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

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2021 May 21; 27(19): 2251-2433

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 27 Number 19 May 21, 2021

## EDITORIAL



## FRONTIER



## REVIEW



## MINIREVIEWS



## ORIGINAL ARTICLE

### Retrospective Cohort Study

*World Journal of Gastroenterology*

**Weekly Volume 27 Number 19 May 21, 2021**

## Contents

*World Journal of Gastroenterology*

**Weekly Volume 27 Number 19 May 21, 2021**

# Contents

## ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, Pietro Fusaroli, MD, Associate Professor, Department of Medical and Surgical Sciences, University of Bologna/Hospital of Imola, Via Montericco 4, Imola (BO) 40026, Italy. pietro.fusaroli@unibo.it

## 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 indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Scopus. The 2020 edition of Journal Citation Report® cites the 2019 impact factor (IF) for *WJG* as 3.665; IF without journal self cites: 3.534; 5-year IF: 4.048; Ranking: 35 among 88 journals in gastroenterology and hepatology; and Quartile category: Q2. The *WJG*'s CiteScore for 2019 is 7.1 and Scopus CiteScore rank 2019: Gastroenterology is 17/137.

## RESPONSIBLE EDITORS FOR THIS ISSUE

**Production Editor:** *Yu-Jie Ma*; **Production Department Director:** *Xiang Li*; **Editorial Office Director:** *Ze-Mao Gong*.

**NAME OF JOURNAL**
*World Journal of Gastroenterology*

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

**LAUNCH DATE**
October 1, 1995

**FREQUENCY**
Weekly

**EDITORS-IN-CHIEF**
Andrzej S Tarnawski, Subrata Ghosh

**EDITORIAL BOARD MEMBERS**
http://www.wjgnet.com/1007-9327/editorialboard.htm

**PUBLICATION DATE**
May 21, 2021

**COPYRIGHT**
© 2021 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**
https://www.wjgnet.com/bpg/gerinfo/204

**GUIDELINES FOR ETHICS DOCUMENTS**
https://www.wjgnet.com/bpg/GerInfo/287

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**
https://www.wjgnet.com/bpg/gerinfo/240

**PUBLICATION ETHICS**
https://www.wjgnet.com/bpg/GerInfo/288

**PUBLICATION MISCONDUCT**
https://www.wjgnet.com/bpg/gerinfo/208

**ARTICLE PROCESSING CHARGE**
https://www.wjgnet.com/bpg/gerinfo/242

**STEPS FOR SUBMITTING MANUSCRIPTS**
https://www.wjgnet.com/bpg/GerInfo/239

**ONLINE SUBMISSION**
https://www.f6publishing.com

© 2021 Baishideng Publishing Group Inc. All rights reserved. 7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2251-2256

DOI: 10.3748/wjg.v27.i19.2251

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

EDITORIAL

# Celiac Disease in Asia beyond the Middle East and Indian subcontinent: Epidemiological burden and diagnostic barriers

Dimitri Poddighe, Diyora Abdukhakimova

**ORCID number:** Dimitri Poddighe 0000-0001-6431-9334; Diyora Abdukhakimova 0000-0003-2427-7015.

**Author contributions:** Poddighe D conceived and wrote the manuscript; Abdukhakimova D provided intellectual contribution and contributed to the literature research.

**Supported by** Nazarbayev University Faculty Development Competitive Research Grant 2020-2022, No. 240919FD3912.

**Conflict-of-interest statement:** The authors have no conflicts of interest to declare.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited

**Dimitri Poddighe, Diyora Abdukhakimova,** Department of Medicine, School of Medicine, Nazarbayev University, Nur-Sultan 010000, Kazakhstan

**Dimitri Poddighe,** Clinical Academic Department of Pediatrics, Research Center for Maternal and Child Health, University Medical Center, Nur-Sultan 010000, Kazakhstan

