the Dutch work situation was used.

Daily functioning

The SF-36 Physical functioning subscale was used to assess daily functioning and daily activities.

Control data

GSRS outcomes were compared to those of a group of healthy controls (n = 215) which we used previously as a control group for the Maastricht Irritable Bowel Syndrome Cohort[17]. Outcomes of the EQ-5D-5L and the SF-36 were compared to norm scores for the Dutch population[13,18,19]. SNAQ, PHQ-9 and GAD-7 were compared with previously defined cut-off values. For data on work participation, Dutch population control data were used[20].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26.0. For the scales, internal consistency was tested with McDonald's Omega. Zero-order Pearson correlations and t-tests were used to assess associations and differences. A P value < 0.05 was considered significant.

RESULTS

Patient characteristics

The group consisted of 235 chronic GI-HPB patients, mean age 50.2 years (SD 17.4), with more women than men (68.1%). The distribution among the subgroups was IBD 31.1% (n = 73), HPB 17.9% (n = 42) and NGM 51% (n = 120). Mean disease

duration was 9.5 years (SD 10.3).

In 31.8% there were no comorbidities present, 19.7% had one comorbidity, 18.4% had two, and 30.1% had three or more. Almost everyone had a somatic comorbidity; 1.8% had only a mental comorbidity.

Positive health by dimension

Bodily functions: GSRS scores for all five domains were significantly ($P < 0.001$) higher in GI-HPB patients compared to healthy controls (Table 1).

Based on SNAQ data, moderate malnutrition (score 2) was present in 55 (23.9%) GI-HPB patients, while 50 (21.7%) had a score of ≥ 3 (severe malnutrition).

Mental well-being

A mental disorder had previously been diagnosed in 26 of the 235 patients (11%). Depressive symptoms (PHQ score ≥ 10) were present in 34.9% and anxiety symptoms (GAD-7 score ≥ 10) in 21.6% of the GI-HPB patients (Table 2). Regarding the Mental health subscale of the SF-36, the mean score of the GI-HPB group was significantly ($P < 0.001$) reduced compared to the Dutch population norm score.

Meaningfulness

The GI-HPB group had a DEQ Meaningfulness subscale mean group score of 2.85 (SD 0.84; range 0-5). A higher score points to a greater impact of the chronic disorder on meaningfulness.

Quality of life

EQ-5D-5L scores were significantly ($P < 0.001$) reduced in the GI-HPB group compared to the Dutch population norm scores (Table 3). The Vitality and General perception of health subscales of the SF-36 were significantly ($P < 0.001$) lower in the GI-HPB group compared to the Dutch population.

Participation

The scores in the SF-36 subscales related to social participation were significantly ($P < 0.001$) lower compared to the Dutch population norm scores (Table 4).

From the group of 235 GI-HPB patients, 81% was of working age (18-66 years). Of them, 54% was actively working, compared to 70% for the Dutch population ($P < 0.001$). Of those not working, the percentage of GI-HPB patients with a disability benefit was 36%.

Daily functioning

The GI-HPB group scored significantly ($P = 0.001$) lower on the SF-36 Physical functioning subscale compared to the Dutch population.

Correlations between PH dimensions

Correlations between the dimensions were weak (0.2-0.4) or very weak (0.0-0.2). Correlations between the various QOL parameters (all subscales of SF-36, EQ-5D-5L and EQ-VAS) were moderate to strong (0.4-0.7), however. Patient characteristics (disease duration, number of comorbidities, *etc*.) showed very weak to weak correlations with the various PH dimensions. Higher GAD and PHQ scores indicating anxiety or depressive symptoms had a moderate negative correlation (-0.4 to -0.6) with all the quality-of-life parameters except for SF mental health, where the correlation was strong. Meaningfulness was the only parameter to show very weak correlations with all the other dimensions (0.0-0.2).

DISCUSSION

To arrive at an integrated assessment of patients with chronic disorders that includes their needs and wishes, it is important to know more about the patients than just their physical condition. We have described and quantified the health status of a group of patients with chronic GI-HPB disorders according to the six dimensions of PH by using an integrative and quantitative approach. To our knowledge, this study is the first to report on PH status in patients with chronic disorders using validated instruments for the various dimensions of PH. The results show that all dimensions of the health status of patients with chronic GI-HPB disorders are substantially impaired.

The group of 235 GI-HPB patients was divided into three subgroups. We focused on the complete GI-HPB group, not on comparisons between subgroups. Of the 235 GI-HPB patients, 68% had comorbidities. In the Dutch population, comorbidity is present in 57% of subjects[1]. In chronic disorders of other organ systems, similar percentages of comorbidity have been observed to our GI-HPB group[21-23]. Age, gender, BMI, disease duration and number of comorbidities revealed only weak correlations with the scores on the various PH dimensions.

Bodily functions

In GI-HPB patients, GSRS were significantly higher in all symptom clusters compared to a historical control group. GSRS scores in our chronic GI-HPB patients were in the range of those previously reported in patients with IBD or irritable bowel syndrome. One limitation is that the GSRS only measures actual symptoms of the past seven days; it does not cover a longer timeframe.

Table 1 Data on bodily function scores in the 235 patients with chronic gastrointestinal and hepato-pancreatico-biliary disorders

Bodily functions	GI-HPB patients	Healthy controls
Patient characteristics	*n* = 235	*n* = 215
Age, years	50.2 (17.4)[b]	44.5 (10.0)
Sex, female	68.1%	61.4%
BMI, kg/m^2	25.6 (6.3)[a]	24.0 (3.8)
GSRS, mean (SD)		
Reflux	2.24 (1.68)[b]	1.22 (0.53)
Abdominal pain	3.15 (1.39)[b]	1.65 (0.71)
Indigestion	3.48 (1.47)[b]	2.00 (0.89)
Diarrhoea	3.02 (1.72)[b]	1.41 (0.63)
Constipation	2.82 (1.61)[b]	1.61 (0.85)
SNAQ, *n* (%)		
0-1	125 (54.3)	
2: Moderately malnourished	55 (23.9)	
≥ 3: Severely malnourished	50 (21.7)	

[a]$P < 0.01$, indicate that characteristics of the gastrointestinal and hepato-pancreatico-biliary patients are significantly different from the Maastricht irritable bowel syndrome healthy control group with respect to age and body mass index and Gastrointestinal Symptom Rating Scale symptom scores.
[b]$P < 0.001$, indicate that characteristics of the gastrointestinal and hepato-pancreatico-biliary patients are significantly different from the Maastricht irritable bowel syndrome healthy control group with respect to age and body mass index and Gastrointestinal Symptom Rating Scale symptom scores.
SNAQ: Short Nutritional Assessment Questionnaire; GI-HPB: Gastrointestinal and hepato-pancreatico-biliary; GSRS: Gastrointestinal Symptom Rating Scale.

Table 2 Mental well-being scored with patient health questionnaire-9, generalized anxiety disorder-7 and short form-36 mental health subscale, measured in 235 patients with chronic gastrointestinal and hepato-pancreatico-biliary disorders, *n* (%)

Mental well-being	GI-HPB patients, *n* = 235	Norm score of Dutch population
PHQ total		
0–9	149 (65.1)	
10–max	80 (34.9)	
GAD 7 total		
0–9	178 (78.4)	
10–max	49 (21.6)	
SF-36		
Mental Health, mean (SD)	68.5 (21.2)[b]	76.8

[b]$P < 0.001$.
Data on Short Form-36 Mental Health are given as mean (SD). PHQ-9: Patient Health Questionnaire; GAD-7: Generalized Anxiety Disorder; SF-36: Short Form-36; GI-HPB: Gastrointestinal and hepato-pancreatico-biliary.

The SNAQ score is used as a screening instrument for malnutrition in the (pre)hospital setting. In this setting, 27% of GI-HPB patients was previously found to be severely malnourished[24]. In the outpatient setting, severe malnutrition is much less frequent: Around 10% has been reported in non-specified, chronically diseased patients. Surprisingly, in our outpatient setting, 22% of the chronic GI-HPB patients was severely malnourished and 24% moderately malnourished. Thus, almost half of our chronic GI-HPB population is in need of nutritional guidance/support. A substantial percentage of these patients would not have been identified without the use of the SNAQ.

Mental well-being

Symptoms of depression and anxiety were more frequent in chronic GI-HPB patients compared to the Dutch population: 34.9% and 21.6% compared to 8.5% and 15%, respectively[25]. For patients with chronic diseases of other organ systems,

Table 3 Quality of life measured with EuroQol and the short form-36 vitality and general perception of health subscales in 235 patients with chronic gastrointestinal and hepato-pancreatico-biliary disorders and norm scores for the Dutch population

Quality of life	GI-HPB patients, *n* = 235	Norm score of Dutch population
EQ-5D-5L		
VAS, mean (SD)	58.7 (23.1)[b]	81.0
Crosswalk, value mean (SD)	0.66 (0.26)[b]	0.91
SF-36		
Vitality, mean (SD)	42.2 (26.2)[b]	67.4
General perception of health, mean (SD)	38.4 (21.3)[b]	72.7

[b]$P < 0.001$.

EQ-5D-5L: EuroQol; VAS: Visual Analogue Scale; SF-36: Short Form-36; GI-HPB: Gastrointestinal and hepato-pancreatico-biliary.

Table 4 Participation scored by the short form-36 social functioning, physical role limitations and emotional role limitations subscales, and by work status in a group of 235 patients with chronic gastrointestinal and hepato-pancreatico-biliary disorders

Participation	GI-HPB patients, *n* = 235	Norm score of Dutch population
SF-36, mean (SD)		
Social functioning	60.1 (30.5)[b]	86.9
Physical role limitations	39.9 (43.2)[b]	79.4
Emotional role limitations	68.7 (42.6)[b]	84.1
Work, *n* (%)		
Working age (18-66 years)	191 (81.3)	
At work if working age (18-66 years)	103 (54)[b]	70%

[b]$P < 0.001$.

GI-HPB: Gastrointestinal and hepato-pancreatico-biliary; SF-36: Short Form-36.

prevalence data on depressive symptoms of 20%-30% and on anxiety symptoms of 16%-32% have been observed[26-28]. Depressive symptoms appear to be more prevalent in our chronic GI-HPB population compared to other chronically diseased populations. Because mental disorders can influence GI-HPB symptoms, it is important to identify them. In a large proportion of GI-HPB patients, mental symptoms were not recognized, although they are present to an extent that treatment should be considered. We recommend more systematic screening for depression and anxiety disorder in patients with chronic GI-HPB disorders and, when indicated, initiating targeted treatment.

Meaningfulness

The DEQ is designed to measure three disease experiences, including meaningfulness. A meaningful life is associated with positive functioning: Life satisfaction, enjoyment of work, happiness, general positive affect, hope and a higher level of well-being. Our GI-HPB group scored a mean of 2.85 on this scale. For comparison, a mean score of 3.16 has been reported in the recent literature for patients with sarcoidosis, a chronic pulmonary disorder[14].

We anticipated that lower scores of physical, mental and social well-being would be associated with higher scores of meaningfulness, pointing to a greater impact of the disease on well-being and functioning. Meaningfulness showed only a very weak, insignificant correlation with all other dimensions of PH. For an integrated, multidimensional approach to chronically diseased patients, it is essential to be informed about a patient's opinion and rating of their life's meaningfulness. In this respect, the DEQ is a first step that deserves further evaluation.

Quality of life

The GI-HPB patients scored significantly lower on all QOL parameters compared to the Dutch norm population. QOL scores in the GI-HPB group are comparable to those found in various chronic disorders of other organ systems[29-31].

Social participation

Scores for the three SF-36 subscales related to social participation were all significantly lower compared to the Dutch population as a whole but were in the range of patients with chronic diseases in other organ systems.

Work participation was significantly lower in the GI-HPB group compared to the Dutch norm population (54% *vs* 71%) but in the range of 40%-60% observed in patients with chronic diseases in other organ systems[32-34]. Of the GI-HPB patients who were not working, only 36% received a disability benefit. Compared to other high-income countries, the percentage of disabled people living in poverty is higher in the Netherlands due to a high threshold for the disability benefit. Early recognition of problems in work participation is essential in order to refer patients for advice and support, and thus prevent prolonged sick leave, resulting in a non-working status with disability pension or unemployment.

Daily functioning

For daily functioning (SF-36 Physical functioning subscale), the GI-HPB group scored significantly worse compared to the Dutch norm population, while the score is in the range of patients with chronic disorders in other organ systems[30,35,36].

Strengths and limitations

One strength of our study is that it bridges the gap between conceptualisation and implementation of PH by using validated questionnaires to quantify each dimension in an integrated fashion. In this way, a broad and personalised picture of a patient's well-being was obtained. It allowed us to generate data for individual follow-up, for comparison between groups and for scientific purposes. Although confirmation is needed from other GI-HPB centres and from patients with chronic disorders of other organ systems, our data on PH in chronic GI-HPB patients provide a unique and valuable insight. Our group is fairly representative of the chronic GI-HPB patient population as a whole, with a somewhat higher percentage of patients with chronic NGM or functional disorders because we function as a national NGM centre of expertise.

A limitation for generalisation to other patient groups is that we used an organ-specific instrument, the GSRS, for physical symptom assessment. Assessment of PH in chronic disorders of other organ systems will require other organ-specific or more general health-oriented instruments for physical symptom assessment that cover longer periods of time than the past week or month.

In general, correlations between the various dimensions were relatively weak. Therefore, an inventory of PH should contain instruments to score all the separate dimensions. For an individual patient, attention should be paid to all dimensions to obtain an overall picture.

Implications

In the guidance and management of patients with chronic disorders, use of the PH concept may be helpful. Improving the physical condition of a patient is essential but is not the ultimate goal. It serves as a first step towards better mental and social well-being, daily functioning, work participation and a meaningful life.

CONCLUSION

In conclusion, describing and quantifying PH revealed that the health status of patients with chronic GI-HPB disorders is substantially impaired in all dimensions. Regarding QOL, social and work participation, daily functioning and meaningfulness, the scores are in the range of patients with chronic disorders in other organ systems, while depressive symptoms and malnutrition were more prevalent in patients with chronic GI-HPB disorders. Intercorrelation scores between the six dimensions were very weak to weak, compelling us to quantify each domain separately. We have shown that quantifying PH in patients with chronic disorders can be successfully performed in standard care using validated instruments.

FOOTNOTES

Author contributions: Masclee AAM, de Rijk AE, Wijnands KAP and Wolter N designed the research study; Lemlijn-Slenter AHWM and Wolter N collected data; data analyses were performed by van der Hamsvoort G, van Iperen LP and Lemlijn-Slenter AHWM; Lemlijn-Slenter AHWM, de Rijk AE and Masclee AAM wrote the manuscript; All authors have read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Medical Ethics Committee in a written statement specifying that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study (METC 2019-1324, Maastricht UMC +, Maastricht).

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

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers.

Country of origin: Netherlands

ORCID number: Anja HWM Lemlijn-Slenter 0009-0002-2672-1706.

S-Editor: Li L
L-Editor: A
P-Editor: Chen YX

REFERENCES

1 **RIVM**. Chronische aandoeningen en multimorbiditeit, leeftijd en geslacht Netherlands 2022. Nov 29, 2022 [cited 21 June 2024]. Available from: https://www.vzinfo.nl/chronische-aandoeningen-en-multimorbiditeit/leeftijd-en-geslacht
2 **De Boer AG**, Bennebroek Evertsz' F, Stokkers PC, Bockting CL, Sanderman R, Hommes DW, Sprangers MA, Frings-Dresen MH. Employment status, difficulties at work and quality of life in inflammatory bowel disease patients. *Eur J Gastroenterol Hepatol* 2016; **28**: 1130-1136 [PMID: 27340897 DOI: 10.1097/MEG.0000000000000685]
3 **Schreiber S**, Panés J, Louis E, Holley D, Buch M, Paridaens K. National differences in ulcerative colitis experience and management among patients from five European countries and Canada: an online survey. *J Crohns Colitis* 2013; **7**: 497-509 [PMID: 22921464 DOI: 10.1016/j.crohns.2012.07.027]
4 **Huber M**, Knottnerus JA, Green L, van der Horst H, Jadad AR, Kromhout D, Leonard B, Lorig K, Loureiro MI, van der Meer JW, Schnabel P, Smith R, van Weel C, Smid H. How should we define health? *BMJ* 2011; **343**: d4163 [PMID: 21791490 DOI: 10.1136/bmj.d4163]
5 **Institute for Positive Health**. Procesnotitie Doorontwikkeling Mijn Positieve Gezondheid. Dec 16, 2021. [cited 21 June 2024]. Available from: https://www.iph.nl/assets/uploads/2021/12/20211119-procesnotitie-doorontwikkeling-spinnenweb-als-gespreksinstrument.pdf
6 **Lemlijn-Slenter AHWM**, Wijnands KAP, de Rijk A, Masclee AAM. Mapping positive health in patients with gastrointestinal disorders. *BMJ Open* 2021; **11**: e052688
7 **Linden M**, Linden U, Goretzko D, Gensichen J. Prevalence and pattern of acute and chronic multimorbidity across all body systems and age groups in primary health care. *Sci Rep* 2022; **12**: 272 [PMID: 34997129 DOI: 10.1038/s41598-021-04256-x]
8 **Svedlund J**, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci* 1988; **33**: 129-134 [PMID: 3123181 DOI: 10.1007/BF01535722]
9 **Kruizenga HM**, Seidell JC, de Vet HC, Wierdsma NJ, van Bokhorst-de van der Schueren MA. Development and validation of a hospital screening tool for malnutrition: the short nutritional assessment questionnaire (SNAQ). *Clin Nutr* 2005; **24**: 75-82 [PMID: 15681104 DOI: 10.1016/j.clnu.2004.07.015]
10 **Kroenke K**, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001; **16**: 606-613 [PMID: 11556941 DOI: 10.1046/j.1525-1497.2001.016009606.x]
11 **Spitzer RL**, Kroenke K, Williams JB, Löwe B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006; **166**: 1092-1097 [PMID: 16717171 DOI: 10.1001/archinte.166.10.1092]
12 **Kroenke K**, Spitzer RL, Williams JB, Löwe B. The Patient Health Questionnaire Somatic, Anxiety, and Depressive Symptom Scales: a systematic review. *Gen Hosp Psychiatry* 2010; **32**: 345-359 [PMID: 20633738 DOI: 10.1016/j.genhosppsych.2010.03.006]
13 **Zee van der KI**. Het meten van de algemene gezondheidstoestand met de RAND-36, een handleiding. Groningen: Noordelijk Centrum voor Gezondheidsvraagstukken, 1993: 28
14 **Galardi M**, Houkes I, de Rijk A. Validation of the disease experience questionnaire for working age adults with chronic conditions. *European Journal of Public Health* 2020; **30**: Supplement_5 [DOI: 10.1093/eurpub/ckaa166.1108]
15 **M Versteegh M**, M Vermeulen K, M A A Evers S, de Wit GA, Prenger R, A Stolk E. Dutch Tariff for the Five-Level Version of EQ-5D. *Value Health* 2016; **19**: 343-352 [PMID: 27325326 DOI: 10.1016/j.jval.2016.01.003]
16 **Reilly MC**, Bracco A, Ricci JF, Santoro J, Stevens T. The validity and accuracy of the Work Productivity and Activity Impairment questionnaire--irritable bowel syndrome version (WPAI:IBS). *Aliment Pharmacol Ther* 2004; **20**: 459-467 [PMID: 15298641 DOI: 10.1111/j.1365-2036.2004.02091.x]
17 **Mujagic Z**, Ludidi S, Keszthelyi D, Hesselink MA, Kruimel JW, Lenaerts K, Hanssen NM, Conchillo JM, Jonkers DM, Masclee AA. Small intestinal permeability is increased in diarrhoea predominant IBS, while alterations in gastroduodenal permeability in all IBS subtypes are largely attributable to confounders. *Aliment Pharmacol Ther* 2014; **40**: 288-297 [PMID: 24943095 DOI: 10.1111/apt.12829]
18 **Aaronson NK**, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, Sprangers MA, te Velde A, Verrips E. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. *J Clin Epidemiol* 1998; **51**: 1055-1068 [PMID: 9817123 DOI: 10.1016/s0895-4356(98)00097-3]
19 **Janssen B**, Szende A. Population Norms for the EQ-5D. 2013 Sep 26. In: Self-Reported Population Health: An International Perspective based on EQ-5D [Internet]. Dordrecht (NL): Springer; 2014– [PMID: 29787189]
20 **Statistics Netherlands**. Werkenden. 2023. [cited 21 June 2024]. Available from: https://www.cbs.nl/nl-nl/visualisaties/dashboard-arbeidsmarkt/werkenden
21 **Sharma A**, Zhao X, Hammill BG, Hernandez AF, Fonarow GC, Felker GM, Yancy CW, Heidenreich PA, Ezekowitz JA, DeVore AD. Trends in Noncardiovascular Comorbidities Among Patients Hospitalized for Heart Failure: Insights From the Get With The Guidelines-Heart Failure Registry. *Circ Heart Fail* 2018; **11**: e004646 [PMID: 29793934 DOI: 10.1161/CIRCHEARTFAILURE.117.004646]
22 **Lee WC**, Lee YT, Li LC, Ng HY, Kuo WH, Lin PT, Liao YC, Chiou TT, Lee CT. The Number of Comorbidities Predicts Renal Outcomes in Patients with Stage 3–5 Chronic Kidney Disease. *J Clin Med* 2018; **7** [PMID: 30486496 DOI: 10.3390/jcm7120493]