**Corresponding author:** Dimitri Poddighe, MD, MSc, PhD-Eq, Associate Professor, Director, Doctor, Department of Medicine, School of Medicine, Nazarbayev University, Kerei-Zhanibek Str. 5/1, Nur-Sultan 010000, Kazakhstan. dimitri.poddighe@nu.edu.kz

## Abstract

Celiac Disease (CD) had been considered uncommon in Asia for a long time. However, several studies suggested that, in the Indian subcontinent and Middle East countries, CD is present and as prevalent as in Western countries. Outside these Asian regions, the information about the epidemiology of CD is still lacking or largely incomplete for different and variable reasons. Here, we discuss the epidemiological aspects and the diagnostic barriers in several Asian regions including China, Japan, Southeast Asia and Russia/Central Asia. In some of those regions, especially Russia and Central Asia, the prevalence of CD is very likely to be underestimated. Several factors may, to a different extent, contribute to CD underdiagnosis (and, thus, underestimation of its epidemiological burden), including the poor disease awareness among physicians and/or patients, limited access to diagnostic resources, inappropriate use or interpretation of the serological tests, absence of standardized diagnostic and endoscopic protocols, and insufficient expertise in histopathological interpretation.

**Key Words:** Celiac disease; Epidemiology; Prevalence; Asia; China; Japan; Russia; Central Asia; HLA-DQB1; Diagnostic barriers

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** This editorial discusses the main epidemiological characteristics of Celiac Disease in Asia outside the Indian subcontinent and Middle East countries. Indeed, information about the epidemiology of Celiac Disease is still lacking or largely incomplete in those Asian regions (China, Japan, South-East Asia, and Russia/Centra

manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Kazakhstan

**Peer-review report's scientific quality classification**

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

**Received:** January 17, 2021
**Peer-review started:** January 17, 2021
**First decision:** February 23, 2021
**Revised:** February 25, 2021
**Accepted:** April 13, 2021
**Article in press:** April 13, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Mijandrusic-Sincic B, Yilmaz Y
**S-Editor:** Gao CC
**L-Editor:** Filipodia
**P-Editor:** Liu JH

Asia), where the disease prevalence is likely be underestimated. Factors contributing to diagnostic difficulties in these Asian regions are discussed.

**Citation:** Poddighe D, Abdukhakimova D. Celiac Disease in Asia beyond the Middle East and Indian subcontinent: Epidemiological burden and diagnostic barriers. *World J Gastroenterol* 2021; 27(19): 2251-2256
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2251.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2251

## INTRODUCTION

The global prevalence of Celiac Disease (CD) is estimated to be around 1% in the general population. However, this estimation is based on epidemiological studies that mainly come from the European and South/North American continents[1,2]. CD had been considered to be uncommon in Asia for a long time, but several studies published in the previous two decades demonstrated that CD is present and is as prevalent in the Indian subcontinent and in the Middle East, as it is in Western countries[3-5].

A few years ago, a meta-analysis by Singh *et al*[6] reported that the pooled prevalence of CD in Asia was around 0.5% without any significant difference between children and adults. Except for one study from Malaysia, all the clinical studies were from the Middle East (Turkey, $n$ = 5; Iran, $n$ = 4; Israel, $n$ = 2; Saudi Arabia, $n$ = 2; Jordan, $n$ = 1) and India ($n$ = 3). A recent study estimated that the prevalence of CD might be as high as 1% even in those Asian countries, based on an assessment of the HLA-DQB1*02 allelic frequencies and wheat consumption in the general population[7]. Indeed, the HLA-DQB1*02 allelic variant is the major HLA-related CD predisposing allele, and the appropriate HLA genetic background is as necessary (but not sufficient) as the dietary gluten intake to trigger of the immunopathological process underlying CD[8,9]. In India, this estimate was confirmed by a community-based study by Makharia *et al*[10], who found a CD prevalence of 1.04% in the north of India, where both the dietary habits and *HLA-DQ* genetic background favor the development of CD. A study from Iran including 1,500 healthy school children serologically screened for CD, led to a biopsy-confirmed diagnosis of CD in 0.6% of this pediatric cohort. As most of the cases appeared to be silent, it is reasonable to expect a higher prevalence in the general population[11]. The situation may be the same in Turkey, where Tatar *et al*[12] screened 2,000 healthy blood donors for CD markers and found that 1.3% ($n$ = 26) were positive, and 12 donors (0.6%) were diagnosed with CD after biopsy. Considering that most of the serologically positive donors ($n$ = 14) could not be contacted or refused endoscopy and all the diagnosed cases had been silent, the overall CD prevalence is probably greater.

For various reasons, epidemiological information about CD in other Asian regions is incomplete. The available data from China, Japan, Southeast Asia, and Russia/Central Asia are discussed below.

## CHINA

In the last decade, more studies investigating the epidemiology of CD have been published from China than from the other Asian regions discussed here. In 2011, Wang *et al*[13] published a national multicenter study describing 14 Chinese children histologically diagnosed with CD after serological screening because of chronic diarrhea. Yuan *et al*[14] reported that the presence of DQB1*0201 allele was not negligible in the Chinese population, with an overall frequency of 10.5%. The highest frequency of HLA-DQB1*02 was > 20%-25% in the northwestern region, where non-Chinese ethnic minorities (*e.g.*, Kazakh and Uygur ethnicities) are present in the population. Recently, the same authors reported a CD seropositivity rate of 2.19% in a cross-sectional study including 19,778 Chinese adolescents and young adults[15].

Dietary exposure to gluten has increased over the past 50 years in the Chinese population. Wheat has become the second most consumed staple food, after rice. China is one of the world's largest producers and consumers of wheat, especially in

the northern regions. Therefore, CD is an emerging disease in this country; and, despite the lack of epidemiological studies with a complete diagnostic definition, the awareness of this disease and its potential impact on health should be increased in such a large population[14]. In 2019, Chen and Li[16] reported that lack of awareness among the population and health professionals have contributed to the hidden epidemiological burden of CD in China. They also reported that local lack of resources could limit the access to standardized tissue acquisition and histological evaluation. Consequently, in most studies, seropositive individuals could not undergo the duodenal biopsy needed for the confirmation of a CD diagnosis.

## JAPAN

In Japan, the epidemiological burden of CD is extremely low. In 2018, Fukunaga *et al*[17] described only two biopsy-based CD diagnoses in a group of 2,055 people including 2,008 asymptomatic individuals and 47 adults complaining of chronic abdominal symptoms, which corresponds to < 0.1% prevalence. That finding is consistent with the very low frequency of the HLA-DQ2/DQ8 immunotype. Indeed, in a study including 371 unrelated healthy apheresis blood donors, the HLA-DQB1*02 allele frequency was reported to be < 1% even though the DQB1*03:02 allele is relatively common (10.8%)[18]. Although the dietary exposure to gluten has been increasing in Japan, the consumption of wheat is still relatively low (it is estimated to be approximately one-third of that consumed in Western countries)[17].

The same research group reported a CD seropositivity rate (based on anti-tissue transglutaminase (tTG) immunoglobulin (Ig) A antibody) of 0.19% in 2,005 Japanese adults tested in 2008-2013. That result is consistent with another study that reported a positivity rate of 0.2% (based on a 10 U/mL titer cutoff) in 2014-2016[17,19]. Because of the presumed low prevalence, in Japanese clinical practice, CD may be rarely considered by physicians who manage patients with chronic abdominal symptoms[17].

It must be emphasized that the available studies were mostly, if not completely, focused on asymptomatic/healthy people. The actual prevalence of CD seropositivity and diagnosis may thus be higher than that reported so far. Nevertheless, Hokari and Higashiyama[20] observed that Japanese physicians are unlikely to overlook CD, as the endoscopic assessment is well-established in Japan for patients complaining of chronic abdominal symptoms. This procedure is performed by well-trained endoscopists and the histopathological appearance of CD mucosa is well-known. Therefore, the low epidemiological burden of CD in Japan is considered to be real: it is consistent with the immunogenetic background and dietary habits, and there are no major diagnostic barriers. However, this situation may change in the near future if wheat consumption increases.