23 **Kreuter M**, Ehlers-Tenenbaum S, Palmowski K, Bruhwyler J, Oltmanns U, Muley T, Heussel CP, Warth A, Kolb M, Herth FJ. Impact of Comorbidities on Mortality in Patients with Idiopathic Pulmonary Fibrosis. *PLoS One* 2016; **11**: e0151425 [PMID: 27023440 DOI: 10.1371/journal.pone.0151425]

24 **Kruizenga H**, van Keeken S, Weijs P, Bastiaanse L, Beijer S, Huisman-de Waal G, Jager-Wittenaar H, Jonkers-Schuitema C, Klos M, Remijnse-Meester W, Witteman B, Thijs A. Undernutrition screening survey in 564,063 patients: patients with a positive undernutrition screening score stay in hospital 1.4 d longer. *Am J Clin Nutr* 2016; **103**: 1026-1032 [PMID: 26961930 DOI: 10.3945/ajcn.115.126615]

25 **ten Have M**, Tuithof M, van Dorsselaer S, Schouten F, de Graaf R. NEMESIS Kerncijfers psychische aandoeningen Vóórkomen van psychische aandoeningen Utrecht2022. Nov 30, 2022. [cited 19 September 2023]. Available from: https://cijfers.trimbos.nl/nemesis/kerncijfers-psychische-aandoeningen/voorkomen-psychische-aandoeningen/#12maands

26 **Costa FMD**, Martins SPV, Moreira ECTD, Cardoso JCMS, Fernandes LPNS. Anxiety in heart failure patients and its association with socio-demographic and clinical characteristics: a cross-sectional study. *Porto Biomed J* 2022; **7**: e177 [PMID: 36186120 DOI: 10.1097/j.pbj.0000000000000177]

27 **Pérez-García LF**, Silveira LH, Moreno-Ramírez M, Loaiza-Félix J, Rivera V, Amezcua-Guerra LM. Frequency of depression and anxiety symptoms in Mexican patients with rheumatic diseases determined by self-administered questionnaires adapted to the Spanish language. *Rev Invest Clin* 2019; **71**: 91-97 [PMID: 31056610 DOI: 10.24875/RIC.18002698]

28 **Takita Y**, Takeda Y, Fujisawa D, Kataoka M, Kawakami T, Doorenbos AZ. Depression, anxiety and psychological distress in patients with pulmonary hypertension: a mixed-methods study. *BMJ Open Respir Res* 2021; **8** [PMID: 33926959 DOI: 10.1136/bmjresp-2021-000876]

29 **Choi HS**, Yang DW, Rhee CK, Yoon HK, Lee JH, Lim SY, Kim YI, Yoo KH, Hwang YI, Lee SH, Park YB. The health-related quality-of-life of chronic obstructive pulmonary disease patients and disease-related indirect burdens. *Korean J Intern Med* 2020; **35**: 1136-1144 [PMID: 32066224 DOI: 10.3904/kjim.2018.398]

30 **Wolfe F**, Michaud K, Li T, Katz RS. EQ-5D and SF-36 quality of life measures in systemic lupus erythematosus: comparisons with rheumatoid arthritis, noninflammatory rheumatic disorders, and fibromyalgia. *J Rheumatol* 2010; **37**: 296-304 [PMID: 20032098 DOI: 10.3899/jrheum.090778]

31 **Yarlioglu AM**, Oguz EG, Gundogmus AG, Atilgan KG, Sahin H, Ayli MD. The relationship between depression, anxiety, quality of life levels, and the chronic kidney disease stage in the autosomal dominant polycystic kidney disease. *Int Urol Nephrol* 2023; **55**: 983-992 [PMID: 36184721 DOI: 10.1007/s11255-022-03375-2]

32 **de Jong RW**, Boezeman EJ, Chesnaye NC, Bemelman FJ, Massy ZA, Jager KJ, Stel VS, de Boer AGEM. Work status and work ability of patients receiving kidney replacement therapy: results from a European survey. *Nephrol Dial Transplant* 2022; **37**: 2022-2033 [PMID: 34643706 DOI: 10.1093/ndt/gfab300]

33 **Leso V**, Romano R, Santocono C, Caruso M, Iacotucci P, Carnovale V, Iavicoli I. The impact of cystic fibrosis on the working life of patients: A systematic review. *J Cyst Fibros* 2022; **21**: 361-369 [PMID: 34470710 DOI: 10.1016/j.jcf.2021.08.011]

34 **Sokka T**, Kautiainen H, Pincus T, Verstappen SM, Aggarwal A, Alten R, Andersone D, Badsha H, Baecklund E, Belmonte M, Craig-Müller J, da Mota LM, Dimic A, Fathi NA, Ferraccioli G, Fukuda W, Géher P, Gogus F, Hajjaj-Hassouni N, Hamoud H, Haugeberg G, Henrohn D, Horslev-Petersen K, Ionescu R, Karateew D, Kuuse R, Laurindo IM, Lazovskis J, Luukkainen R, Mofti A, Murphy E, Nakajima A, Oyoo O, Pandya SC, Pohl C, Predeteanu D, Rexhepi M, Rexhepi S, Sharma B, Shono E, Sibilia J, Sierakowski S, Skopouli FN, Stropuviene S, Toloza S, Valter I, Woolf A, Yamanaka H; QUEST-RA. Work disability remains a major problem in rheumatoid arthritis in the 2000s: data from 32 countries in the QUEST-RA study. *Arthritis Res Ther* 2010; **12**: R42 [PMID: 20226018 DOI: 10.1186/ar2951]

35 **Cruz MC**, Andrade C, Urrutia M, Draibe S, Nogueira-Martins LA, Sesso Rde C. Quality of life in patients with chronic kidney disease. *Clinics (Sao Paulo)* 2011; **66**: 991-995 [PMID: 21808864 DOI: 10.1590/S1807-59322011000600012]

36 **Huber A**, Oldridge N, Höfer S. International SF-36 reference values in patients with ischemic heart disease. *Qual Life Res* 2016; **25**: 2787-2798 [PMID: 27318487 DOI: 10.1007/s11136-016-1316-4]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3428-3446

DOI: 10.3748/wjg.v30.i28.3428

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Effects of elafibranor on liver fibrosis and gut barrier function in a mouse model of alcohol-associated liver disease

Aritoshi Koizumi, Kosuke Kaji, Norihisa Nishimura, Shohei Asada, Takuya Matsuda, Misako Tanaka, Nobuyuki Yorioka, Yuki Tsuji, Koh Kitagawa, Shinya Sato, Tadashi Namisaki, Takemi Akahane, Hitoshi Yoshiji

Specialty type: Gastroenterology and hepatology

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

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade B, Grade C, Grade C

Novelty: Grade A, Grade A, Grade B, Grade C

Creativity or Innovation: Grade A, Grade B, Grade B, Grade C

Scientific Significance: Grade A, Grade A, Grade B, Grade B

P-Reviewer: Feng X; Pavlidis TE; Zhang XF

Received: February 26, 2024

Revised: May 31, 2024

Accepted: June 20, 2024

Published online: July 28, 2024

Processing time: 148 Days and 17.4 Hours

Aritoshi Koizumi, Kosuke Kaji, Norihisa Nishimura, Shohei Asada, Takuya Matsuda, Misako Tanaka, Nobuyuki Yorioka, Yuki Tsuji, Koh Kitagawa, Shinya Sato, Tadashi Namisaki, Takemi Akahane, Hitoshi Yoshiji, Department of Gastroenterology, Nara Medical University, Kashihara 634-8521, Japan

Corresponding author: Kosuke Kaji, MD, PhD, Chief, Lecturer, Department of Gastroenterology, Nara Medical University, Shijo-cho 840, Kashihara 634-8521, Japan.
kajik@naramed-u.ac.jp

Abstract

BACKGROUND

Alcohol-associated liver disease (ALD) is a leading cause of liver-related morbidity and mortality, but there are no therapeutic targets and modalities to prevent ALD-related liver fibrosis. Peroxisome proliferator activated receptor (PPAR) α and δ play a key role in lipid metabolism and intestinal barrier homeostasis, which are major contributors to the pathological progression of ALD. Meanwhile, elafibranor (EFN), which is a dual PPARα and PPARδ agonist, has reached a phase III clinical trial for the treatment of metabolic dysfunction-associated steatotic liver disease and primary biliary cholangitis. However, the benefits of EFN for ALD treatment is unknown.

AIM

To evaluate the inhibitory effects of EFN on liver fibrosis and gut-intestinal barrier dysfunction in an ALD mouse model.

METHODS

ALD-related liver fibrosis was induced in female C57BL/6J mice by feeding a 2.5% ethanol (EtOH)-containing Lieber-DeCarli liquid diet and intraperitoneally injecting carbon tetrachloride thrice weekly (1 mL/kg) for 8 weeks. EFN (3 and 10 mg/kg/day) was orally administered during the experimental period. Histological and molecular analyses were performed to assess the effect of EFN on steatohepatitis, fibrosis, and intestinal barrier integrity. The EFN effects on HepG2 lipotoxicity and Caco-2 barrier function were evaluated by cell-based assays.

RESULTS

The hepatic steatosis, apoptosis, and fibrosis in the ALD mice model were

significantly attenuated by EFN treatment. EFN promoted lipolysis and β-oxidation and enhanced autophagic and antioxidant capacities in EtOH-stimulated HepG2 cells, primarily through PPARα activation. Moreover, EFN inhibited the Kupffer cell-mediated inflammatory response, with blunted hepatic exposure to lipopolysaccharide (LPS) and toll like receptor 4 (TLR4)/nuclear factor kappa B (NF-κB) signaling. EFN improved intestinal hyperpermeability by restoring tight junction proteins and autophagy and by inhibiting apoptosis and proinflammatory responses. The protective effect on intestinal barrier function in the EtOH-stimulated Caco-2 cells was predominantly mediated by PPARδ activation.

CONCLUSION

EFN reduced ALD-related fibrosis by inhibiting lipid accumulation and apoptosis, enhancing hepatocyte autophagic and antioxidant capacities, and suppressing LPS/TLR4/NF-κB-mediated inflammatory responses by restoring intestinal barrier function.

Key Words: Liver fibrosis; Ethanol; Gut barrier function; Apoptosis; Autophagy; Peroxisome proliferator activated receptor

Core Tip: Peroxisome proliferator activated receptor (PPAR) α and δ play a key role in lipid metabolism and intestinal barrier homeostasis, which are major contributors to alcohol-associated liver disease (ALD) pathogenesis. This study elucidates the preventive effect of elafibranor, a dual PPARα/δ agonist from ALD-related liver fibrosis induced by ethanol plus carbon tetrachloride in mice. This effect is involved in multifaceted regulatory functions: (1) Suppression of lipid accumulation and improvement of autophagy in hepatocytes, which reduced apoptosis and enhanced antioxidant activities; and (2) Inhibition of toll like receptor 4 pathway with blockade of hepatic influx of lipopolysaccharide by repairing intestinal barrier integrity. This regimen represents a potential strategy against ALD-related liver fibrosis.

Citation: Koizumi A, Kaji K, Nishimura N, Asada S, Matsuda T, Tanaka M, Yorioka N, Tsuji Y, Kitagawa K, Sato S, Namisaki T, Akahane T, Yoshiji H. Effects of elafibranor on liver fibrosis and gut barrier function in a mouse model of alcohol-associated liver disease. *World J Gastroenterol* 2024; 30(28): 3428-3446

URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3428.htm

DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3428

INTRODUCTION

Alcohol-associated liver disease (ALD), which is a notorious harmful consequence of excessive alcohol consumption, places an enormous burden worldwide[1-4]. ALD represents a spectrum of liver injury, ranging from hepatic steatosis to more advanced stages with fibrosis progression, including steatohepatitis, cirrhosis, and even hepatocellular carcinoma [1-4]. Currently, approximately 25% of cirrhosis-related mortality worldwide are causally related with alcohol consumption[1-4]. Therefore, identification of therapeutic targets and modalities to prevent ALD-related liver fibrosis is urgent.

ALD causes liver fibrosis through a multifaceted mechanism, which includes lipid accumulation, apoptosis, and proinflammatory response. In the process of ethanol (EtOH) metabolism, EtOH and acetaldehyde are continuously oxidized by alcohol dehydrogenase and aldehyde dehydrogenase, respectively, to generate large amounts of reduced nicotinamide adenine dinucleotide[5,6]. These changes shift liver metabolism toward the reductive synthesis of lipids[5,6]. Moreover, excessive EtOH consumption augments the activity of sterol regulatory element-binding protein-1c, which is the master regulator of fatty acid synthesis, and dampens the activity of peroxisome proliferator activated receptor (PPAR) α, which controls fatty acid breakdown[5-8]. Consequently, fatty acids, which are esterified into triglycerides (TGs), accumulate in hepatocytes as lipid droplets[5-8].

The gut–liver crosstalk is also functionally involved in the pathophysiology of ALD[4-6,9,10]. Excessive alcohol consumption compromises the integrity of the intestinal barrier by disrupting tight junctions, resulting in the transfer of gut-derived lipopolysaccharides (LPS) to liver tissue; thereafter, LPS is recognized by toll like receptor 4 (TLR4), which is expressed on the liver parenchyma and innate immune cells[9-11]. The LPS/TLR4 axis triggers the downstream activation of nuclear factor kappa B (NF-κB), which is a master regulator of the proinflammatory response in ALD[9-11].

PPARα and PPARδ have been known to represent promising therapeutic targets in chronic liver disease, including ALD[12,13]. PPARα is abundantly expressed in metabolically active tissues, such as the liver, heart, kidneys, and intestines. As described above, PPARα activation is functionally associated with the pathways of hepatic lipid metabolism, including fatty acid oxidation, elongation, and desaturation and TG synthesis and breakdown[8]. Moreover, PPARα plays a key role in protecting against oxidative stress by increasing the expression of antioxidant enzymes[14,15]. Therefore, PPARα is a potential target for detoxifying EtOH-induced hepatotoxicity, thereby, preventing ALD progression. Meanwhile, PPARδ has been implicated in lipid metabolism and energy homeostasis in various organs, including the liver[16]. In a recent animal study on metabolic dysfunction-associated steatohepatitis (MASH) models, PPARδ was

shown to reduce hepatic steatosis through autophagy-mediated fatty acid oxidation[17]. Moreover, because of its intestinal expression, PPARδ activation can increase the proliferation of intestinal epithelial cells and suppress macrophage-derived inflammation[18]. Consistently, another study found that a selective PPARδ agonist attenuated EtOH-induced liver injury and improved gut barrier function in mice[19]. However, the efficacy of each monoagonist of PPARα or PPARδ alone in inhibiting liver fibrosis development in ALD appears to be limited.

Elafibranor (EFN), which is a dual PPARα/PPARδ agonist, has been developed reached a phase III clinical trial for MASH[20]. Although EFN demonstrated a modest effect on the histological resolution of MASH, it had no beneficial effect on fibrosis[20]. Meanwhile, a recent phase III clinical trial on primary biliary cholangitis has shown that EFN treatment resulted in significantly greater improvements in the relevant biochemical indicators of cholestasis[21]. However, the effect of EFN on ALD-related fibrosis remains unclear. In the current study, we sought to elucidate the benefits of EFN-mediated dual activation of PPARα/PPARδ on ALD-related liver fibrosis, particularly its the effects on lipotoxicity, oxidative stress, and intestinal barrier function.

MATERIALS AND METHODS

Animals and compounds

Ten-week-old female and male C57BL/6J mice (CLEA Japan, Osaka, Japan) were caged with free access to food and water, under controlled temperature (23 ± 3 °C) and humidity (50% ± 20%) and a 12-hour light/dark cycle. This study was reviewed and approved by the ethics committee of Nara Medical University (No. 13130) and was performed in accordance with the Guide for Care and Use of Laboratory Animals of the National Research Council. EFN (code name GFT505) was purchased from MedChemExpress (Monmouth Junction, NJ, United States). GW7647 and GSK3787 (Abcam, Cambridge, United Kingdom) were used as selective antagonists of PPARα and PPARδ, respectively.

In vivo experimental protocol

Female mice (n = 40) were randomly divided into four experimental groups (n = 10 each) and underwent treatment for 8 weeks (Figure 1A). The control group was fed a non-EtOH normal liquid diet (Research Diets, New Brunswick, NJ, United States) and received intraperitoneal injections of corn oil (Nacalai Tesque, Kyoto, Japan) three times weekly. Lactose hydrate (FUJIFILM, Wako Pure Chemical Corporation, Osaka, Japan) was administered as the vehicle for EFN. The three ALD mice groups were fed a 2.5% (v/v) EtOH-containing Lieber–DeCarli liquid diet (research diets); received intraperitoneal injection of carbon tetrachloride (CCl_4) (FUJIFILM, Wako Pure Chemical Corporation) three times a week (1 mL/kg); and received daily gavage of either: (1) Lactose hydrate as the vehicle; (2) Low dose EFN (3 mg/kg); or (3) High dose EFN (10 mg/kg)[22]. Additionally, male mice (n = 24) were randomly divided into four experimental groups (n = 6 each) and underwent treatment similar to female mice. After 8 weeks of experimental intervention, mice were anesthetized by intravenous injection of 150 mg/kg sodium pentobarbital and euthanized. Blood was drawn from the aorta, and the liver and ileum were removed immediately after sacrifice.

Cell culture

The human hepatocellular cell line HepG2 and the human activated hepatic stellate cell (HSC) line LX-2 were obtained from the Japanese Collection of Research Bioresources Cell Bank (Osaka, Japan) and Merck KGaA (Darmstadt, Germany), respectively. The human intestinal epithelial cell line Caco-2 was purchased from Riken BRC (Ibaraki, Japan). The cells were suspended in Dulbecco's modified Eagle medium (Nacalai tesque), which was supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, United States), 1% penicillin-streptomycin, 1% nonessential amino acids, and 25 mmol/L glucose and incubated at 37 °C in a 5% CO_2 air environment. All assays for the Caco-2 cells were performed after 10-20 passages. Depending on each assay, the cells were incubated with EtOH (0-50 mmol/L), EFN (0-30 μM), GW7647 (10 μM), and GSK3787 (10 μM).

Biochemical analysis

Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma glutamyl transferase (GGT) were measured using Mouse enzyme-linked immunosorbent assay (ELISA) kits for each enzyme (Abcam).

Histological and immunohistochemical analyses

Paraffin sections (4 μm) of mouse liver and ileum tissues were prepared with hematoxylin and eosin staining and Sirius-Red staining. Two pathologists independently evaluated liver pathology scores by randomly magnifying 10 fields from each slide by 400 × according to previously reported criteria[23]. Cell apoptosis in liver sections was measured by the TdT-mediated dUTP Nick End Labeling (TUNEL) assay using an in situ apoptosis detection kit (Takara Bio Inc., Kusatsu, Japan) according to the manufacturer's instructions.

Immunohistochemical detection of Ki67, α-smooth muscle actin (SMA), and F4/80 (Supplementary Table 1) was performed on paraffin liver sections (4 μm), and subsequent sections were exposed to HRP-antibody colored with DAB. Paraffin ileum sections (4 μm) were prepared for immunofluorescence, incubated with primary antibody overnight, followed by the secondary antibody, and then mounted with 4',6-diamidino-2-phenylindole. The primary antibodies included zonula occludens-1 (ZO-1), occludin, and claudin-2 (Supplementary Table 1). The secondary antibodies included Alexa Fluor-conjugated secondary antibodies (1:200; Thermo Fisher Scientific). Semiquantitative analysis was performed using ImageJ software version 64 (National Institutes of Health, Bethesda, MD, United States).