## SOUTHEAST ASIA

Few clinical studies assessed the epidemiological burden of CD in this Asian region[21]. In most countries of Southeast Asia, the allele frequency of HLA-DQB1*02 is estimated to be < 10%-15%[6]. The low dietary intake of gluten also contributes to the low prevalence of CD. In Vietnam, CD autoimmunity was assessed in 1,961 children and around 1% were found positive for anti-tTG IgA[22]. In Thailand, CD serology was assessed in 46 children with type 1 diabetes mellitus and only one child was positive for anti-tTG IgA. Considering an expected prevalence of > 5% (and up to 10%) in such a risk group, the prevalence of CD is assumed to be low in Thai children and in the general population[23,24].

In Malaysia, Yap *et al*[25] reported a relatively high CD seroprevalence of 1.25% in healthy young adults. This finding might be explained by the fact that the Malaysian population includes three main ethnicities (*i.e.* Malay, Chinese, and Indian), but HLA genotyping was not performed in that study. These authors noted that CD is underdiagnosed in Malaysia and discussed the diagnostic barriers in this country. Lack of medical awareness of CD (resulting in a low rate of request for CD serology tests), the use of less sensitive CD serological markers, inappropriate application of endoscopic protocols, and under-recognition of mild CD histopathological patterns, were all considered to be contributing factors.