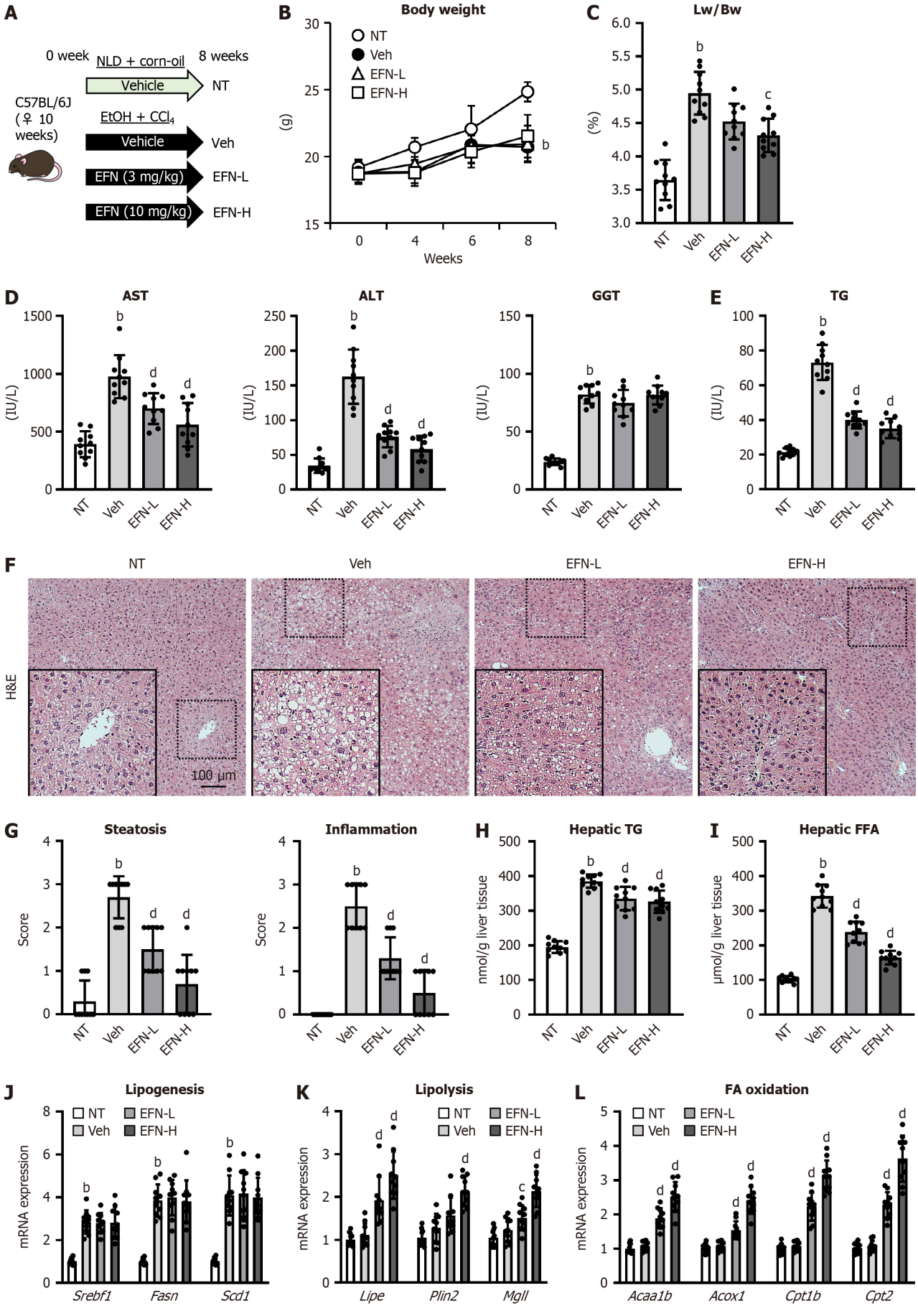


Figure 1 Elafibranor on steatohepatitis and lipid accumulation in the alcohol-associated liver disease mice. A: *In vivo* experimental design; B:

Changes in the body weights during the experimental period (n = 10); C: Liver/body weight at the end of experiment (n = 10); D: Serum levels of aspartate aminotransferase, alanine aminotransferase and gamma glutamyl transferase (n = 10); E: Serum triglyceride level (n = 10); F: Representative microphotographs of hematoxylin and eosin of the livers in the experimental mice; G: Hepatic pathological scores for steatosis and inflammation. Localized magnified images in the lower left corner of each picture (n = 10); H: Hepatic triglyceride content; I: Hepatic free fatty acid concentration (n = 10); J-L: Hepatic mRNA level of the markers related to lipogenesis (J), lipolysis (K), fatty acid oxidation (L) (n = 10). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction. Quantitative values are indicated as fold changes to the values of non-therapeutic group. Data are the mean ± SD. $^{b}P < 0.01$ *vs* non-therapeutic group; $^{c}P < 0.05$ *vs* vehicle-treated alcohol-associated liver disease group; $^{d}P < 0.01$ *vs* vehicle-treated alcohol-associated liver disease group, significant difference between groups by Student's *t*-test. NT: Non-therapeutic group; Veh: Vehicle-treated alcohol-associated liver disease group; EFN-L: Elafiblanor (3 mg/kg/day)-treated alcohol-associated liver disease group; EFN-H: Elafibranor (10 mg/kg/day)-treated alcohol-associated liver disease group; NLD: Normal liquid diet; EtOH: Ethanol; CCL_4: Carbon tetrachloride; EFN: Elafiblanor; Lw: Liver weight; Bw: Body weight; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transferase; TG: Triglyceride; H&E: Hematoxylin and eosin; FFA: Free fatty acid; FA: Fatty acid.

Real-time quantitative polymerase chain reaction

Total RNA was isolated from mouse liver and ileum tissue specimens and whole cell lysates using Trizol kit (Sigma-Aldrich, Inc, St. Louis, MO, United States) according to the manufacturer's instruction. The RNA was cleaned using the QiagenRNeasy miniRNA cleanup kit (Qiagen, Valencia, CA, United States). The concentration of total RNA was performed by measuring the absorbance of RNA sample solutions at 260 nm by using a NanoDrop ND-1000 UV-Vis (Thermo Fisher Scientific). Total RNA (1.0 μg) was reverse transcribed using iScript cDNA reverse transcription kits (Bio-Rad, Hercules, CA, United States) according to the manufacturer's instructions. Quantitative polymerase chain reaction was performed using the Fast Start Universal SYBR Green Master Mix (Applied Biosystems, Foster City, CA, United States). Real-time quantitative polymerase chain reaction was performed using Applied Biosystems® 7500 Real-Time PCR Systems (Thermo Fisher Scientific) with corresponding primers (Supplementary Table 2). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was simultaneously assayed as a loading control. Data was analyzed using a $2^{-\Delta\Delta CT}$ method.

Western blotting

According to the standard protocol, proteins were isolated from the mouse liver and ileum tissues in RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO, United States) plus Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). The protein concentrations were measured by Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific) and normalized to 2.5 μg/μL. Protein samples were separated by sodium-dodecyl sulfate gel electrophoresis (Thermo Fisher Scientific) and transferred to an Invitrolon™ polyvinylidene fluoride membrane (Thermo Fisher Scientific). After sealing with 5% skimmed milk, the membranes were successively incubated overnight at 4 °C with diluted primary antibodies, including LC3, IκBα, p-NFκB, NF-κB, Bcl-2, and Mcl-1 (Supplementary Table 1). The next day, the membrane was washed and incubated with Amersham ECL IgG and HRP-linked F(ab)2 fragment (1:5000, Cytiva, Tokyo, Japan) as secondary antibodies. Chemiluminescence was detected using a Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, United States) with Bright™ CL1500 Imaging System (Thermo Fisher Scientific). Densitometric analysis was performed using ImageJ software version 64 (NIH).

TG and free fatty acid concentrations

The TG content in mouse serum, liver tissue, and cultured HepG2 was measured using Triglyceride-Glo™ Assay (Promega, Madison, WI, United States), following the manufacturer's instructions. The free fatty acid content in mouse liver tissue was determined using the Free Fatty Acid Assay Kit (Abcam), according to the manufacturer's instructions. Protein content was normalized using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific).

Cleaved caspase-3 levels and caspase-3/7 activity

To assess *in vivo* cell apoptosis, the cleaved caspase-3 concentrations in mouse liver and ileum tissues were measured using the Cleaved-Caspase-3 (D175) ELISA Kit (Raybiotech, Norcross, GA, United States), according to the manufacturer's instructions. Protein content was normalized using Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific). *In vitro* apoptosis in the HepG2 and Caco-2 cells (1×10^6 cells) was determined using the Caspase-Glo 3/7 Assay System (Promega), according to the manufacturer's instructions.

Superoxide dismutase 1 and catalase levels

The levels of the antioxidant enzymes superoxide dismutase 1 (SOD1) and catalase (CAT) in mouse liver tissue were measured using a Mouse Superoxide Dismutase 1 ELISA Kit (Abcam) and a Mouse Catalase ELISA Kit (CUSABIO, Houston, TX, United States), respectively, following the manufacturers' instructions. Both enzyme levels were also determined in the cultured HepG2 cells using the Human Superoxide Dismutase 1 ELISA Kit and Human Catalase ELISA Kit (Abcam), respectively, following the manufacturers' instructions.

Hydroxyproline and tissue inhibitor of metalloproteinase 1 levels and matrix metalloproteinase activity

The mouse liver tissue concentrations of hydroxyproline and tissue inhibitor of metalloproteinase 1 (TIMP1) and activity of matrix metalloproteinase (MMP) 2, MMP9, and MMP13 were assessed using a Hydroxyproline Assay Kit (Cell Biolabs, San Diego), Mouse TIMP1 ELISA Kit (Abcam) and SensoLyte 490 MMP2, MMP9, and MMP-13 Assay Kits (AnaSpec, Fremont, CA, United States), respectively, following the manufacturers' instructions.

Fluorescein isothiocyanate-dextran intestinal permeability assay

The intestinal permeability was assessed by the *in vivo* fluorescein isothiocyanate (FITC)-dextran permeability assay using additional experimental mice groups (n = 10 for each group)[24]. After a 12-hour fast, the mice were administered a dose of FITC-dextran (4 kDa) (Sigma-Aldrich) (600 mg/kg) dissolved in sterile phosphate-buffered saline by oral cannulation. Then, 2.5 hours after FITC–dextran administration, approximately 200 μL of blood was drawn *via* the portal vein and centrifuged for 15 minutes at 3500 × g at 4 °C. The plasma FITC-dextran concentrations were assessed in a plate reader with an excitation wavelength of 490 nm and an emission wavelength of 520 nm.

Cell viability and proliferation assays

HepG2 or LX-2 cells were seeded in 96-well plates (5 × 10^4 cells/well) with Dulbecco's modified Eagle medium containing 10% fetal bovine serum for 24 hours. Thereafter, the cells were exposed with/without EtOH (50 mmol/L) and in different concentrations of EFN (0-30 μM). Cell viability was determined using WST-8 Assay Kit (Abcam), according to the manufacturer's protocol.

Transepithelial electrical resistance

To evaluate the epithelial barrier function in Caco-2 cells, transepithelial electrical resistance (TEER) was measured using a Millicell-ERS device (Millipore Corp., Bedford, MA, United States), as previously described[25]. The result was multiplied by the effective membrane area to obtain the final TEER value. The electrical resistance was expressed in units of Ω/cm^2 using the surface area of the Trans-well insert.

Statistical analyses

Data are presented as mean ± SD. Statistical significance was analyzed with a 2-sided Student's t-test or one-way analysis of variance, followed by Bonferroni's multiple comparison test, as appropriate using GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, United States). Statistical significance was defined as a $P < 0.05$. Additional methods can be found online in the Supplementary material.

RESULTS

Effect of EFN on the activation of hepatic PPARα/δ signaling in the ALD mice

Figure 1A displays the *in vivo* study design to assess the effects of EFN on EtOH + CCl_4-induced ALD-related liver injury in mice. Initially, we verified the effect of EFN on the activation of PPARα and PPARδ signaling in the liver and intestinal tissues of ALD mice. In the ALD mice, the hepatic expression of *PPARA* was decreased and treatment with EFN increased its expression (Supplementary Figure 1A). PPARα plays a key role in the regulation of lipid metabolism in the liver of ALD by phospholipase A2 (PLA2)/cyclooxygenase (COX)-2 pathway[26]. Consistently, treatment with EFN increased the hepatic level of PLA2 and COX-2 in parallel with increased hepatic *PPARA* expression in the ALD mice (Supplementary Figure 1B). Meanwhile, the hepatic expression of *PPARD* was also decreased in the ALD mice but treatment with EFN did not alter the hepatic expression of *PPARD* as well as its target gene, *Cyp2b10* (Supplementary Figure 1C and D)[27].

EFN reduced steatohepatitis and lipid accumulation in the ALD mice

Compared with the control female mice, the ALD mice demonstrated inhibited body weight gain, and treatment with both low and high doses of EFN did not significantly alter the body weight loss in the ALD mice (Figure 1B). Meanwhile, the ALD mice showed marked hepatomegaly, which was attenuated by treatment with high-dose EFN (Figure 1C). The serum levels of AST, ALT, and GGT were markedly elevated in the ALD mice, and treatment with EFN significantly reduced the levels of AST and ALT but did not affect the GGT level (Figure 1D). As shown in Figure 1E, the ALD mice exhibited hypertriglyceridemia, which was suppressed by EFN treatment. Histological analysis revealed hepatic steatosis and necroinflammation in the ALD mice, and these histological changes were attenuated by EFN treatment (Figure 1F and G). In accordance with the attenuation of hepatic steatosis, EFN treatment significantly reduced the hepatic levels of TG and free fatty acid (Figure 1H and I). These inhibitory effects of EFN on steatohepatitis were also observed in male mice as well as female mice (Supplementary Figure 2A-D).

Next, we evaluated the effect of EFN on lipid metabolism in the liver of the ALD mice. EFN did not change the hepatic mRNA levels of the lipogenesis-related markers, including *Srebf1*, *Fasn*, and *Scd1* (Figure 1J). Meanwhile, treatment with EFN significantly increased the hepatic expressions of the markers related with lipolysis (*i.e.*, *Lipe*, *Plin2*, and *Mgll*) and fatty acid oxidation (*i.e.*, *Acaa1b*, *Acox1*, *Cpt1b*, and *Cpt2*) (Figure 1K and L).

EFN suppressed hepatocyte apoptosis by improving autophagy and antioxidant activity in the ALD mice

We next assessed the effect of EFN on hepatocyte cell death in the ALD mice. In accordance with the progression of steatosis and necroinflammation, the number of TUNEL-positive apoptotic hepatocytes were higher, whereas the number of Ki67-positive proliferative hepatocytes were lower in the ALD mice than in the control mice (Figure 2A and B). EFN treatment significantly decreased the apoptosis and increased hepatocyte proliferation in the ALD mice (Figure 2A and B). Reflecting hepatocyte apoptosis, the level of cleaved caspase-3 in the liver tissue increased in the ALD mice, and this was suppressed by EFN treatment (Figure 2C). The autophagy-related markers, including hepatic *LC3-II* levels, and the expression of *Atg7* and *Beclin-1* were decreased in the ALD mice, indicating that EtOH + CCl_4 administration impaired autophagy in mice (Figure 2D and E). To support this inference, the ALD mice showed an increase in the hepatic

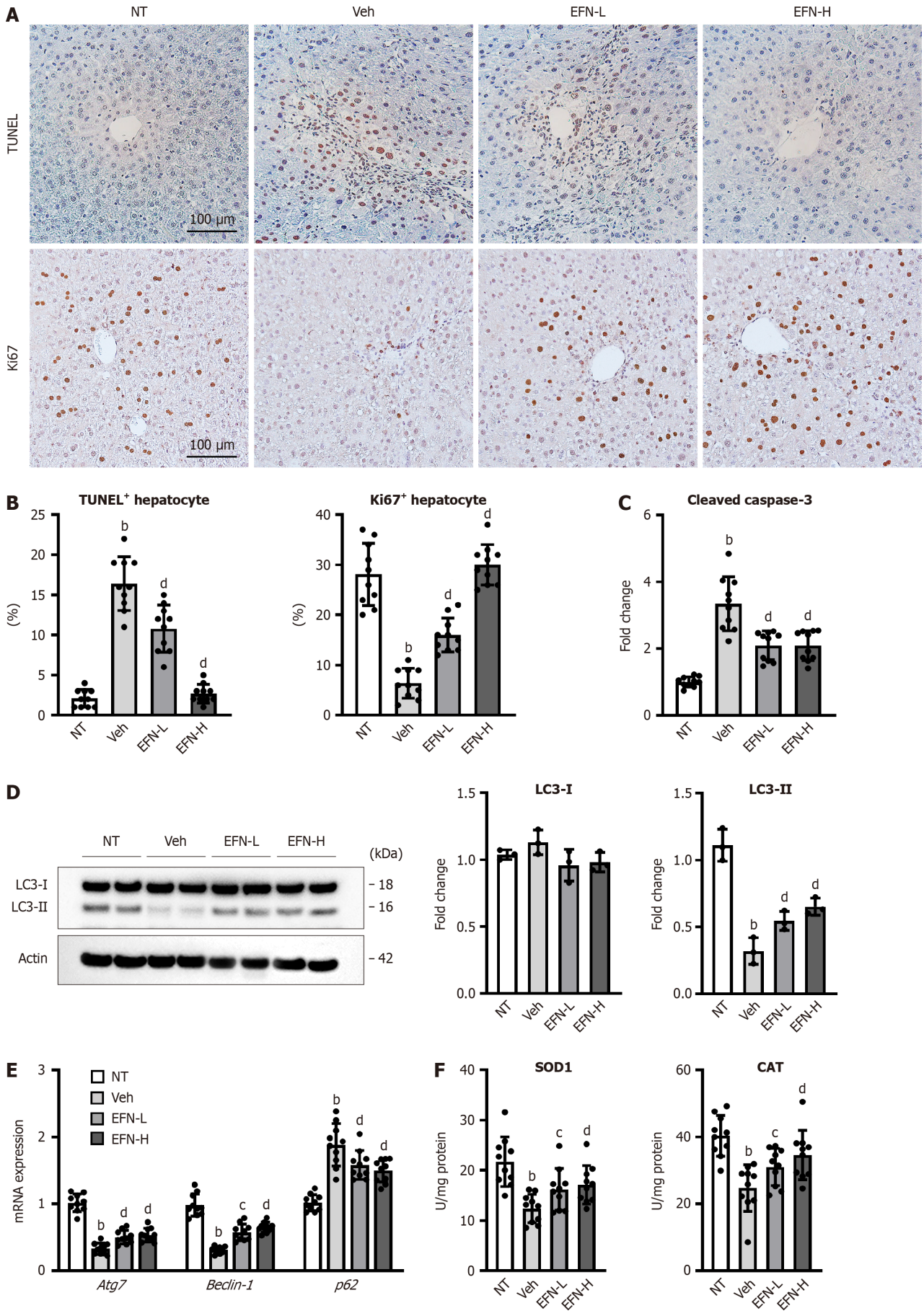


Figure 2 Elafibranor on hepatocyte cell death, autophagy and oxidative stress in the alcohol-associated liver disease mice. A:

Representative microphotographs of TdT-mediated dUTP Nick End Labeling (TUNEL) and Ki67 staining of the livers in the experimental mice; B: Quantification of TUNEL-positive hepatocytes and Ki67-positive hepatocytes in high-power field (n = 10); C: Cleaved caspase-3 level in the liver tissue (n = 10); D: Western blot for LC3-1 and 2 protein level in the liver tissue. Actin was used as an internal control (n = 3); E: Hepatic mRNA level of the markers related to autophagy (n = 10); F: Hepatic level of antioxidant enzymes, superoxide dismutase 1 and catalase (n = 10). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction (E and F). Quantitative values are indicated as fold changes to the values of non-therapeutic group (C-E). Data are the mean ± SD. $^{b}P < 0.01$ *vs* non-therapeutic group; $^{c}P < 0.05$ *vs* vehicle-treated alcohol-associated liver disease group; $^{d}P < 0.01$ *vs* vehicle-treated alcohol-associated liver disease group, significant difference between groups by Student's *t*-test. NT: Non-therapeutic group; Veh: Vehicle-treated alcohol-associated liver disease group; EFN-L: Elafiblanor (3 mg/kg/day)-treated alcohol-associated liver disease group; EFN-H: Elafibranor (10 mg/kg/day)-treated alcohol-associated liver disease group; TUNEL: TdT-mediated dUTP Nick End Labeling; SOD: Superoxide dismutase; CAT: Catalase.

expression of *p62*, which is a predictor of autophagy flux and is inversely correlated with autophagy activity (Figure 2E). Treatment with EFN induced the upregulation of *LC3-II*, *Atg7*, and *Beclin-1* and downregulation of *p62* (Figure 2D and E). Moreover, EFN treatment increased the hepatic levels of antioxidant markers, including SOD1 and CAT, in the ALD mice (Figure 2F).

EFN exerted an antifibrotic effect in the ALD mice

The ALD mice exhibited fibrotic livers as identified by Sirius-Red staining of liver sections (Figure 3A). Treatment with EFN markedly reduced hepatic fibrosis at both doses (3 and 10 mg/kg), as well as the number of α-SMA^{+} myofibroblasts, which was increased in ALD mice (Figure 3A and B). Semiquantitative analysis showed that the degree of liver fibrosis and HSC expansion was reduced to less than 50% after treatment with EFN, especially at a high dose (Figure 3B). Similar to what was observed in the effect on steatohepatitis, the inhibition of fibrosis by EFN was observed not only in female mice but also in male mice (Supplementary Figure 3A and B). In accordance with these changes in the histological features, EFN treatment reduced the hepatic content of hydroxyproline in the ALD mice (Figure 3C). Moreover, the ALD mice had increased hepatic mRNA expressions of profibrotic genes (*i.e.*, *Acta2*, *Col1a1*, and *Tgfb1*), and this effect was suppressed by EFN treatment (Figure 3D). We also assessed the activity of MMPs in regulating extracellular matrix homeostasis. As shown in Figure 3E, the ALD mice exhibited increased activity of hepatic MMP2, MMP9, and MMP13, and this effect was reduced by EFN treatment. These effects of EFN on MMP activity were associated with a decrease in hepatic TIMP-1 levels (Figure 3F).

EFN protected against EtOH-stimulated steatosis and apoptosis in HepG2 cells but did not directly affect LX-2 cells

Following the ameliorative effect of EFN on alcoholic liver injury in mice, we analyzed its effects on hepatocytes and activated HSCs using HepG2 and LX-2 cells. EtOH exposure increased the TG levels in HepG2 cells, and EFN effectively suppressed this effect in a dose-dependent manner (Figure 4A). Regarding the markers of lipid metabolism, EFN did not change the mRNA expression of *SREBF1*, but it increased those of *LIPE*, *CPT1B*, and *CPT2* in the EtOH-exposed HepG2 cells (Figure 4B). Interestingly, the effect of EFN on lipid accumulation was negated by pretreatment with GW7647 but was not changed by GSK3787 (Figure 4B), suggesting that this effect was mainly mediated by PPARα activation. As shown in Figure 4C-E, EFN dose-dependently suppressed the decline in cell viability and increased caspase-3/7 activity in EtOH-stimulated HepG2 cells. We also found that EFN improved autophagic activity, as indicated by the upregulation of *Atg7* and *Beclin-1* and the downregulation of *p62* in the EtOH-stimulated HepG2 cells (Figure 4F). Moreover, EFN treatment increased the production of antioxidant markers in HepG2 cells (Figure 4G). These effects on cell survival, autophagy, and oxidative stress were predominantly inhibited by pretreatment with GW7647 (Figure 4D-G). In contrast, EFN did not affect cell proliferation or profibrogenic activity in the EtOH-stimulated LX-2 cells (Figure 4H and I).

EFN inhibited macrophage activation and TLR4/NF-κB signaling in the liver of ALD mice

The inflammatory status in the ALD mice liver was examined based on the EFN-induced improvement in hepatic inflammation and fibrosis. F4/80^{+} macrophages infiltration was observed in the liver of the ALD mice, which was attenuated by EFN treatment (Figure 5A and B). In the pathogenesis of ALD progression, the LPS/TLR4 pathway plays a key role in the hepatic activation of macrophages[9-11]. As shown in Figure 5C and D, the ALD mice showed an increase in hepatic mRNA levels of LPS-binding protein (*Lbp*), a reactant that mediates innate immune responses triggered by LPS, and *Tlr4* and its coreceptor *Cd14*, which can recognize LPS. Notably, the upregulation of these genes was significantly suppressed by EFN treatment (Figure 5C and D).

In ALD mice, these increased mRNA expression levels were accompanied by decreased IκBα protein levels and augmented phosphorylation of NF-κB, indicating IκBα degradation and NF-κB phosphorylation (Figure 5E and F). EFN treatment attenuated the TLR4-mediated activation of NF-κB in the ALD mice, consistent with the reduced hepatic overload of LPS (Figure 5E and F). Consequently, EFN treatment reduced the hepatic expression of the proinflammatory cytokines tumor necrosis factor-alpha (*Tnfa*), interleukin-6 (*Il6*), and *Il1b* and the chemokine *Ccl2*, which were increased in the ALD mice (Figure 5G and H).

EFN protected the gut barrier integrity and restored intestinal autophagic activity in the ALD mice

Given that EFN reduced the hepatic *Lbp* expression (Figure 5C), we focused on the effect of EFN on intestinal barrier integrity related to LPS influx into the liver. Intestinal *PPARA* and *PPARD* expressions were decreased in the ALD mice, and treatment with EFN did not alter the intestinal expression of *PPARA* but increased that of *PPARD* (Supplementary Figure 4A and B). Next, we validated the selected gene expression which is related to PPARδ-mediated gut barrier

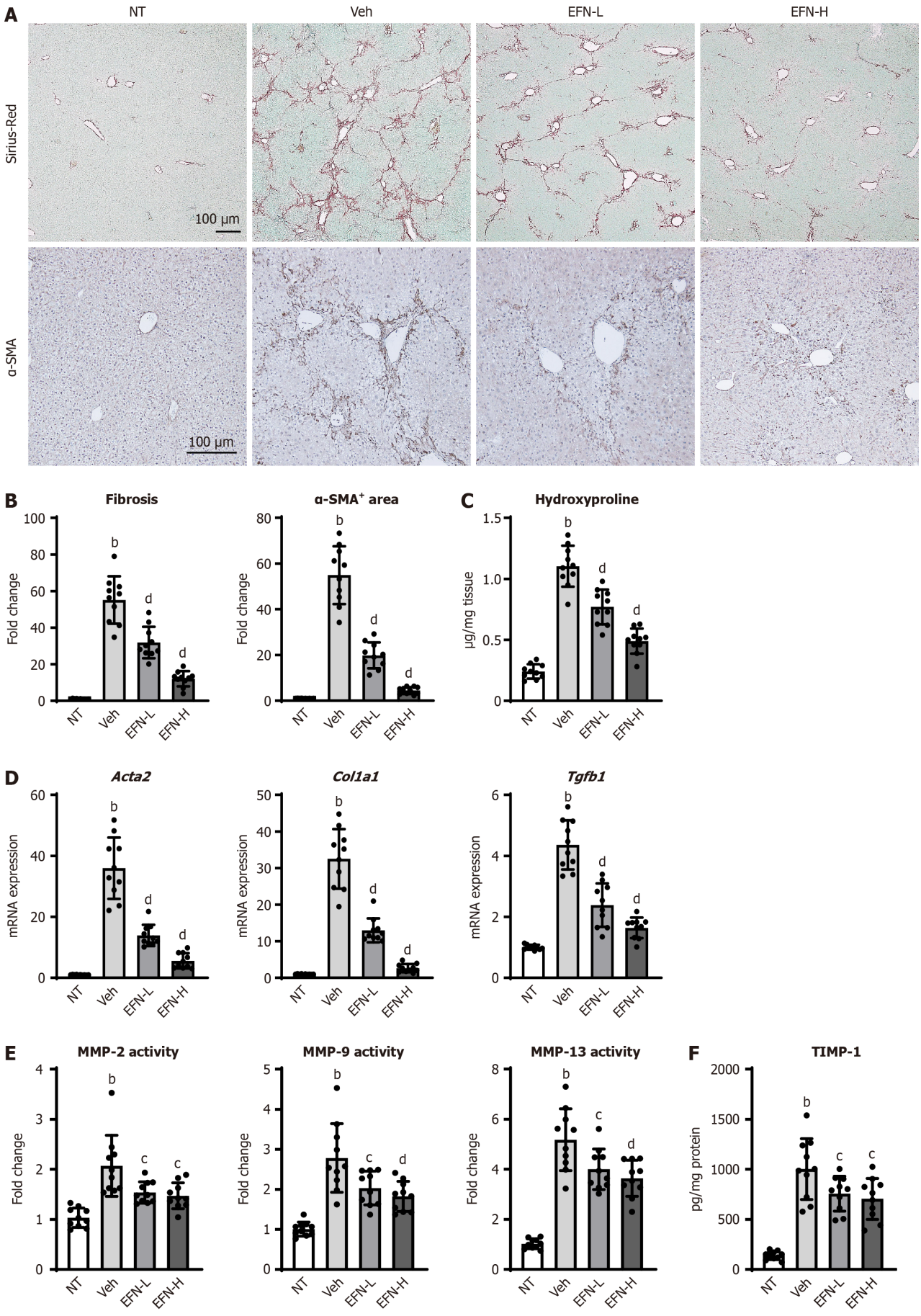


Figure 3 Elafibranor on hepatic fibrosis development in the alcohol-associated liver disease mice. A: Representative microphotographs of sirius-

red and α-smooth muscle actin (SMA) staining of the livers in the experimental mice; B: Quantification of sirius-red stained fibrotic area and α-SMA-positive area in high-power field (n = 10); C: Hepatic concentration of hydroxyproline (n = 10); D: Hepatic mRNA level of profibrotic markers (n = 10); E: Hepatic activity of matrix metalloproteinases (MMP)-2, MMP-9, and MMP-13 (n = 10); F: Hepatic level of tissue inhibitor of metalloproteinase 1 (n = 10). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction (D). Quantitative values are indicated as fold changes to the values of non-therapeutic group (B, D and E). Data are the mean ± SD. $^{b}P < 0.01$ *vs* non-therapeutic group; $^{c}P < 0.05$ *vs* vehicle-treated alcohol-associated liver disease group; $^{d}P < 0.01$ *vs* vehicle-treated alcohol-associated liver disease group, significant difference between groups by Student's *t*-test. NT: Non-therapeutic group; Veh: Vehicle-treated alcohol-associated liver disease group; EFN-L: Elafiblanor (3 mg/kg/day)-treated alcohol-associated liver disease group; EFN-H: Elafibranor (10 mg/kg/day)-treated alcohol-associated liver disease group; α-SMA: α-smooth muscle actin; MMP: Matrix metalloproteinases; TIMP1: Tissue inhibitor of metalloproteinase 1.

homeostasis[19]. In the ALD mice, intestinal expression of *Ftcd* and *Sox9* that are involved in reducing tight junction proteins (TJPs), increasing inflammation, and inhibiting proliferation of epithelial cells were increased, and those of *Dhrs9*, *FoxM1*, *S100G*, and *Mgl2* that promote gut barrier function and have anti-inflammatory properties, were decreased (Supplementary Figure 4C and D). Notably, these changes in gene expression in ALD mice were significantly attenuated by treatment with EFN (Supplementary Figure 4C and D). Figure 6A displays a marked decrease in the intestinal expression of TJPs, including ZO-1, occludin, and claudin-2, in the ALD mice. Quantitative analysis revealed a reduced TJP expression to less than 25% in the ALD mice, compared with that in the control mice (Figure 6B). Notably, treatment with EFN effectively suppressed the loss of TJP expression in the ALD mice (Figure 6A and B). Consistently, the intestinal mRNA levels of *Zo1*, *Ocln*, and *Cldn2* in the ALD mice were decreased by EFN treatment (Figure 6C). Along with the decrease in TJP expression, plasma levels of FITC-dextran, which leaked from the intestinal tract into the portal vein, were elevated in the ALD mice (Figure 6D). In accordance with the restoration of TJP expression, EFN treatment inhibited the leak of FITC-dextran from the intestine, as indicated by the decrease in its portal levels (Figure 6D).

To analyze the mechanism underlying the improvement of intestinal permeability, we further evaluated the effect of EFN on apoptosis and inflammation in the intestines of the ALD mice. As shown in Figure 6E, the level of cleaved caspase-3 was elevated, indicating promoted apoptosis in the intestine of the ALD mice. Concomitantly, the intestinal expressions of the antiapoptotic markers Bcl-2 and Mcl-1 decreased (Figure 6F). We found that the promotion of epithelial apoptosis was accompanied by autophagy dysfunction, as indicated by the downregulation of LC3-II; decrease in *Atg5*, *Atg7*, and *Beclin-1*; and increase in *p62* mRNA expressions in the intestine of the ALD mice (Figure 6F and G). Remarkably, EFN treatment suppressed this impaired autophagy-induced epithelial apoptosis (Figure 6E-G). Moreover, treatment with EFN decreased the expression of the M1 macrophage markers *Tnfa*, *Il1b*, and *Nos2* and increased the expression of the M2 macrophage marker *Arg* in the intestine of the ALD mice (Figure 6H).

EFN directly improved EtOH-stimulated apoptosis and permeability in Caco-2 cells

We further investigated the direct effects of EFN on intestinal barrier function using Caco-2 human intestinal cells. EtOH stimulation dose-dependently deprived the Caco-2 cells of TJP expression and the results showed that EtOH stimulation at 50 mmol/L induced the greatest decrease in TJP expression (Figure 7A). Consequently, TEER, which is a vital indicator of epithelial cellular barrier integrity, was decreased by EtOH stimulation (Figure 7B). Treatment with EFN dose-dependently restored TJP expression and attenuated the TEER decline in the EtOH (50 mmol/L)-stimulated Caco-2 cells (Figure 7C and D). These effects of EFN on intestinal barrier function were inhibited by GW7647 and GSK3787 at a significantly stronger degree by the latter (Figure 7C and D). These results suggested that the effect of EFN on intestinal barrier function appeared to be predominantly mediated by PPARδ activation rather than by PPARα activation. Reflecting the effect on the ALD mice, EFN significantly reduced caspase-3/7 activity, which was elevated after EtOH stimulation of the Caco-2 cells (Figure 7E). This antiapoptotic effect of EFN was accompanied by an improvement in autophagic activity, as indicated by the increased expressions of *ATG5*, *ATG7*, and *BECLIN-1* and the decreased expression of *p62* (Figure 7F). Consistent with its action on intestinal barrier function, EFN was suggested to exert its effects mainly through PPARδ agonism.

DISCUSSION

ALD is based on two conditions, including hepatic injury and addictive disorder. Alcohol cessation is the cornerstone of treatment and should be recommended to all patients with ALD. In fact, abstinence from excessive alcohol consumption has been shown to improve clinical outcomes and prognosis, even in patients with ALD-related liver cirrhosis[28-30]. However, for most patients who seek treatment, considering sobriety as an acceptable, desirable, or realistic treatment goal is difficult[28-30]. The present study demonstrated that EFN, which is a dual PPARα/PPARδ agonist, effectively prevented hepatic steatosis, inflammation, and fibrosis in an EtOH + CCl_4-induced ALD mice model. We determined the following multifaceted underlying mechanism that was mediated by PPARα/PPARδ activation: (1) Suppression of lipid accumulation and improvement of autophagy in hepatocytes, which reduced apoptosis and enhanced antioxidant activities; and (2) Blockade of hepatic influx of LPS by repairing intestinal barrier integrity.

In response to alcohol abuse, lipid accumulation in hepatocytes is the initial stage of ALD[4-8]. Excessive alcohol intake increases the cytosolic NADH/NAD^+ ratio in hepatocytes and impairs mitochondrial fatty acid β-oxidation, resulting in lipid accumulation[4-8,31]. Our gene expression analysis suggested that the inhibitory effect of EFN on lipid accumulation was based on the augmentation of lipolysis and fatty acid oxidation rather than on the inhibition of lipogenesis.

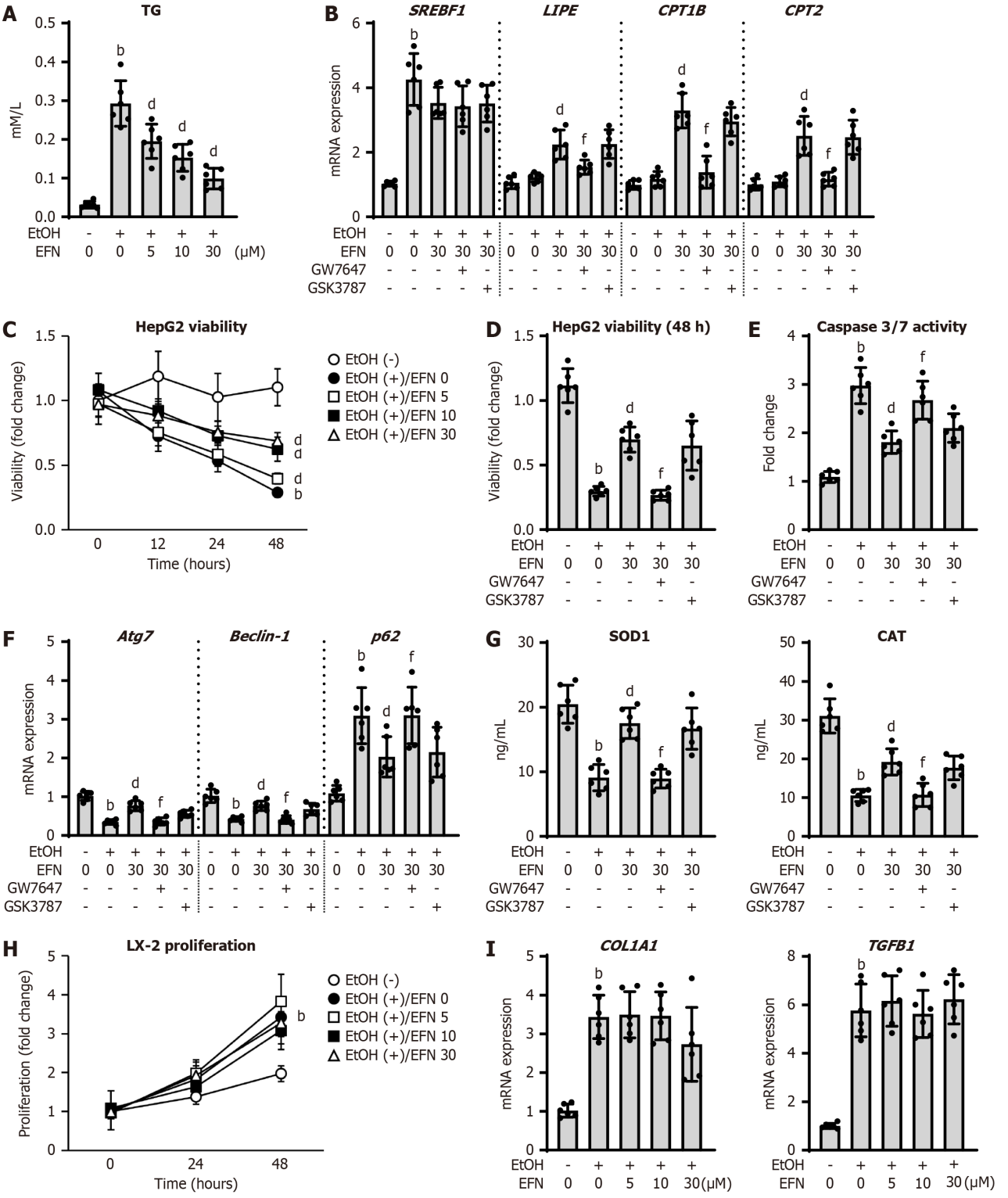


Figure 4 Elafibranor on the ethanol-stimulated human hepatocytes and human hepatic stellate cells. A: Intracellular triglyceride content in HepG2 cells (n = 6); B: Intracellular mRNA level of the markers related to lipid metabolism in HepG2 cells (n = 6); C: Chronological change in HepG2 cell viability by treatment with ethanol (EtOH) and/or elafibtanor (EFN) (n = 6); D: Effect of a selective antagonists of peroxisome proliferator activated receptor (PPAR)α (GW7647) or PPARδ (GSK3787) on EtOH and EFN-treated HepG2 cell viability (incubation for 48 hours) (n = 6); E: Intracellular caspase 3/7 activity in HepG2 cells (n = 6); F: Intracellular mRNA level of the markers related to autophagy in HepG2 cells (n = 6); G: Intracellular levels of superoxide dismutase 1 and catalase in HepG2 cells (n = 6). HepG2 cells were incubated with (A and C) EtOH (0 or 50 mmol/L) and EFN (0, 5, 10, 30 μM) for 24 hours (A) or 0, 12, 24, and 48 hours (C), EtOH (0 or 50 mmol/L) and EFN (0 or 30 μM) for 48 hour following pretreatment with GW7647 (10 μM) or GSK3787 (10 μM) for 6 hours (B, D-G); H: Chronological change in LX-2 cell proliferation by treatment with EtOH and/or EFN (n = 6); I: Intracellular mRNA level of the profibrotic markers in LX-2 cells (n = 6). LX-2 cells were incubated with EtOH (0 or 50 mmol/L) and EFN (0, 5, 10, and 30 μM) for 0, 24, 48 hours (H) or 24 hours (I). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction (B, F and I). Quantitative values are indicated as fold changes to the values of EtOH (-)/EFN (0 μM)-treated group (B-F, H and I). Data are the mean ± SD. $^{b}P < 0.01$ *vs* ethanol (-)/elafibtanor (0 μM)-treated group; $^{d}P < 0.01$ *vs* ethanol (+)/elafibtanor (0 μM)-treated group; $^{f}P < 0.01$ *vs* ethanol (+)/elafibtanor (30 μM)-treated group. EtOH: Ethanol; EFN: Elafibtanor; SOD: Superoxide dismutase; CAT: Catalase; TG: Triglyceride.

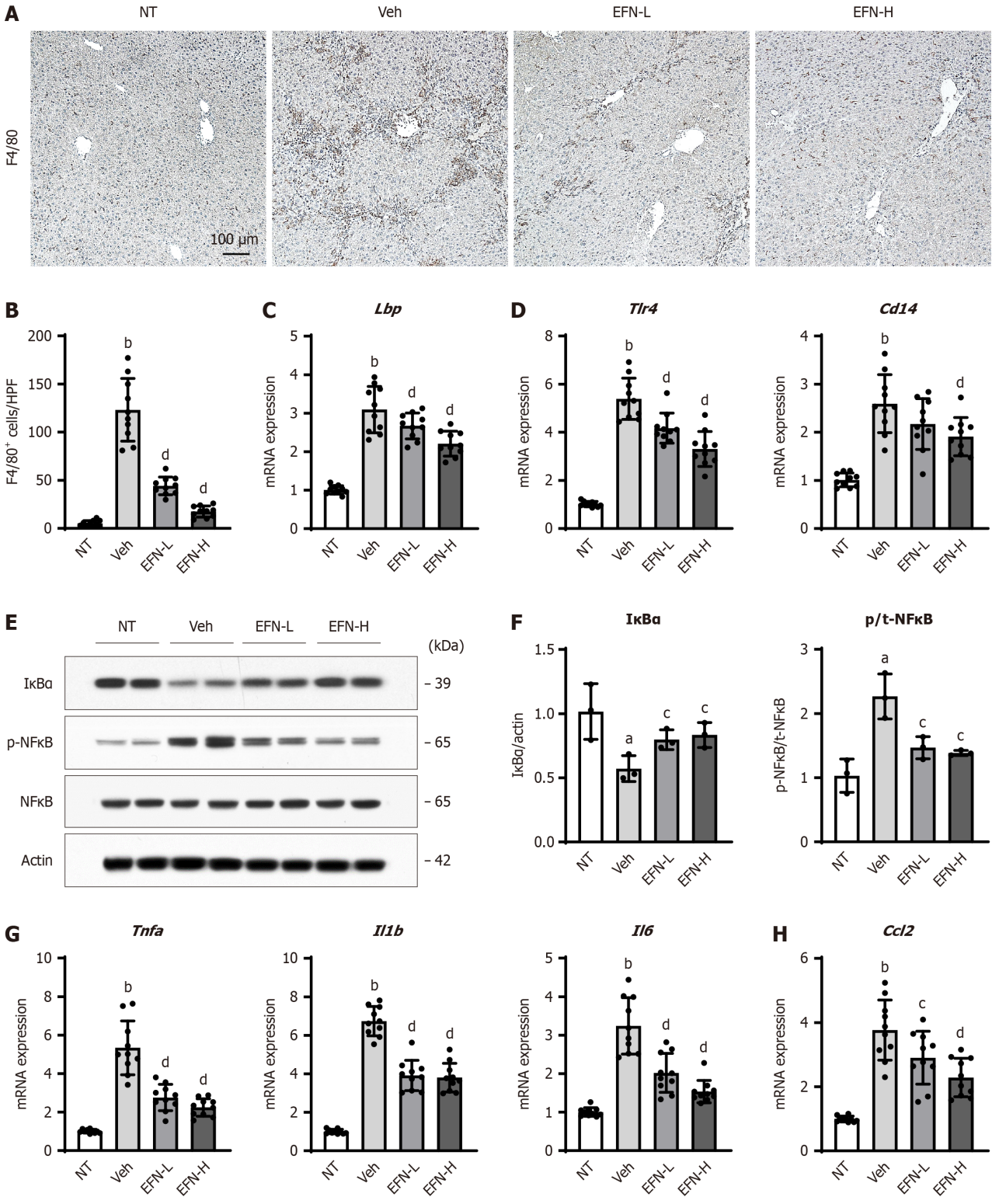
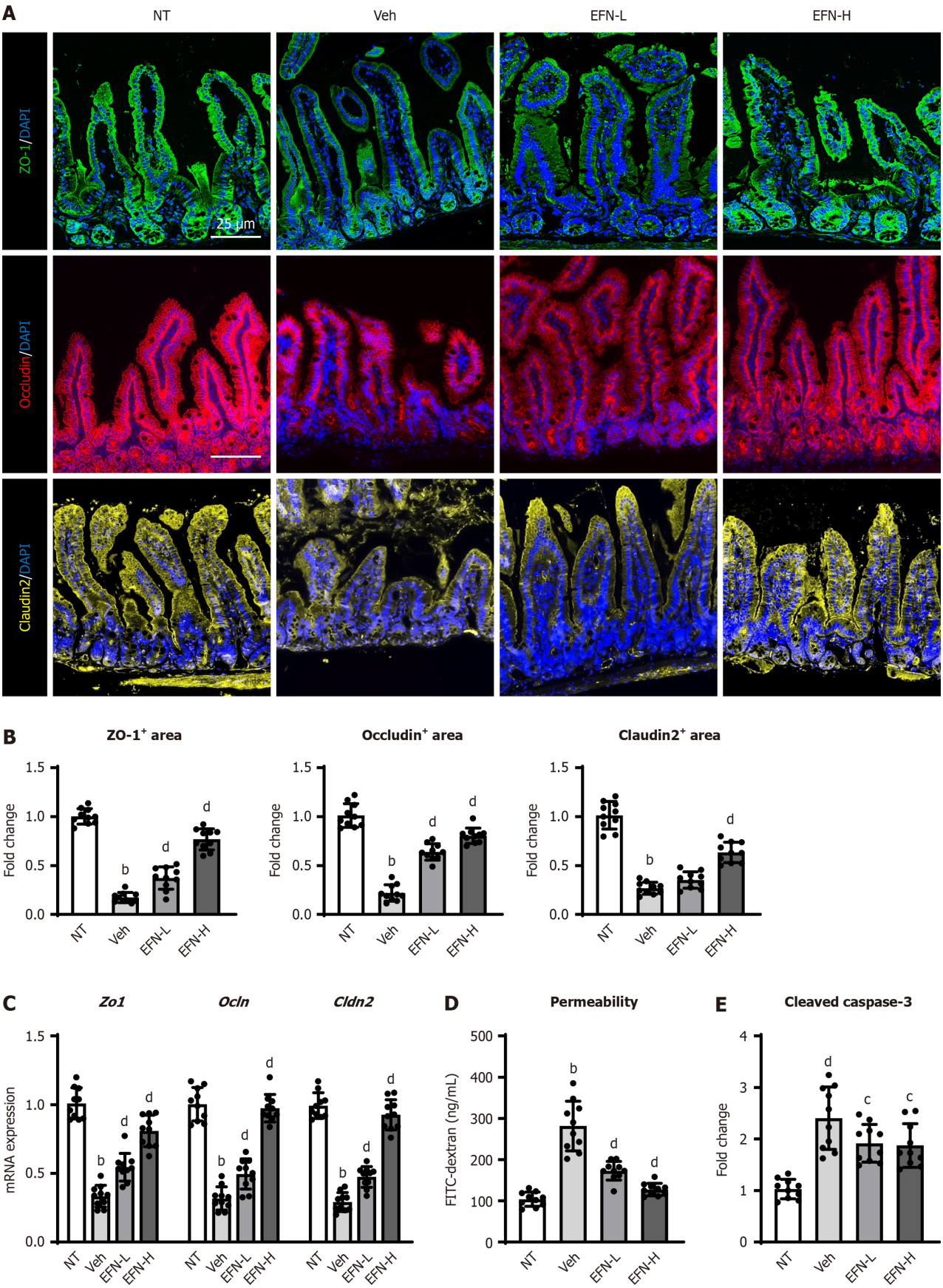


Figure 5 Elafibranor on Kupffer cell-mediated inflammatory response in the alcohol-associated liver disease mice. A: Representative microphotographs of F4/80 staining of the livers in the experimental mice; B: Quantification of F4/80-positive cells in high-power field (n = 10); C and D: Hepatic mRNA level of lipopolysaccharide-binding protein (C), toll like receptor 4 and CD14 (D) (n = 10); E: Western blot for the protein expression of IκBα, p-nuclear factor kappa B (NFκB) and NF-κB in the liver tissue. Actin was used as an internal control; F: Quantification of the protein level of IκBα and the ratio of NF-κB phosphorylation based on western blotting (n = 10); G and H: Hepatic mRNA level of tumor necrosis factor-alpha, interleukin 1β (*Il1b*), and *Il6* (G), and *Ccl2* (H) (n = 10). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction (C, D, G and H). Quantitative values are indicated as fold changes to the values of non-therapeutic group. Data are the mean ± SD. $^{a}P < 0.05$ *vs* non-therapeutic group; $^{b}P < 0.01$ *vs* non-therapeutic group; $^{c}P < 0.05$ *vs* vehicle-treated alcohol-associated liver disease group; $^{d}P < 0.01$ *vs* vehicle-treated alcohol-associated liver disease group, significant difference between groups by Student's *t*-test. NT: Non-therapeutic group; Veh: Vehicle-treated alcohol-associated liver disease group; EFN-L: Elafiblanor (3 mg/kg/day)-treated alcohol-associated liver disease group; EFN-H: Elafibranor (10 mg/kg/day)-treated alcohol-associated liver disease group; NF-κB: Nuclear factor kappa B; Lbp: Lipopolysaccharide-binding protein; TLR: Toll like receptor; TNF: Tumor necrosis factor; IL: Interleukin.


A
NT
Veh
EFN-L
EFN-H
ZO-1/DAPI
Occludin/DAPI
Claudin2/DAPI
25 μm
B
ZO-1+ area
Occludin+ area
Claudin2+ area
Fold change
C
Zo1
Ocln
Cldn2
mRNA expression
D
Permeability
FITC-dextran (ng/mL)
E
Cleaved caspase-3
Fold change

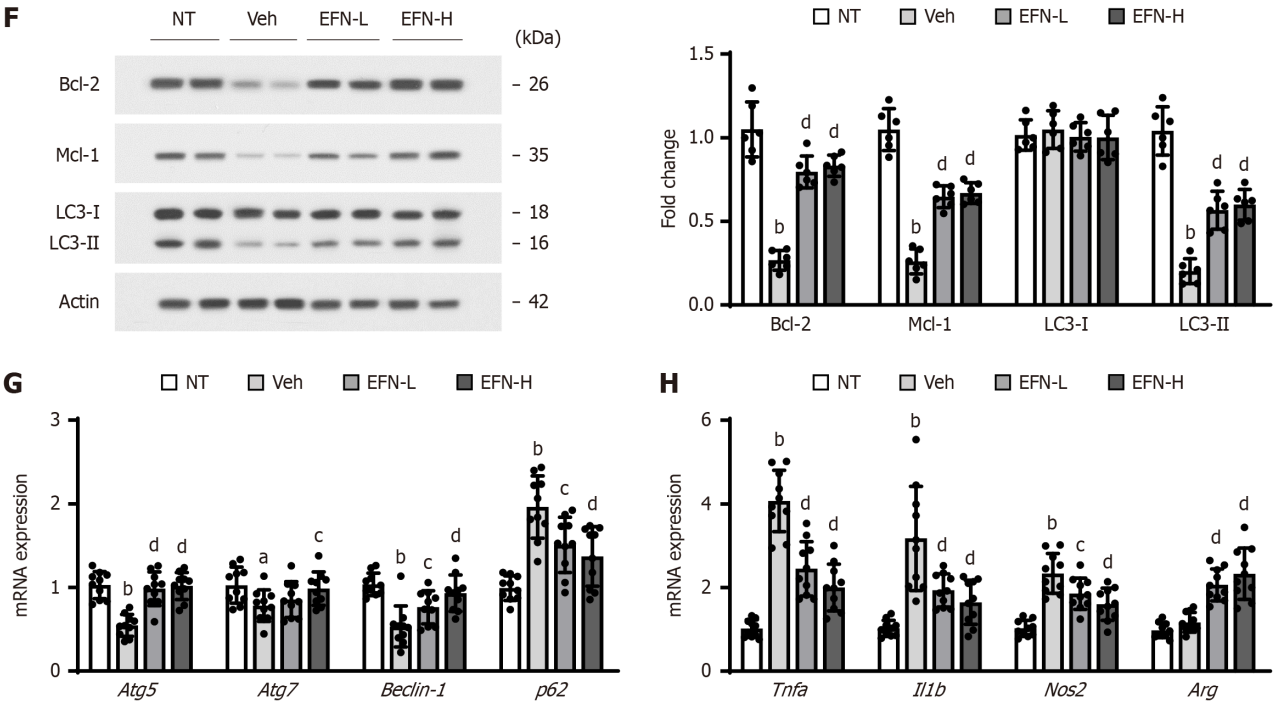


Figure 6 Elafibranor on intestinal barrier function in the alcohol-associated liver disease mice. A: Representative microphotographs of ileum sections immunofluorescent stained with tight junction proteins (TJPs) including zonula occludens-1 (ZO-1), occludin and claudin-2; B: Quantitation of ZO-1, occludin and claudin-2 immunopositive area in high-power field (n = 10); C: Intestinal mRNA levels of TJPs (n = 10); D: Blood levels of fluorescein isothiocyanate-dextran (4 kDa) 4 hours after oral administration (n = 3); E: Cleaved caspase-3 level in the ileum tissue (n = 10); F: Western blot for the protein expression of Bcl-2, Mcl-1 and LC3-1 and 2 in the ileum tissue. Actin was used as an internal control; G and H: Intestinal mRNA level of the markers related to autophagy (G) and macrophage activation (H) (n = 10). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction (C, G, and H). Quantitative values are indicated as fold changes to the values of non-therapeutic group (B, C, E-H). Data are the mean ± SD. $^{a}P < 0.05$ *vs* non-therapeutic group; $^{b}P < 0.01$ *vs* non-therapeutic group; $^{c}P < 0.05$ *vs* vehicle-treated alcohol-associated liver disease group; $^{d}P < 0.01$ *vs* vehicle-treated alcohol-associated liver disease group, significant difference between groups by Student's *t*-test. NT: Non-therapeutic group; Veh: Vehicle-treated alcohol-associated liver disease group; EFN-L: Elafiblanor (3 mg/kg/day)-treated alcohol-associated liver disease group; EFN-H: Elafibranor (10 mg/kg/day)-treated alcohol-associated liver disease group; ZO-1: Zonula occludens-1; TNF: Tumor necrosis factor; IL: Interleukin; FITC: Fluorescein isothiocyanate.

Moreover, several studies have shown that autophagy protects the liver from alcohol-induced injury[32,33]. Autophagy is known to promote cell survival by supplying nutrients during starvation and by selectively scavenging damaged organelles, such as mitochondria[34]. Proper autophagy, such as mitophagy and lipid autophagy, assists in improving alcohol-induced liver dysfunction, which leads to apoptosis secondary to damaged mitochondria and reactive oxygen species accumulation in hepatocytes[29,35]. In the current study, EFN treatment attenuated hepatocyte apoptosis, with improved autophagy and enhanced antioxidant capacity, in accordance with reduced lipid accumulation in the liver of the ALD mice. Notably, the cell-based assay elucidated that these effects on EtOH-exposed HepG2 cells were predominantly mediated by PPARα activation. Downregulation and/or dysfunction of PPARα is involved in the development of ALD[36]. Kong *et al*[37] reported that pharmacological activation of PPARα attenuated steatohepatitis by increasing lipid oxidation and downregulating proinflammatory factors in ALD models. In addition, the hepatic expression and transcriptional activity of PPARα are closely associated with the induction of autophagy by directly increasing the expression of several autophagy genes, such as *LC3B*[38]. Moreover, a recent animal study has shown that PPARα activation reversed murine alcoholic liver injury and increased the levels of antioxidant enzymes, including CAT and SOD1[39,40]. We used HepG2 cells as the hepatocyte-like cells for *in vitro* study. HepG2 cells are used to identify the effects of alcohol on human hepatocyte-like cells due to the expression of ADH4, which metabolizes EtOH[41-43]. Moreover, HepG2 cells have been used to evaluate the PPAR-mediated pharmacological effect (including EFN) against hepatocyte injury[44-46]. Therefore, there are no major obstacles to the use of HepG2 cells as human hepatocyte-like cells in the present study. On the other hand, since HepG2 cells are essentially a hepatocellular carcinoma cell line, analysis of the effects of EFN using primary cultured hepatocytes would be an issue for future study.

In some studies, a PPARα agonist was shown to ameliorate alcoholic liver injury, but its effect on liver fibrosis is unknown or limited. Our findings demonstrated a marked inhibitory effect of EFN on liver fibrosis in the ALD mice. EFN did not show a direct effect on LX-2 cells, which are activated HSCs, but it inhibited hepatic LPS/TLR4 pathway and proinflammatory response, which both play crucial roles in the development of ALD-related fibrosis. Therefore, we focused on the effect of EFN on intestinal barrier function, which functionally regulates LPS influx to the liver. We and another group have reported that EtOH + CCl_4-treated ALD mice showed intestinal barrier disruption and downregulation of intestinal TJPs, including ZO-1, occludin, and claudin-2[25,47,48]. EFN effectively restored the expression of TJPs, resulting in reduced intestinal permeability and hepatic LPS influx in the ALD mice. Moreover, we confirmed the protective effect of EFN on intestinal barrier function in EtOH-exposed Caco-2 cells. A previous report showed that EFN

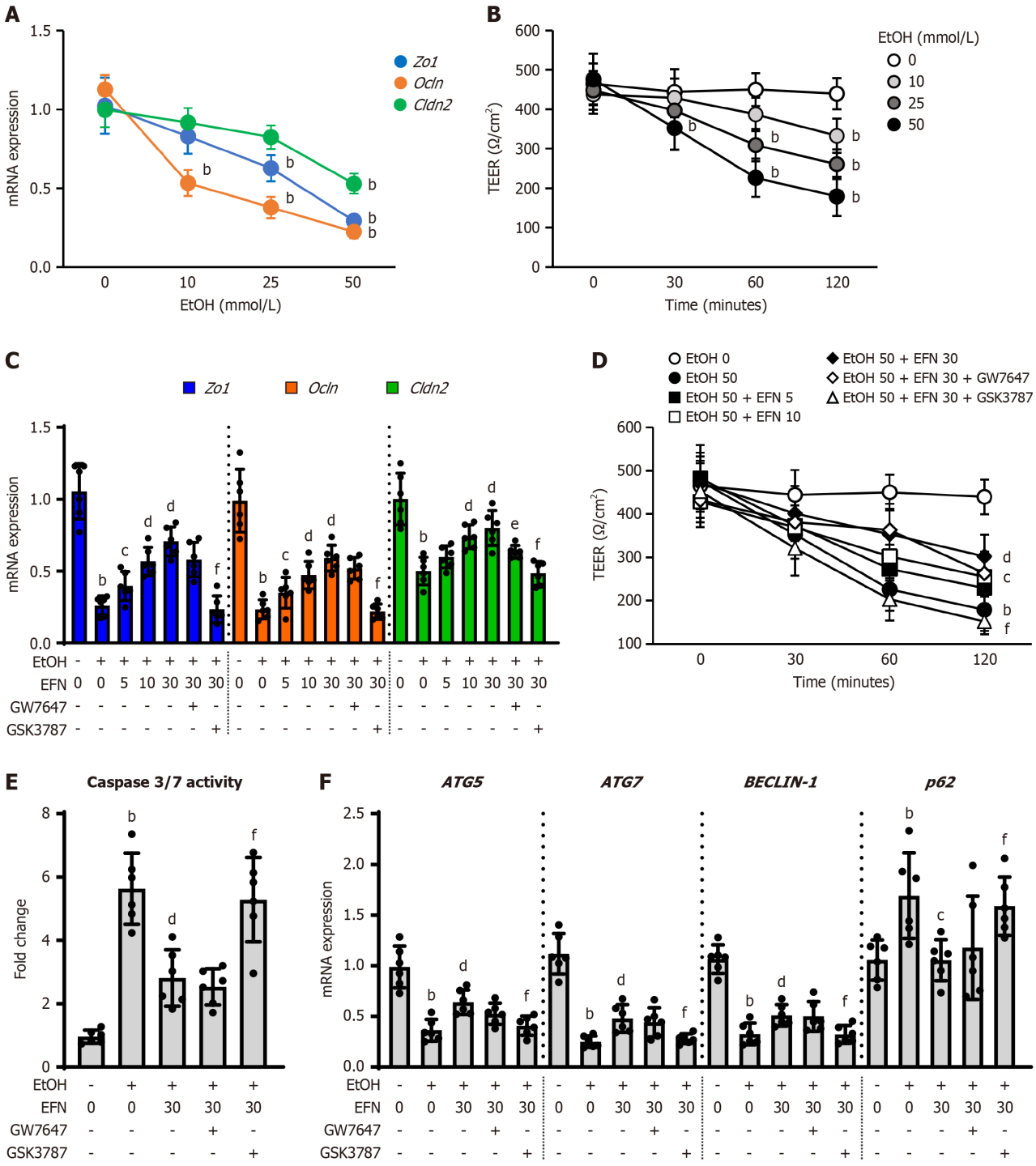


Figure 7 Elafibranor on the ethanol-stimulated human intestinal epithelial cells. A: Intracellular mRNA levels of tight junction proteins (TJPs) including zonula occludens-1 (ZO-1), Ocln, and Cldn2 in ethanol (EtOH)-stimulated Caco-2 cells (n = 6); B: Integrity of the epithelial cellular barrier in EtOH-stimulated Caco-2 cells determined as transepithelial electrical resistance (TEER) (n = 6). Cells were incubated with different concentration of EtOH (0, 10, 25, and 50 mmol/L) for 120 minutes (A) and 0, 30, 60, and 120 minutes (B); C: Effect of elafibranor (EFN) on the TJPs mRNA expression in the EtOH-stimulated Caco-2 cells (n = 6); D: Effect of EFN on the TEER in the EtOH-stimulated Caco-2 cells (n = 6). Cells were incubated with EtOH (0 or 50 mmol/L) and EFN (0, 5, 10, 30 μM) for 120 minutes (C) or 0, 30, 60, and 120 minutes (D) following pretreatment with GW7647 (10 μM) or GSK3787 (10 μM) for 15 minutes; E: Effect of EFN on the intracellular caspase 3/7 activity in the EtOH-stimulated Caco-2 cells (n = 6); F: Effect of EFN on mRNA expression of the markers related to autophagy in the EtOH-stimulated Caco-2 cells (n = 6). Cells were incubated with EtOH (0 or 50 mmol/L) and EFN (0 or 30 μM) for 48 hours following pretreatment with a peroxisome proliferator activated receptor (PPAR)α antagonist, GW7647 (10 μM) or a PPARδ antagonist, GSK3787 (10 μM) for 6 hours (E and F). Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control for real-time quantitative polymerase chain reaction (A, C, and F). Quantitative values are indicated as fold changes to the values of EtOH (-)/EFN (0 μM)-treated group (A, C, E, and F). Data are the mean ± SD. $^{b}P < 0.01$ *vs* ethanol (-)/elafibranor (0 μM)-treated group; $^{c}P < 0.05$ *vs* ethanol (+)/elafibranor (0 μM)-treated group; $^{d}P < 0.01$ *vs* ethanol (+)/elafibranor (0 μM)-treated group; $^{e}P < 0.05$ *vs* ethanol (+)/elafibranor (30 μM)-treated group; $^{f}P < 0.01$ *vs* ethanol (+)/elafibranor (30 μM)-treated group. EtOH: Ethanol; EFN: Elafibtanor; TEER: Transepithelial electrical resistance; ZO-1: Zonula occludens-1.

restored intestinal integrity in a mouse model of nonalcoholic steatohepatitis; this may be relevant to the findings of the present study[46]. Moreover, a recent animal study has demonstrated that EFN increased the *Beclin-1* and *LC3-II* levels and autophagy flux and decreased the *p62* and caspase levels in the gut of a different ALD model[49]. Interestingly, in our study, the effects of EFN on intestinal barrier function were mainly mediated by PPARδ activation. Several mechanisms are involved in the regulatory effect of PPARδ activation on intestinal barrier function. PPARδ activation can suppress macrophage-driven inflammation by downregulating the intestinal expressions of proinflammatory mediators, including monocyte chemotactic protein-1 and IL-1β, and upregulating the expression of various anti-inflammatory genes [19]. Furthermore, PPARδ activation was reported to augment antiapoptotic pathways in intestinal epithelial cells[49] and was suggested to enhance autophagy by increasing Beclin-1 and LC3II expressions in several types of cells[50]. These findings supported our results on the prevention of intestinal barrier disruption through EFN-mediated PPARδ activation.

Our findings showed that EFN could affect hepatocytes and intestinal epithelial cells by activating PPARα and PPARδ, respectively. However, some studies have reported that PPARα activation affects intestinal epithelial cells[51,52]. PPARα activation ameliorated chemical-induced colitis and enhanced intestinal barrier function in a rodent model of inflammatory bowel disease[51]. In Caco-2 cells, treatment with the PPARα activator fenofibrate protected barrier function, attenuated junctional flexure, and increased Claudin-1 expression after exposure to high glucose levels or inflammatory cytokines[52]. Meanwhile, PPARδ has also been implicated in lipid metabolism and energy homeostasis in the liver[53]. Recent clinical studies demonstrated that treatment with PPARδ agonists reduced the hepatic fat content in overweight patients with mixed dyslipidemia[54,55]. Likewise, Tong *et al*[17] reported that the pharmacological and genetic activation of PPARδ had a beneficial effect in attenuating hepatic steatosis by activating autophagy in the hepatocytes of obese mice. Despite this reported evidence, our *in vitro* study on EtOH-stimulated HepG2 or Caco-2 cells suggested that the effects of PPARα on intestinal barrier function and PPARδ on hepatocytes were limited. However, the effectiveness of EFN might have differed, depending on the experimental model; we did not identify the mechanism for the imbalanced effect of EFN. Therefore, further studies using different ALD models are needed to focus on the EFN-mediated effects of PPARα on the intestine and of PPARδ on hepatocytes.

In addition to the aforementioned limitation, the role of EFN in bile acid metabolism was not fully examined in the current study. A PPARα agonist was reported to inhibit the expression of farnesoid X receptor target genes, thereby, reducing hepatic bile acid levels[56]. Furthermore, a PPARδ agonist was found to reduce bile acid accumulation in the liver and small intestine, leading to attenuated EtOH-induced liver disease in mice[19]. Because the regulation of bile acid is closely associated with both lipid metabolism and intestinal barrier homeostasis, further analysis of the relationship between the EFN effect on bile acid metabolism and its ameliorative effect on alcoholic liver injury using the present model would be important. Second, our results showed that EFN suppressed the LPS/TLR4 signaling in the liver tissue of ALD mice, but the status of TLR4 activation were not specifically evaluated at the macrophage level. It has been recognized that LPS/TLR4 pathway also plays a key role in HSC activation[57]. Thus, further studies are needed to prove that the reduction of LPS influx into the liver by EFN mainly affects the activation of macrophages, including analysis of macrophages and HSCs isolated from experimental mouse models.

Third, we found that EFN had a preventive effect on ALD alongside EtOH + CCl_4 exposure. In practice, however, pharmacologic treatment is usually given when liver fibrosis has already developed. Thus, further investigation is needed using a model of drug administration at the stage of advanced cirrhosis.

CONCLUSION

Taken together, EFN appeared to prevent the development of liver fibrosis in EtOH + CCl_4-induced ALD mice. Notably, EFN can exert dual pharmacological actions by activating PPARα, which mediated the inhibition of lipid accumulation and apoptosis and the enhanced autophagic activity and antioxidative capacity of hepatocytes, and PPARδ, which mediated the protection of intestinal barrier function, resulting in suppression of the LPS/TLR4/NF-κB signaling pathway in the liver. Although the safety of EFN has been proven in clinical trials on primary biliary cholangitis, our results suggested that this drug may eventually emerge as a viable treatment option for ALD.

FOOTNOTES

Author contributions: Koizumi A and Kaji K contributed to the data curation; Koizumi A, Nishimura N, Asada S, Matsuda T, Tanaka M, Yorioka N, and Tsuji Y were involved in the investigation of this manuscript; Koizumi A, Nishimura N, and Kitagawa K participated in the formal analysis; Koizumi A, Kaji K, and Namisaki T contributed to the methodology of this study; Koizumi A prepared the writing-original draft; Kaji K and Yoshiji H took part in the conceptualization and resources of this article; Koizumi A, Akahane T, and Yoshiji H contributed to the supervision of this manuscript; Kaji K participated in the validation; Kaji K and Kitagawa K were involved in the visualization; Kaji K, Nishimura N, Asada S, Matsuda T, Tanaka M, Yorioka N, Tsuji Y, Kitagawa K, Sato S, Namisaki T, Akahane T, and Yoshiji H contributed to the writing-review and editing of this manuscript; Sato S contributed to the software.

Institutional review board statement: The study was reviewed and approved by the Institutional review board of Nara Medical University, Kashihara, Japan (authorization numbers: 13130).

Institutional animal care and use committee statement: All animal experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals of the National Research Council.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country of origin: Japan

ORCID number: Kosuke Kaji 0000-0002-1822-6759; Norihisa Nishimura 0000-0002-6295-3283; Yuki Tsuji 0000-0001-7625-4208; Koh Kitagawa 0000-0001-5794-1512; Shinya Sato 0000-0003-3049-3443; Tadashi Namisaki 0000-0002-3158-5318; Takemi Akahane 0000-0002-6675-0475; Hitoshi Yoshiji 0000-0002-5243-8544.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Zheng XM

REFERENCES

1 **Julien J**, Ayer T, Bethea ED, Tapper EB, Chhatwal J. Projected prevalence and mortality associated with alcohol-related liver disease in the USA, 2019-40: a modelling study. *Lancet Public Health* 2020; **5**: e316-e323 [PMID: 32504584 DOI: 10.1016/S2468-2667(20)30062-1]
2 **Asrani SK**, Mellinger J, Arab JP, Shah VH. Reducing the Global Burden of Alcohol-Associated Liver Disease: A Blueprint for Action. *Hepatology* 2021; **73**: 2039-2050 [PMID: 32986883 DOI: 10.1002/hep.31583]
3 **Devarbhavi H**, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol* 2023; **79**: 516-537 [PMID: 36990226 DOI: 10.1016/j.jhep.2023.03.017]
4 **Mackowiak B**, Fu Y, Maccioni L, Gao B. Alcohol-associated liver disease. *J Clin Invest* 2024; **134** [PMID: 38299591 DOI: 10.1172/JCI176345]
5 **Gao B**, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: 10.1053/j.gastro.2011.09.002]
6 **Wu X**, Fan X, Miyata T, Kim A, Cajigas-Du Ross CK, Ray S, Huang E, Taiwo M, Arya R, Wu J, Nagy LE. Recent Advances in Understanding of Pathogenesis of Alcohol-Associated Liver Disease. *Annu Rev Pathol* 2023; **18**: 411-438 [PMID: 36270295 DOI: 10.1146/annurev-pathmechdis-031521-030435]
7 **You M**, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (SREBP). *J Biol Chem* 2002; **277**: 29342-29347 [PMID: 12036955 DOI: 10.1074/jbc.M202411200]
8 **Meng FG**, Zhang XN, Liu SX, Wang YR, Zeng T. Roles of peroxisome proliferator-activated receptor α in the pathogenesis of ethanol-induced liver disease. *Chem Biol Interact* 2020; **327**: 109176 [PMID: 32534989 DOI: 10.1016/j.cbi.2020.109176]
9 **Szabo G**, Bala S. Alcoholic liver disease and the gut-liver axis. *World J Gastroenterol* 2010; **16**: 1321-1329 [PMID: 20238398 DOI: 10.3748/wjg.v16.i11.1321]
10 **Albillos A**, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol* 2020; **72**: 558-577 [PMID: 31622696 DOI: 10.1016/j.jhep.2019.10.003]
11 **Rao R**. Endotoxemia and gut barrier dysfunction in alcoholic liver disease. *Hepatology* 2009; **50**: 638-644 [PMID: 19575462 DOI: 10.1002/hep.23009]
12 **Tanaka N**, Aoyama T, Kimura S, Gonzalez FJ. Targeting nuclear receptors for the treatment of fatty liver disease. *Pharmacol Ther* 2017; **179**: 142-157 [PMID: 28546081 DOI: 10.1016/j.pharmthera.2017.05.011]
13 **Königshofer P**, Brusilovskaya K, Petrenko O, Hofer BS, Schwabl P, Trauner M, Reiberger T. Nuclear receptors in liver fibrosis. *Biochim Biophys Acta Mol Basis Dis* 2021; **1867**: 166235 [PMID: 34339839 DOI: 10.1016/j.bbadis.2021.166235]
14 **Toyama T**, Nakamura H, Harano Y, Yamauchi N, Morita A, Kirishima T, Minami M, Itoh Y, Okanoue T. PPARalpha ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats. *Biochem Biophys Res Commun* 2004; **324**: 697-704 [PMID: 15474484 DOI: 10.1016/j.bbrc.2004.09.110]
15 **Nan YM**, Wang RQ, Fu N. Peroxisome proliferator-activated receptor α, a potential therapeutic target for alcoholic liver disease. *World J Gastroenterol* 2014; **20**: 8055-8060 [PMID: 25009377 DOI: 10.3748/wjg.v20.i25.8055]
16 **Magadum A**, Engel FB. PPARβ/δ: Linking Metabolism to Regeneration. *Int J Mol Sci* 2018; **19** [PMID: 29996502 DOI: 10.3390/ijms19072013]
17 **Tong L**, Wang L, Yao S, Jin L, Yang J, Zhang Y, Ning G, Zhang Z. PPARδ attenuates hepatic steatosis through autophagy-mediated fatty acid oxidation. *Cell Death Dis* 2019; **10**: 197 [PMID: 30814493 DOI: 10.1038/s41419-019-1458-8]
18 **Gupta RA**, Wang D, Katkuri S, Wang H, Dey SK, DuBois RN. Activation of nuclear hormone receptor peroxisome proliferator-activated receptor-delta accelerates intestinal adenoma growth. *Nat Med* 2004; **10**: 245-247 [PMID: 14758356 DOI: 10.1038/nm993]
19 **Chu H**, Jiang L, Gao B, Gautam N, Alamoudi JA, Lang S, Wang Y, Duan Y, Alnouti Y, Cable EE, Schnabl B. The selective PPAR-delta agonist seladelpar reduces ethanol-induced liver disease by restoring gut barrier function and bile acid homeostasis in mice. *Transl Res* 2021; **227**: 1-14 [PMID: 32553670 DOI: 10.1016/j.trsl.2020.06.006]
20 **Westerouen Van Meeteren MJ**, Drenth JPH, Tjwa ETTL. Elafibranor: a potential drug for the treatment of nonalcoholic steatohepatitis

(NASH). *Expert Opin Investig Drugs* 2020; **29**: 117-123 [PMID: 31523999 DOI: 10.1080/13543784.2020.1668375]

21 **Kowdley KV**, Bowlus CL, Levy C, Akarca US, Alvares-da-Silva MR, Andreone P, Arrese M, Corpechot C, Francque SM, Heneghan MA, Invernizzi P, Jones D, Kruger FC, Lawitz E, Mayo MJ, Shiffman ML, Swain MG, Valera JM, Vargas V, Vierling JM, Villamil A, Addy C, Dietrich J, Germain JM, Mazain S, Rafailovic D, Taddé B, Miller B, Shu J, Zein CO, Schattenberg JM; ELATIVE Study Investigators' Group; ELATIVE Study Investigators' Group. Efficacy and Safety of Elafibranor in Primary Biliary Cholangitis. *N Engl J Med* 2024; **390**: 795-805 [PMID: 37962077 DOI: 10.1056/NEJMoa2306185]

22 **Staels B**, Rubenstrunk A, Noel B, Rigou G, Delataille P, Millatt LJ, Baron M, Lucas A, Tailleux A, Hum DW, Ratziu V, Cariou B, Hanf R. Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology* 2013; **58**: 1941-1952 [PMID: 23703580 DOI: 10.1002/hep.26461]

23 **Lu JW**, Wang H, Yan-Li J, Zhang C, Ning H, Li XY, Zhang H, Duan ZH, Zhao L, Wei W, Xu DX. Differential effects of pyrrolidine dithiocarbamate on TNF-alpha-mediated liver injury in two different models of fulminant hepatitis. *J Hepatol* 2008; **48**: 442-452 [PMID: 18215436 DOI: 10.1016/j.jhep.2007.10.014]

24 **Matheus VA**, Monteiro L, Oliveira RB, Maschio DA, Collares-Buzato CB. Butyrate reduces high-fat diet-induced metabolic alterations, hepatic steatosis and pancreatic beta cell and intestinal barrier dysfunctions in prediabetic mice. *Exp Biol Med (Maywood)* 2017; **242**: 1214-1226 [PMID: 28504618 DOI: 10.1177/1535370217708188]

25 **Shibamoto A**, Kaji K, Nishimura N, Kubo T, Iwai S, Tomooka F, Suzuki J, Tsuji Y, Fujinaga Y, Kawaratani H, Namisaki T, Akahane T, Yoshiji H. Vitamin D deficiency exacerbates alcohol-related liver injury *via* gut barrier disruption and hepatic overload of endotoxin. *J Nutr Biochem* 2023; **122**: 109450 [PMID: 37777163 DOI: 10.1016/j.jnutbio.2023.109450]

26 **Xu Y**, Denning KL, Lu Y. PPARα agonist WY-14,643 induces the PLA2/COX-2/ACOX1 pathway to enhance peroxisomal lipid metabolism and ameliorate alcoholic fatty liver in mice. *Biochem Biophys Res Commun* 2022; **613**: 47-52 [PMID: 35526488 DOI: 10.1016/j.bbrc.2022.04.132]

27 **Koga T**, Yao PL, Goudarzi M, Murray IA, Balandaram G, Gonzalez FJ, Perdew GH, Fornace AJ Jr, Peters JM. Regulation of Cytochrome P450 2B10 (CYP2B10) Expression in Liver by Peroxisome Proliferator-activated Receptor-β/δ Modulation of SP1 Promoter Occupancy. *J Biol Chem* 2016; **291**: 25255-25263 [PMID: 27765815 DOI: 10.1074/jbc.M116.755447]

28 **Rabiee A**, Mahmud N, Falker C, Garcia-Tsao G, Taddei T, Kaplan DE. Medications for alcohol use disorder improve survival in patients with hazardous drinking and alcohol-associated cirrhosis. *Hepatol Commun* 2023; **7** [PMID: 36972386 DOI: 10.1097/HC9.0000000000000093]

29 **Diaz LA**, Winder GS, Leggio L, Bajaj JS, Bataller R, Arab JP. New insights into the molecular basis of alcohol abstinence and relapse in alcohol-associated liver disease. *Hepatology* 2023 [PMID: 37862466 DOI: 10.1097/HEP.0000000000000645]

30 **Gratacós-Ginès J**, Bruguera P, Pérez-Guasch M, López-Lazcano A, Borràs R, Hernández-Évole H, Pons-Cabrera MT, Lligoña A, Bataller R, Ginès P, López-Pelayo H, Pose E. Medications for alcohol use disorder promote abstinence in alcohol-associated cirrhosis: Results from a systematic review and meta-analysis. *Hepatology* 2024; **79**: 368-379 [PMID: 37625154 DOI: 10.1097/HEP.0000000000000570]

31 **Jeon S**, Carr R. Alcohol effects on hepatic lipid metabolism. *J Lipid Res* 2020; **61**: 470-479 [PMID: 32029510 DOI: 10.1194/jlr.R119000547]

32 **Chao X**, Wang S, Zhao K, Li Y, Williams JA, Li T, Chavan H, Krishnamurthy P, He XC, Li L, Ballabio A, Ni HM, Ding WX. Impaired TFEB-Mediated Lysosome Biogenesis and Autophagy Promote Chronic Ethanol-Induced Liver Injury and Steatosis in Mice. *Gastroenterology* 2018; **155**: 865-879.e12 [PMID: 29782848 DOI: 10.1053/j.gastro.2018.05.027]

33 **Babuta M**, Furi I, Bala S, Bukong TN, Lowe P, Catalano D, Calenda C, Kodys K, Szabo G. Dysregulated Autophagy and Lysosome Function Are Linked to Exosome Production by Micro-RNA 155 in Alcoholic Liver Disease. *Hepatology* 2019; **70**: 2123-2141 [PMID: 31090940 DOI: 10.1002/hep.30766]

34 **Picca A**, Faitg J, Auwerx J, Ferrucci L, D'Amico D. Mitophagy in human health, ageing and disease. *Nat Metab* 2023; **5**: 2047-2061 [PMID: 38036770 DOI: 10.1038/s42255-023-00930-8]

35 **Salete-Granado D**, Carbonell C, Puertas-Miranda D, Vega-Rodríguez VJ, García-Macia M, Herrero AB, Marcos M. Autophagy, Oxidative Stress, and Alcoholic Liver Disease: A Systematic Review and Potential Clinical Applications. *Antioxidants (Basel)* 2023; **12** [PMID: 37507963 DOI: 10.3390/antiox12071425]

36 **Nakajima T**, Kamijo Y, Tanaka N, Sugiyama E, Tanaka E, Kiyosawa K, Fukushima Y, Peters JM, Gonzalez FJ, Aoyama T. Peroxisome proliferator-activated receptor alpha protects against alcohol-induced liver damage. *Hepatology* 2004; **40**: 972-980 [PMID: 15382117 DOI: 10.1002/hep.20399]

37 **Kong L**, Ren W, Li W, Zhao S, Mi H, Wang R, Zhang Y, Wu W, Nan Y, Yu J. Activation of peroxisome proliferator activated receptor alpha ameliorates ethanol induced steatohepatitis in mice. *Lipids Health Dis* 2011; **10**: 246 [PMID: 22208561 DOI: 10.1186/1476-511X-10-246]

38 **Lee JM**, Wagner M, Xiao R, Kim KH, Feng D, Lazar MA, Moore DD. Nutrient-sensing nuclear receptors coordinate autophagy. *Nature* 2014; **516**: 112-115 [PMID: 25383539 DOI: 10.1038/nature13961]

39 **Inoue I**, Goto S, Matsunaga T, Nakajima T, Awata T, Hokari S, Komoda T, Katayama S. The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma increase Cu2+,Zn2+-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. *Metabolism* 2001; **50**: 3-11 [PMID: 11172467 DOI: 10.1053/meta.2001.19415]

40 **Yue R**, Chen GY, Xie G, Hao L, Guo W, Sun X, Jia W, Zhang Q, Zhou Z, Zhong W. Activation of PPARα-catalase pathway reverses alcoholic liver injury *via* upregulating NAD synthesis and accelerating alcohol clearance. *Free Radic Biol Med* 2021; **174**: 249-263 [PMID: 34390780 DOI: 10.1016/j.freeradbiomed.2021.08.005]

41 **Donohue TM**, Osna NA, Clemens DL. Recombinant Hep G2 cells that express alcohol dehydrogenase and cytochrome P450 2E1 as a model of ethanol-elicited cytotoxicity. *Int J Biochem Cell Biol* 2006; **38**: 92-101 [PMID: 16181800 DOI: 10.1016/j.biocel.2005.07.010]

42 **Pocharedddy S**, Edenberg HJ. Chronic alcohol exposure alters gene expression in HepG2 cells. *Alcohol Clin Exp Res* 2012; **36**: 1021-1033 [PMID: 22150570 DOI: 10.1111/j.1530-0277.2011.01677.x]

43 **Gao L**, Shan W, Zeng W, Hu Y, Wang G, Tian X, Zhang N, Shi X, Zhao Y, Ding C, Zhang F, Liu K, Yao J. Carnosic acid alleviates chronic alcoholic liver injury by regulating the SIRT1/ChREBP and SIRT1/p66shc pathways in rats. *Mol Nutr Food Res* 2016; **60**: 1902-1911 [PMID: 27125489 DOI: 10.1002/mnfr.201500878]

44 **Chen WM**, Shaw LH, Chang PJ, Tung SY, Chang TS, Shen CH, Hsieh YY, Wei KL. Hepatoprotective effect of resveratrol against ethanol-induced oxidative stress through induction of superoxide dismutase in vivo and in vitro. *Exp Ther Med* 2016; **11**: 1231-1238 [PMID: 27073428 DOI: 10.3892/etm.2016.3077]

45 **Tao Z**, Zhang L, Wu T, Fang X, Zhao L. Echinacoside ameliorates alcohol-induced oxidative stress and hepatic steatosis by affecting SREBP1c/FASN pathway *via* PPARα. *Food Chem Toxicol* 2021; **148**: 111956 [PMID: 33378712 DOI: 10.1016/j.fct.2020.111956]

46 **Hakeem AN**, Kamal MM, Tawfiq RA, Abdelrahman BA, Hammam OA, Elmazar MM, El-Khatib AS, Attia YM. Elafibranor modulates ileal macrophage polarization to restore intestinal integrity in NASH: Potential crosstalk between ileal IL-10/STAT3 and hepatic TLR4/NF-κB axes. *Biomed Pharmacother* 2023; **157**: 114050 [PMID: 36462310 DOI: 10.1016/j.biopha.2022.114050]

47 **Inamura T**, Miura S, Tsuzuki Y, Hara Y, Hokari R, Ogawa T, Teramoto K, Watanabe C, Kobayashi H, Nagata H, Ishii H. Alteration of intestinal intraepithelial lymphocytes and increased bacterial translocation in a murine model of cirrhosis. *Immunol Lett* 2003; **90**: 3-11 [PMID: 14611901 DOI: 10.1016/j.imlet.2003.05.002]

48 **Fujimoto Y**, Kaji K, Nishimura N, Enomoto M, Murata K, Takeda S, Takaya H, Kawaratani H, Moriya K, Namisaki T, Akahane T, Yoshiji H. Dual therapy with zinc acetate and rifaximin prevents from ethanol-induced liver fibrosis by maintaining intestinal barrier integrity. *World J Gastroenterol* 2021; **27**: 8323-8342 [PMID: 35068872 DOI: 10.3748/wjg.v27.i48.8323]

49 **Li TH**, Yang YY, Huang CC, Liu CW, Tsai HC, Lin MW, Tsai CY, Huang SF, Wang YW, Lee TY, Huang YH, Hou MC, Lin HC. Elafibranor interrupts adipose dysfunction-mediated gut and liver injury in mice with alcoholic steatohepatitis. *Clin Sci (Lond)* 2019; **133**: 531-544 [PMID: 30602573 DOI: 10.1042/CS20180873]

50 **Gou Q**, Jiang Y, Zhang R, Xu Y, Xu H, Zhang W, Shi J, Hou Y. PPARδ is a regulator of autophagy by its phosphorylation. *Oncogene* 2020; **39**: 4844-4853 [PMID: 32439863 DOI: 10.1038/s41388-020-1329-x]

51 **Basso PJ**, Sales-Campos H, Nardini V, Duarte-Silva M, Alves VBF, Bonfá G, Rodrigues CC, Ghirotto B, Chica JEL, Nomizo A, Cardoso CRB. Peroxisome Proliferator-Activated Receptor Alpha Mediates the Beneficial Effects of Atorvastatin in Experimental Colitis. *Front Immunol* 2021; **12**: 618365 [PMID: 34434187 DOI: 10.3389/fimmu.2021.618365]

52 **Crakes KR**, Pires J, Quach N, Ellis-Reis RE, Greathouse R, Chittum KA, Steiner JM, Pesavento P, Marks SL, Dandekar S, Gilor C. Fenofibrate promotes PPARα-targeted recovery of the intestinal epithelial barrier at the host-microbe interface in dogs with diabetes mellitus. *Sci Rep* 2021; **11**: 13454 [PMID: 34188162 DOI: 10.1038/s41598-021-92966-7]

53 **Liu S**, Hatano B, Zhao M, Yen CC, Kang K, Reilly SM, Gangl MR, Gorgun C, Balschi JA, Ntambi JM, Lee CH. Role of peroxisome proliferator-activated receptor {delta}/{beta} in hepatic metabolic regulation. *J Biol Chem* 2011; **286**: 1237-1247 [PMID: 21059653 DOI: 10.1074/jbc.M110.138115]

54 **Risérus U**, Sprecher D, Johnson T, Olson E, Hirschberg S, Liu A, Fang Z, Hegde P, Richards D, Sarov-Blat L, Strum JC, Basu S, Cheeseman J, Fielding BA, Humphreys SM, Danoff T, Moore NR, Murgatroyd P, O'Rahilly S, Sutton P, Willson T, Hassall D, Frayn KN, Karpe F. Activation of peroxisome proliferator-activated receptor (PPAR)delta promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* 2008; **57**: 332-339 [PMID: 18024853 DOI: 10.2337/db07-1318]

55 **Bays HE**, Schwartz S, Littlejohn T 3rd, Kerzner B, Krauss RM, Karpf DB, Choi YJ, Wang X, Naim S, Roberts BK. MBX-8025, a novel peroxisome proliferator receptor-delta agonist: lipid and other metabolic effects in dyslipidemic overweight patients treated with and without atorvastatin. *J Clin Endocrinol Metab* 2011; **96**: 2889-2897 [PMID: 21752880 DOI: 10.1210/jc.2011-1061]

56 **Pineda Torra I**, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B. Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene *via* activation of the farnesoid X receptor. *Mol Endocrinol* 2003; **17**: 259-272 [PMID: 12554753 DOI: 10.1210/me.2002-0120]

57 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332 [PMID: 17952090 DOI: 10.1038/nm1663]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3447-3451

DOI: 10.3748/wjg.v30.i28.3447

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LETTER TO THE EDITOR

Exploring non-invasive diagnostics for metabolic dysfunction-associated fatty liver disease

Biao Qu, Zheng Li

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 B
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Kotlyarov S

Received: April 24, 2024
Revised: July 1, 2024
Accepted: July 8, 2024
Published online: July 28, 2024
Processing time: 90 Days and 8.4 Hours

Biao Qu, Department of Clinical Pharmacology, The Second Hospital of Anhui Medical University, Hefei 230601, Anhui Province, China

Zheng Li, Jiangsu Engineering Research Center of Cardiovascular Drugs Targeting Endothelial Cells, College of Health Sciences, School of Life Sciences, Jiangsu Normal University, Xuzhou 221000, Jiangsu Province, China

Corresponding author: Zheng Li, PhD, Lecturer, Pharmacist, Jiangsu Engineering Research Center of Cardiovascular Drugs Targeting Endothelial Cells, College of Health Sciences, School of Life Sciences, Jiangsu Normal University, No. 101 Shanghai Road, Xuzhou 221000, Jiangsu Province, China. lizhengcpu@163.com

Abstract

The population with metabolic dysfunction-associated fatty liver disease (MAFLD) is increasingly common worldwide. Identification of people at risk of progression to advanced stages is necessary to timely offer interventions and appropriate care. Liver biopsy is currently considered the gold standard for the diagnosis and staging of MAFLD, but it has associated risks and limitations. This has spurred the exploration of non-invasive diagnostics for MAFLD, especially for steatohepatitis and fibrosis. These non-invasive approaches mostly include biomarkers and algorithms derived from anthropometric measurements, serum tests, imaging or stool metagenome profiling. However, they still need rigorous and widespread clinical validation for the diagnostic performance.

Key Words: Metabolic dysfunction-associated fatty liver disease; Non-invasive diagnostics; Circulating biomarkers; Imaging biomarkers; Stool microbial biomarkers

Core Tip: Metabolic dysfunction-associated fatty liver disease (MAFLD) is a burdensome public health problem. The diagnostic assessment of MAFLD is an important step for timely management. Extensive effort and encouraging progress have been made to establish non-invasive tests to diagnose steatohepatitis and fibrosis.

Citation: Qu B, Li Z. Exploring non-invasive diagnostics for metabolic dysfunction-associated fatty liver disease. *World J Gastroenterol* 2024; 30(28): 3447-3451
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3447.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3447

TO THE EDITOR

We read with great interest the article by Trinks *et al*[1] on the omics-based biomarkers as diagnostic tools for metabolic dysfunction-associated fatty liver disease (MAFLD). Due to its global epidemic, MAFLD becomes a burdensome public health problem[2]. The development of steatohepatitis, especially liver fibrosis, is most strongly associated with poorer long-term outcomes and increased incidence of liver-related mortality[3-5]. It is in this context that identification of people at risk of progression to advanced stages is necessary to timely offer interventions and appropriate care. Currently, liver biopsy is still the reference standard for diagnosis and staging of MAFLD. However, it is an invasive approach with poor compliance and a small but appreciable risk of complications[6]. Besides, it is also expensive, prone to sampling bias, and has high intra- and interobserver variability[6-8]. These inherent limitations have driven the need for non-invasive approaches to replace liver biopsy in severity assessment and risk stratification of patients with MASLD. In this regard, extensive effort and encouraging progress have been made in this filed. There is now increased availability of non-invasive tests, and some become increasingly incorporated into routine clinical practice[9-12]. These non-invasive approaches mostly include biomarkers and algorithms derived from anthropometric measurements[9], serum tests[10], imaging[11], or stool metagenome profiling[12].

Anthropometric measurements

Anthropometric indicators have been used for prediction of MASLD, such as body mass index, abdomen, waist, and chest circumferences, and trunk fat. These indicators are easy to be determined with simple and affordable equipment, thus making them ideal for use in remote areas or in primary clinical practice[9]. Recently, artificial intelligence, such as machine learning and deep learning, has been applied to assist anthropometric diagnostics of MAFLD[13]. However, these indicators are still limited by suboptimal accuracy, especially in detecting fibrosis[13].

Serum biomarkers and related panels

Increased serum triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), or ALT/AST ratio are not accurately predictive of MAFLD severity[14,15]. Related panels derived from these biochemical indicators are also subject to low accuracy and specificity, such as fatty liver index for hepatic steatosis, and Bayesian Argumentation *via* Delphi score (body mass index, AST/ALT ratio, and presence of diabetes) for hepatic fibrosis[16,17]. Despite these limitations, they are still commonly used for screening owing to general applicability. Recently, some novel serum biomarkers have been proposed as promising alternatives for diagnosis of non-alcoholic steatohepatitis (NASH) and fibrosis. Circulating concentrations of cytokeratin-18 (CK-18) fragments were proposed to be the most reliable predictor of steatohepatitis[18], and its combination with other indicators in a biomarker panel could further increase the diagnostic performance[19]. However, it has relatively low power to determine the severity of NASH fibrosis[20]. Serum Pro-C3 and metalloprotease-1 inhibitor, are emerging biomarkers for fibrosis with excellent diagnostic performance[21,22]. Subsequently, several biomarker panels are proposed, such as MACK-3 (HOMA-IR, AST, and CK-18 M30)[23] and ADAPT (age, platelet count, diabetes, and PRO-C3)[24], and their diagnostic performance is evaluated in comparative diagnostic accuracy studies[10,25]. In addition, the application of innovative omics technologies has screened out some novel serum biomarkers[26,27], but their accuracy, reproducibility, and reliability have not yet gone through analytical/biological and clinical cohort validation.

Imaging biomarkers

Conventional ultrasonography is the first-line imaging test for detecting hepatic steatosis[28], and newer quantitative ultrasound-based techniques demonstrate superior performance[29]. As a contrast, magnetic resonance imaging-proton density fat fraction is considered more accurate at quantifying liver fat than ultrasonography[30], and even liver biopsy [31]. Current imaging-based biomarkers have poor diagnostic performance for steatohepatitis, especially distinguishing steatohepatitis from fibrosis[32]. Ultrasound-based measurements of liver stiffness by vibration-controlled transient elastography (VCTE), commercially marketed as FibroScan, have demonstrated very good diagnostic accuracy for advanced fibrosis[33]. Likewise, magnetic resonance elastography (MRE) shows low failure rate in diagnosis of advanced fibrosis[34]. More importantly, MRE outperforms VCTE in diagnostic accuracy for earlier stages of fibrosis[30].

Gut-microbiome-derived biomarkers

Dysregulation of the gut microbiome is implicated in the progression of MAFLD as evidenced by several studies[35,36]. This association could be translated into diagnostic capacity for MAFLD. A latest study characterized gut microbiome compositions using metagenomic sequencing of stool samples from patients with biopsy-proven MAFLD, and established a gut microbiome-based metagenomic signature to differentiate between mild or moderate and advanced fibrosis[12]. These microbial biomarkers achieved robust diagnostic accuracy in small samples, and further studies are needed to validate their clinical utility.

In summary, progress has been made in the identification of novel non-invasive diagnostics for MAFLD, including biomarkers and algorithms integrating biomarkers. Although none of biomarkers achieved the sufficient performance to replace liver biopsy in diagnosis of steatohepatitis and fibrosis, some diagnostics are promising tools for identifying advanced fibrosis.

FOOTNOTES

Author contributions: Li Z designed the article; Qu B and Li Z drafted and revised the article.

Supported by The National Natural Science Foundation of China, No. 82104525.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: China

ORCID number: Zheng Li 0000-0002-2882-6600.

S-Editor: Luo ML
L-Editor: Wang TQ
P-Editor: Zhang L

REFERENCES

1 **Trinks J**, Mascardi MF, Gadano A, Marciano S. Omics-based biomarkers as useful tools in metabolic dysfunction-associated steatotic liver disease clinical practice: How far are we? *World J Gastroenterol* 2024; **30**: 1982-1989 [PMID: 38681130 DOI: 10.3748/wjg.v30.i14.1982]

2 **Estes C**, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018; **67**: 123-133 [PMID: 28802062 DOI: 10.1002/hep.29466]

3 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-397 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]

4 **Dulai PS**, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, Sebastiani G, Ekstedt M, Hagstrom H, Nasr P, Stal P, Wong VW, Kechagias S, Hultcrantz R, Loomba R. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology* 2017; **65**: 1557-1565 [PMID: 28130788 DOI: 10.1002/hep.29085]

5 **Hagström H**, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, Kechagias S. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. *J Hepatol* 2017; **67**: 1265-1273 [PMID: 28803953 DOI: 10.1016/j.jhep.2017.07.027]

6 **Davison BA**, Harrison SA, Cotter G, Alkhouri N, Sanyal A, Edwards C, Colca JR, Iwashita J, Koch GG, Dittrich HC. Suboptimal reliability of liver biopsy evaluation has implications for randomized clinical trials. *J Hepatol* 2020; **73**: 1322-1332 [PMID: 32610115 DOI: 10.1016/j.jhep.2020.06.025]

7 **Ratziu V**, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T; LIDO Study Group. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906 [PMID: 15940625 DOI: 10.1053/j.gastro.2005.03.084]

8 **Merriman RB**, Ferrell LD, Patti MG, Weston SR, Pabst MS, Aouizerat BE, Bass NM. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 874-880 [PMID: 17006934 DOI: 10.1002/hep.21346]

9 **Almeida NS**, Rocha R, Cotrim HP, Daltro C. Anthropometric indicators of visceral adiposity as predictors of non-alcoholic fatty liver disease: A review. *World J Hepatol* 2018; **10**: 695-701 [PMID: 30386462 DOI: 10.4254/wjh.v10.i10.695]

10 **Sanyal AJ**, Shankar SS, Yates KP, Bolognese J, Daly E, Dehn CA, Neuschwander-Tetri B, Kowdley K, Vuppalanchi R, Behling C, Tonascia J, Samir A, Sirlin C, Sherlock SP, Fowler K, Heymann H, Kamphaus TN, Loomba R, Calle RA. Diagnostic performance of circulating biomarkers for non-alcoholic steatohepatitis. *Nat Med* 2023; **29**: 2656-2664 [PMID: 37679433 DOI: 10.1038/s41591-023-02539-6]

11 **Ajmera V**, Loomba R. Imaging biomarkers of NAFLD, NASH, and fibrosis. *Mol Metab* 2021; **50**: 101167 [PMID: 33460786 DOI: 10.1016/j.molmet.2021.101167]

12 **Loomba R**, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, Dulai PS, Caussy C, Bettencourt R, Highlander SK, Jones MB, Sirlin CB, Schnabl B, Brinkac L, Schork N, Chen CH, Brenner DA, Biggs W, Yooseph S, Venter JC, Nelson KE. Gut Microbiome-Based Metagenomic Signature for Non-invasive Detection of Advanced Fibrosis in Human Nonalcoholic Fatty Liver Disease. *Cell Metab* 2019; **30**: 607 [PMID: 31484056 DOI: 10.1016/j.cmet.2019.08.002]

13 **Razmpour F**, Daryabeygi-Khotbehsara R, Soleimani D, Asgharnezhad H, Shamsi A, Bajestani GS, Nematy M, Pour MR, Maddison R, Islam SMS. Application of machine learning in predicting non-alcoholic fatty liver disease using anthropometric and body composition indices. *Sci Rep* 2023; **13**: 4942 [PMID: 36973382 DOI: 10.1038/s41598-023-32129-y]

14 **Younossi ZM**, Loomba R, Anstee QM, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, Serfaty L, Negro F, Caldwell SH, Ratziu V, Corey KE, Friedman SL, Abdelmalek MF, Harrison SA, Sanyal AJ, Lavine JE, Mathurin P, Charlton MR, Goodman ZD, Chalasani

NP, Kowdley KV, George J, Lindor K. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. *Hepatology* 2018; **68**: 349-360 [PMID: 29222917 DOI: 10.1002/hep.29721]

15 **Verma S**, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). *Liver Int* 2013; **33**: 1398-1405 [PMID: 23763360 DOI: 10.1111/liv.12226]

16 **Fedchuk L**, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V; LIDO Study Group. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* 2014; **40**: 1209-1222 [PMID: 25267215 DOI: 10.1111/apt.12963]

17 **McPherson S**, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut* 2010; **59**: 1265-1269 [PMID: 20801772 DOI: 10.1136/gut.2010.216077]

18 **Kwok R**, Tse YK, Wong GL, Ha Y, Lee AU, Ngu MC, Chan HL, Wong VW. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease--the role of transient elastography and plasma cytokeratin-18 fragments. *Aliment Pharmacol Ther* 2014; **39**: 254-269 [PMID: 24308774 DOI: 10.1111/apt.12569]

19 **Shen J**, Chan HL, Wong GL, Choi PC, Chan AW, Chan HY, Chim AM, Yeung DK, Chan FK, Woo J, Yu J, Chu WC, Wong VW. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. *J Hepatol* 2012; **56**: 1363-1370 [PMID: 22314419 DOI: 10.1016/j.jhep.2011.12.025]

20 **Cusi K**, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, Ortiz-Lopez C, Hecht J, Feldstein AE, Webb A, Louden C, Goros M, Tio F. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. *J Hepatol* 2014; **60**: 167-174 [PMID: 23973932 DOI: 10.1016/j.jhep.2013.07.042]

21 **Leeming DJ**, Grove JI, Kaye P, Hoad C, Francis S, Nielsen MJ, Karsdal MA, Guha IN, Aithal GP. Estimation of serum "true collagen type III formation" (Pro-C3) levels as a marker of non-alcoholic steatohepatitis in a prospective cohort. *J Hepatol* 2017; **66**: S154 [DOI: 10.1016/S0168-8278(17)30582-2]

22 **Abdelaziz R**, Elbasel M, Esmat S, Essam K, Abdelaaty S. Tissue Inhibitors of Metalloproteinase-1 and 2 and Obesity Related Non-Alcoholic Fatty Liver Disease: Is There a Relationship. *Digestion* 2015; **92**: 130-137 [PMID: 26329758 DOI: 10.1159/000439083]

23 **Boursier J**, Anty R, Vonghia L, Moal V, Vanwolleghem T, Canivet CM, Michalak S, Bonnafous S, Michielsen P, Oberti F, Iannelli A, Van Gaal L, Patouraux S, Blanchet O, Verrijken A, Gual P, Rousselet MC, Driessen A, Hunault G, Bertrais S, Tran A, Calès P, Francque S. Screening for therapeutic trials and treatment indication in clinical practice: MACK-3, a new blood test for the diagnosis of fibrotic NASH. *Aliment Pharmacol Ther* 2018; **47**: 1387-1396 [PMID: 29577364 DOI: 10.1111/apt.14621]

24 **Daniels SJ**, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A, Karsdal MA, Grove JI, Neil Guha I, Kawaguchi T, Torimura T, McLeod D, Akiba J, Kaye P, de Boer B, Aithal GP, Adams LA, George J. ADAPT: An Algorithm Incorporating PRO-C3 Accurately Identifies Patients With NAFLD and Advanced Fibrosis. *Hepatology* 2019; **69**: 1075-1086 [PMID: 30014517 DOI: 10.1002/hep.30163]

25 **Vali Y**, Lee J, Boursier J, Petta S, Wonders K, Tiniakos D, Bedossa P, Geier A, Francque S, Allison M, Papatheodoridis G, Cortez-Pinto H, Pais R, Dufour JF, Leeming DJ, Harrison SA, Chen Y, Cobbold JF, Pavlides M, Holleboom AG, Yki-Jarvinen H, Crespo J, Karsdal M, Ostroff R, Zafarmand MH, Torstenson R, Duffin K, Yunis C, Brass C, Ekstedt M, Aithal GP, Schattenberg JM, Bugianesi E, Romero-Gomez M, Ratziu V, Anstee QM, Bossuyt PM; Liver Investigation: Testing Marker Utility in Steatohepatitis (LITMUS) consortium investigators. Biomarkers for staging fibrosis and non-alcoholic steatohepatitis in non-alcoholic fatty liver disease (the LITMUS project): a comparative diagnostic accuracy study. *Lancet Gastroenterol Hepatol* 2023; **8**: 714-725 [PMID: 36958367 DOI: 10.1016/S2468-1253(23)00017-1]

26 **Mayo R**, Crespo J, Martínez-Arranz I, Banales JM, Arias M, Mincholé I, Aller de la Fuente R, Jimenez-Agüero R, Alonso C, de Luis DA, Vitek L, Stritesky J, Caballería J, Romero-Gómez M, Martín-Duce A, Mugüerza Huguet JM, Busteros-Moraza JI, Idowu MO, Castro A, Martínez-Chantar ML, Ortiz P, Bruha R, Lu SC, Bedossa P, Noureddin M, Sanyal AJ, Mato JM. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: Results from discovery and validation cohorts. *Hepatol Commun* 2018; **2**: 807-820 [PMID: 30027139 DOI: 10.1002/hep4.1188]

27 **Sanyal AJ**, Williams SA, Lavine JE, Neuschwander-Tetri BA, Alexander L, Ostroff R, Biegel H, Kowdley KV, Chalasani N, Dasarathy S, Diehl AM, Loomba R, Hameed B, Behling C, Kleiner DE, Karpen SJ, Williams J, Jia Y, Yates KP, Tonascia J. Defining the serum proteomic signature of hepatic steatosis, inflammation, ballooning and fibrosis in non-alcoholic fatty liver disease. *J Hepatol* 2023; **78**: 693-703 [PMID: 36528237 DOI: 10.1016/j.jhep.2022.11.029]

28 **Strauss S**, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. *AJR Am J Roentgenol* 2007; **189**: W320-W323 [PMID: 18029843 DOI: 10.2214/AJR.07.2123]

29 **Han A**, Zhang YN, Boehringer AS, Montes V, Andre MP, Erdman JW Jr, Loomba R, Valasek MA, Sirlin CB, O'Brien WD Jr. Assessment of Hepatic Steatosis in Nonalcoholic Fatty Liver Disease by Using Quantitative US. *Radiology* 2020; **295**: 106-113 [PMID: 32013792 DOI: 10.1148/radiol.2020191152]

30 **Park CC**, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, Hooker J, Sy E, Savides MT, Alquiraish MH, Valasek MA, Rizo E, Richards L, Brenner D, Sirlin CB, Loomba R. Magnetic Resonance Elastography vs Transient Elastography in Detection of Fibrosis and Noninvasive Measurement of Steatosis in Patients With Biopsy-Proven Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2017; **152**: 598-607 [PMID: 27911262 DOI: 10.1053/j.gastro.2016.10.026]

31 **Noureddin M**, Lam J, Peterson MR, Middleton M, Hamilton G, Le TA, Bettencourt R, Changchien C, Brenner DA, Sirlin C, Loomba R. Utility of magnetic resonance imaging versus histology for quantifying changes in liver fat in nonalcoholic fatty liver disease trials. *Hepatology* 2013; **58**: 1930-1940 [PMID: 23696515 DOI: 10.1002/hep.26455]

32 **Jayaswal ANA**, Levick C, Selvaraj EA, Dennis A, Booth JC, Collier J, Cobbold J, Tunnicliffe EM, Kelly M, Barnes E, Neubauer S, Banerjee R, Pavlides M. Prognostic value of multiparametric magnetic resonance imaging, transient elastography and blood-based fibrosis markers in patients with chronic liver disease. *Liver Int* 2020; **40**: 3071-3082 [PMID: 32730664 DOI: 10.1111/liv.14625]

33 **Siddiqui MS**, Vuppalanchi R, Van Natta ML, Hallinan E, Kowdley KV, Abdelmalek M, Neuschwander-Tetri BA, Loomba R, Dasarathy S, Brandman D, Doo E, Tonascia JA, Kleiner DE, Chalasani N, Sanyal AJ; NASH Clinical Research Network. Vibration-Controlled Transient Elastography to Assess Fibrosis and Steatosis in Patients With Nonalcoholic Fatty Liver Disease. *Clin Gastroenterol Hepatol* 2019; **17**: 156-163 [PMID: 29705261 DOI: 10.1016/j.cgh.2018.04.043]

34 **Wagner M**, Corcuera-Solano I, Lo G, Esses S, Liao J, Besa C, Chen N, Abraham G, Fung M, Babb JS, Ehman RL, Taouli B. Technical Failure of MR Elastography Examinations of the Liver: Experience from a Large Single-Center Study. *Radiology* 2017; **284**: 401-412 [PMID: 28045604 DOI: 10.1148/radiol.2016160863]

35 **Hoyles L**, Fernández-Real JM, Federici M, Serino M, Abbott J, Charpentier J, Heymes C, Luque JL, Anthony E, Barton RH, Chilloux J, Myridakis A, Martinez-Gili L, Moreno-Navarrete JM, Benhamed F, Azalbert V, Blasco-Baque V, Puig J, Xifra G, Ricart W, Tomlinson C, Woodbridge M, Cardellini M, Davato F, Cardolini I, Porzio O, Gentileschi P, Lopez F, Foufelle F, Butcher SA, Holmes E, Nicholson JK,

Postic C, Burcelin R, Dumas ME. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* 2018; **24**: 1070-1080 [PMID: 29942096 DOI: 10.1038/s41591-018-0061-3]

36 **Sharpton SR**, Ajmera V, Loomba R. Emerging Role of the Gut Microbiome in Nonalcoholic Fatty Liver Disease: From Composition to Function. *Clin Gastroenterol Hepatol* 2019; **17**: 296-306 [PMID: 30196156 DOI: 10.1016/j.cgh.2018.08.065]

World Journal of Gastroenterology

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

World J Gastroenterol 2024 July 28; 30(28): 3452-3455

DOI: 10.3748/wjg.v30.i28.3452

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

LETTER TO THE EDITOR

Impact of neoadjuvant multimodal therapy in the setting of locally advanced hepatocellular carcinoma

Daniele Ferraro, Federica Falaschi, Luca Nazzaro, Giovanni Vennarecci

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 B
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Zhou S

Received: April 29, 2024
Revised: May 28, 2024
Accepted: July 2, 2024
Published online: July 28, 2024
Processing time: 86 Days and 5.2 Hours

Daniele Ferraro, Federica Falaschi, Luca Nazzaro, Giovanni Vennarecci, Hepato-biliary and Liver Transplant Centre, Department of General Surgery and Woman's Health, AORN Antonio Cardarelli, Naples 80131, Italy

Corresponding author: Daniele Ferraro, MD, Consultant in HPB Surgery and Liver Transplantation, Hepato-biliary and Liver Transplant Centre, Department of General Surgery and Woman's Health, AORN Antonio Cardarelli, Naples 80131, Italy.
daniele.ferraro@aocardarelli.it

Abstract

Immunotherapy and the implementation of more aggressive treatment schemes for locally advanced hepatocellular carcinomas have expanded the boundaries of curative options. Because of these advancements, patients who were once considered beyond the aim of a cure are now eligible for liver transplantation and resection.

Key Words: Hepatocellular carcinoma; Immunotherapy; Radioembolization; Chemoembolization; Liver transplantation; Liver resection

Core Tip: The field of treatment for hepatocellular carcinoma is constantly evolving due to the advances in highly effective chemotherapeutic regimens. The possible use of drugs in the neoadjuvant setting as a powerful downstaging tool opens up the possibility of liver transplantation or liver resection for patients once deemed incurable. The use of these drugs in the adjuvant therapy setting could reinforce the results of surgery in the treatment of hepatocellular carcinoma.

Citation: Ferraro D, Falaschi F, Nazzaro L, Vennarecci G. Impact of neoadjuvant multimodal therapy in the setting of locally advanced hepatocellular carcinoma. *World J Gastroenterol* 2024; 30(28): 3452-3455
URL: https://www.wjgnet.com/1007-9327/full/v30/i28/3452.htm
DOI: https://dx.doi.org/10.3748/wjg.v30.i28.3452

TO THE EDITOR

We read with great interest the paper by Wu *et al*[1], who presented a retrospective study comparing the oncological outcomes of two arms of patients affected by hepatocellular carcinoma (HCC) with portal vein tumor thrombosis (PVTT) with or without distant metastasis, who underwent either triple therapy with transarterial chemoembolization (TACE) combined with PD-1 inhibitors and lenvatinib or TACE plus lenvatinib alone.

Despite the limits of the study (*i.e.*, its retrospective nature, limited study population, and different proportions of patients with metastatic disease in the two study groups), we agree with the authors of the study that multimodal therapy for locally advanced HCC shows superior results in terms of local control of the disease, downstaging ability, and prolonged time to progression.

We have already published a case of a patient with locally advanced HCC of segment IX with satellite nodules, alpha-fetoprotein > 3000 ng/mL, left portal vein tumor thrombosis with partial response to TACE, and complete and persistent radiological and biological responses to neoadjuvant therapy with lenvatinib[2]. However, since this publication, treatment options have advanced and expanded. The application of immunotherapy with a PD-1 inhibitor and anti-CTLA-4 has been proven as an effective tool to downstage locally-advanced HCC patients, allowing curative treatments such as salvage liver transplantation and liver resection[3].

Patients who achieve a partial response after conversion therapy benefit from liver resection or liver transplantation as the only curative option remaining. Indeed, it has been shown that surgery after locoregional treatment with a partial response improves overall survival (OS)[4]. Performing surgery after conversion therapy that leads to a complete response, however, remains a matter of debate. The gold standard therapeutic approach is still questionable, and solid scientific evidence is lacking. There is evidence, though, that a watch-and-wait approach might be safe and feasible for these patients[5]. Arguments in favor of surgical resection after complete radiological response include the possibility of persistent microscopic residue only evident on resected specimens and the possibility of *in situ* recurrence after a complete response. Future research on circulating molecular markers could provide an implementation strategy for a watch-and-wait approach in well-selected cases. In our case, the patient remained cancer-free for more than 2 years and was ineligible for liver transplantation as liver function remained stable and the disease did not recur.

When HCC involves the major portal vein branches, we believe radioembolization performs better for local control. There is a better possibility of complete regression of neoplastic thrombosis[6], better control of microvascular invasion [7], and improvement of time-to-progression compared to other transarterial locoregional therapies[8]. Stable and durable response to radioembolization could serve as a tool to test HCC biology in the setting of downstaging prior to liver transplantation. Many groups[7,8], including ours[6], have shown acceptable oncological outcomes of liver transplantation in patients affected by HCC complicated by PVTT.

A mindset change is needed for the issue of treating HCC with PVTT. PVTT can partially or completely respond to radiotherapy (stereotactic body radiation therapy/external beam radiation therapy/selective internal radiation therapy), TACE, proton beam therapy, or systemic therapy, used alone or in combination, thus improving progression-free survival and OS. Soin *et al*[9] showed 5-year OS and recurrence-free survival rates of 53% and 52%, respectively, in patients who successfully downstaged to stable disease. These rates are far superior to the 10% OS at 3-year expected by the Barcelona Clinic Liver Cancer (BCLC) staging and treatment algorithm. These data translate into a huge transplant benefit measured for these categories of HCC patients.

Similar results have been achieved by Serenari *et al*[10] and Assalino *et al*[11] who showed a 5-year OS of 60% in patients who were successfully downstaged and received a liver transplantation. Careful selection of patients with HCC with favorable biology (small tumor sizes, low alpha-fetoprotein level, low tumor grade, low avidity on fluorodeoxy-glucose-positron emission tomography) is warranted to obtain adequate oncological results. In this scenario, a living donor donation may be advantageous because it is favorable for a planned and timely transplant and it avoids depletion of the already scarce pool of deceased donors.

Under these premises, however, it could be postulated that brand new scenarios might be faced in the foreseeable future. With the advent of highly active locoregional and biological therapies, after an appropriate test of time, two questions arise: (1) Would it be possible to label patients within BCLC stage B and C who achieve complete response biologically and radiologically as cured? or (2) Should we proceed to liver transplantation anyway, having achieved just a very good downstaging? The vast majority of HCC patients seen in our clinics nowadays belong to BCLC stage B and C, which is by definition biologically very aggressive. Offering a pre-emptive liver transplant, *i.e.*, without radiologic or biologic evidence of residual/recurrent disease, after an appropriate test of time, might represent the only potentially curative option for these categories of HCC patients. However, as far as allocation policies are concerned, it remains unclear whether these patients that might not have MELD scores sufficient to be listed for transplantation should be prioritized or not and in what measure in a system that provides patients with untreatable HCC or with partial response to bridge therapy with exception points in the waiting list. Regardless of what the right answer will be proven to be, we should start looking at patients with locally advanced HCC or portal neoplastic thrombosis as a focus for our best efforts in order to rescue a portion of them so that they are candidates for curative options, although those are few, according to the principles of treatment stage migration and therapeutic hierarchy.

The results of the IMbrave050 trial[12] demonstrated the ability of the combination of atezolizumab-bevacizumab to improve the recurrence-free survival of patients with resected or ablated HCC. It is advisable to enroll patients with HCC with a high risk of recurrence in prospective and randomized studies to further investigate the role in the adjuvant setting of other lines of immunotherapy, such as durvalumab/tremelimumab. The latter combination of drugs, available in Italy since last April and with areas of application similar to atezolizumab/bevacizumab and lenvatinib, showed promising results in terms of overall survival compared to sorafenib in patients with unresectable HCC, in a recent phase-3 trial[13].

In conclusion, we are facing a rapidly growing body of neoadjuvant systemic treatment schemes that may in the future be used as adjuvant therapies. Robust data on which therapies will stand the test of time and represent the standardized treatment for locally advanced HCC in the neoadjuvant and adjuvant settings are much needed. Undoubtedly, liver transplantation and resection still represent curative options. Their utility is bound to increase as the abovementioned therapies allow locally advanced HCC patients to become eligible for these treatments. Approximately 1700 liver transplants were performed in Italy in 2023. Among those, more than half listed HCC as the main indication. The steady decline of viral etiologies as main indications, along with the implementation of the highly active biologic therapies, will continue to increase the number of liver transplantations performed for HCC.

FOOTNOTES

Author contributions: Ferraro D, Falaschi F, Nazzaro L, and Vennarecci G equally contributed to the study design, research, data analysis, writing, and revision of the letter; and all authors read and approved the final version of the manuscript.

Conflict-of-interest statement: We declare having no conflicts of interest.

Country/Territory of origin: Italy

ORCID number: Daniele Ferraro 0000-0002-1022-8864; Giovanni Vennarecci 0000-0001-9727-4304.

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

REFERENCES

1 **Wu HX**, Ding XY, Xu YW, Yu MH, Li XM, Deng N, Chen JL. Transcatheter arterial chemoembolization combined with PD-1 inhibitors and Lenvatinib for hepatocellular carcinoma with portal vein tumor thrombus. *World J Gastroenterol* 2024; **30**: 843-854 [PMID: 38516240 DOI: 10.3748/wjg.v30.i8.843]

2 **Pirozzi REM**, Di Martino M, Ferraro D, Pisaniello D, Vennarecci G. Systemic therapy with lenvatinib in advanced hepatocellular carcinoma: what to do in the case of a complete radiologic response? *J Gastrointestin Liver Dis* 2023; **32**: 118-119 [PMID: 37004223 DOI: 10.15403/jgld-4811]

3 **Llovet JM**, Pinyol R, Yarchoan M, Singal AG, Marron TU, Schwartz M, Pikarsky E, Kudo M, Finn RS. Adjuvant and neoadjuvant immunotherapies in hepatocellular carcinoma. *Nat Rev Clin Oncol* 2024; **21**: 294-311 [PMID: 38424197 DOI: 10.1038/s41571-024-00868-0]

4 **Tang YL**, Qi XS, Guo XZ. Hepatic Resection after Initial Transarterial Chemoembolization Versus Transarterial Chemoembolization Alone for the Treatment of Hepatocellular Carcinoma: A Meta-analysis of Observational Studies. *Asian Pac J Cancer Prev* 2015; **16**: 7871-7874 [PMID: 26625813 DOI: 10.7314/apjcp.2015.16.17.7871]

5 **Li B**, Wang C, He W, Qiu J, Zheng Y, Zou R, Lin Z, Shi Y, Yuan Y, Zhang R, Zhang C, Chen M, Lau WY, Yuan Y. Watch-and-wait strategy vs. resection in patients with radiologic complete response after conversion therapy for initially unresectable hepatocellular carcinoma: a propensity score-matching comparative study. *Int J Surg* 2024; **110**: 2545-2555 [PMID: 38329081 DOI: 10.1097/JS9.0000000000001155]

6 **Levi Sandri GB**, Ettorre GM, Colasanti M, De Werra E, Mascianà G, Ferraro D, Tortorelli G, Sciuto R, Lucatelli P, Pizzi G, Visco-Comandini U, Vennarecci G. Hepatocellular carcinoma with macrovascular invasion treated with yttrium-90 radioembolization prior to transplantation. *Hepatobiliary Surg Nutr* 2017; **6**: 44-48 [PMID: 28261594 DOI: 10.21037/hbsn.2017.01.08]

7 **Zori AG**, Ismael MN, Limaye AR, Firpi R, Morelli G, Soldevila-Pico C, Suman A, Vogel JD, Lazarowicz M, Geller BS, Toskich B, Gonzalo DH, Johnson MW, Cabrera R. Locoregional Therapy Protocols With and Without Radioembolization for Hepatocellular Carcinoma as Bridge to Liver Transplantation. *Am J Clin Oncol* 2020; **43**: 325-333 [PMID: 32079854 DOI: 10.1097/COC.0000000000000678]

8 **Salem R**, Gordon AC, Mouli S, Hickey R, Kallini J, Gabr A, Mulcahy MF, Baker T, Abecassis M, Miller FH, Yaghmai V, Sato K, Desai K, Thornburg B, Benson AB, Rademaker A, Ganger D, Kulik L, Lewandowski RJ. Y90 Radioembolization Significantly Prolongs Time to Progression Compared With Chemoembolization in Patients With Hepatocellular Carcinoma. *Gastroenterology* 2016; **151**: 1155-1163.e2 [PMID: 27575820 DOI: 10.1053/j.gastro.2016.08.029]

9 **Soin AS**, Bhangui P, Kataria T, Baijal SS, Piplani T, Gautam D, Choudhary NS, Thiagarajan S, Rastogi A, Saraf N, Saigal S. Experience With LDLT in Patients With Hepatocellular Carcinoma and Portal Vein Tumor Thrombosis Postdownstaging. *Transplantation* 2020; **104**: 2334-2345 [PMID: 32032291 DOI: 10.1097/TP.0000000000003162]

10 **Serenari M**, Cappelli A, Cucchetti A, Mosconi C, Strigari L, Monari F, Ravaioli M, Rizzini EL, Fanti S, Golfieri R, Cescon M. Deceased Donor Liver Transplantation After Radioembolization for Hepatocellular Carcinoma and Portal Vein Tumoral Thrombosis: A Pilot Study. *Liver Transpl* 2021; **27**: 1758-1766 [PMID: 34355489 DOI: 10.1002/lt.26257]

11 **Assalino M**, Terraz S, Grat M, Lai Q, Vachharajani N, Gringeri E, Bongini MA, Kulik L, Tabrizian P, Agopian V, Mehta N, Brustia R, Vitali GC, Andres A, Berney T, Mazzaferro V, Compagnon P, Majno P, Cillo U, Chapman W, Zieniewicz K, Scatton O, Toso C. Liver transplantation for hepatocellular carcinoma after successful treatment of macrovascular invasion - a multi-center retrospective cohort study. *Transpl Int* 2020; **33**: 567-575 [PMID: 31994238 DOI: 10.1111/tri.13586]

12 **Qin S**, Chen M, Cheng AL, Kaseb AO, Kudo M, Lee HC, Yopp AC, Zhou J, Wang L, Wen X, Heo J, Tak WY, Nakamura S, Numata K, Uguen T, Hsiehchen D, Cha E, Hack SP, Lian Q, Ma N, Spahn JH, Wang Y, Wu C, Chow PKH; IMbrave050 investigators. Atezolizumab plus bevacizumab versus active surveillance in patients with resected or ablated high-risk hepatocellular carcinoma (IMbrave050): a randomised, open-label, multicentre, phase 3 trial. *Lancet* 2023; **402**: 1835-1847 [PMID: 37871608 DOI: 10.1016/S0140-6736(23)01796-8]

13 **Abou-Alfa GK**, Lau G, Kudo M, Chan SL, Kelley RK, Furuse J, Sukeepaisarnjaroen W, Kang YK, Van Dao T, De Toni EN, Rimassa L, Breder V, Vasilyev A, Heurgué A, Tam VC, Mody K, Thungappa SC, Ostapenko Y, Yau T, Azevedo S, Varela M, Cheng AL, Qin S, Galle PR, Ali S, Marcovitz M, Makowsky M, He P, Kurland JF, Negro A, Sangro B. Tremelimumab plus Durvalumab in Unresectable Hepatocellular Carcinoma. *NEJM Evid* 2022; **1**: EVIDoa2100070 [PMID: 38319892 DOI: 10.1056/EVIDoa2100070]

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