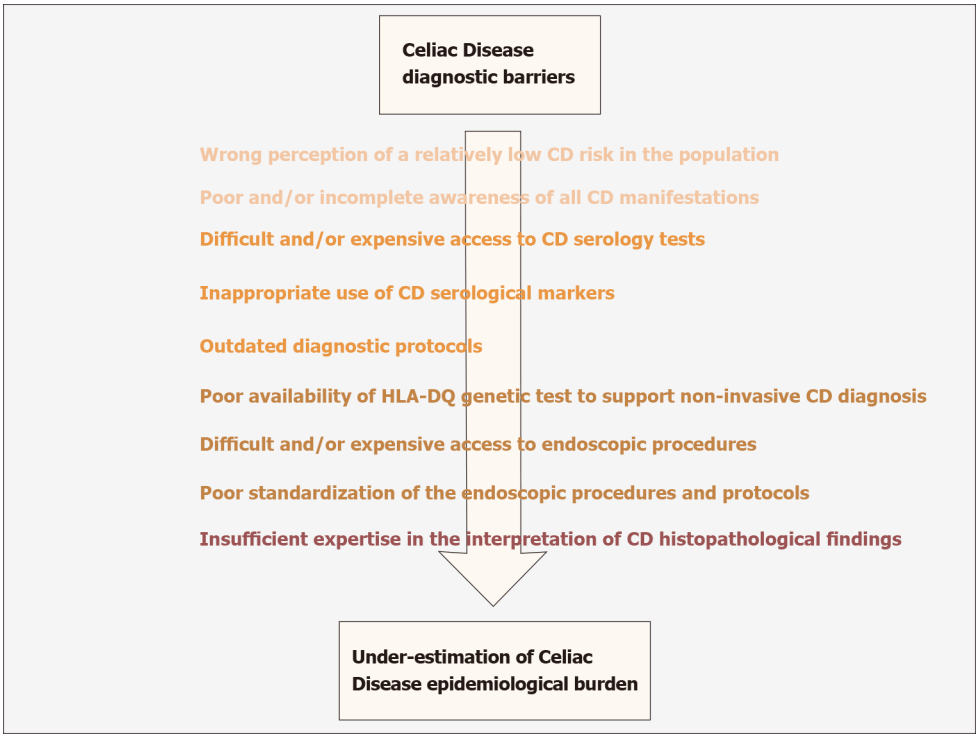



**Figure 1 Schematic overview of CD diagnostic barriers in Kazakhstan** (CD: Celiac Disease).

## RUSSIA AND CENTRAL ASIA

A review by Savvateeva *et al*[26] is the primary English-language publication of the overall and indirect epidemiological evidence of CD in Russia. Most studies considered in this review were conducted from 2000 to 2014 and were published in Russian language. The authors concluded that the prevalence of CD in children has increased in the last few decades and is at least 0.6%, although significant inter-regional variations should be considered, because of the geographical extent of this region. The carrier frequency of HLA-DQ2/DQ8 haplotypes in the Russian population, especially in the western region, seems to be comparable to that in Europe. Savvateeva *et al*[26] reported epidemiological trends and prevalence rates similar to those in Europe, but do not discuss potential barriers hampering CD diagnosis in their country. However, they do declare well-established therapeutical support and follow-up protocols for CD patients in Russia.

Epidemiological data from Central Asia and, more precisely, from Kazakhstan are also mentioned in the aforementioned article by Savvateeva *et al*[26]. They reported a CD prevalence of < 0.4% or one case in every 262 children. However, the diagnostic approach based on anti-gliadin antibodies and the study design were prone to a significant underestimation of the actual prevalence[21,26]. Moreover, our group recently showed that the carrier frequencies of HLA-DQB1*02 and HLA-DQB1*03:02 in Kazakhstani healthy blood donors are 38% and 12.5%, respectively, and these numbers are comparable to those described in Caucasian populations. Considering the high consumption of wheat foods in Kazakhstan, we concluded that it is reasonable to expect that the CD prevalence in this country may be comparable to that reported in Europe[27].

Large well-designed clinical studies are needed to provide a reliable estimate of the CD epidemiological burden in Central Asia. It is likely that CD is underdiagnosed in Kazakhstan. Several barriers currently contribute to the underdiagnosis of CD in this country, including the inappropriate use of serological tests, limited access to diagnostic tools for economic reasons, the absence of standard protocols for endoscopic procedures, and difficulties in histopathological interpretation[27,28]. The combined effects of all those obstacles lead to the underestimation of the diagnostic and epidemiological burden in this country, as summarized in Figure 1. These considerations might also apply in other regions of Central Asia that have even fewer economic resources.

## CONCLUSION

Outside the Indian subcontinent and Middle East countries, the epidemiological burden of CD in Asia is very likely to be underestimated, especially in Russia and Central Asia, where wheat is a staple food and the genetic predisposition to CD is comparable to Europe.

In agreement with other authors[29,30], several factors (that vary by country and regions) can contribute to hamper CD diagnosis and, thus, the estimation of its epidemiological burden. Overall, these factors include poor disease awareness among physicians and/or patients, limited access to diagnostic resources (because of economic and/or organizational and/or geographical reasons), inappropriate use or interpretation of the available serological tests, absence of standardized diagnostic and endoscopic protocols, and insufficient expertise in histopathological interpretation.

## REFERENCES


1 **Lindfors K**, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, Murray JA, Verdu EF, Kaukinen K. Coeliac disease. *Nat Rev Dis Primers* 2019; **5**: 3 [PMID: 30631077 DOI: 10.1038/s41572-018-0054-z]

2 **Lebwohl B**, Sanders DS, Green PHR. Coeliac disease. *Lancet* 2018; **391**: 70-81 [PMID: 28760445 DOI: 10.1016/S0140-6736(17)31796-8]

3 **Ashtari S**, Pourhoseingholi MA, Rostami K, Aghdaei HA, Rostami-Nejad M, Busani L, Tavirani MR, Zali MR. Prevalence of gluten-related disorders in Asia-Pacific region: a systematic review. *J Gastrointestin Liver Dis* 2019; **28**: 95-105 [PMID: 30851178 DOI: 10.15403/jgld.2014.1121.281.sys]

4 **Cummins AG**, Roberts-Thomson IC. Prevalence of celiac disease in the Asia-Pacific region. *J Gastroenterol Hepatol* 2009; **24**: 1347-1351 [PMID: 19702902 DOI: 10.1111/j.1440-1746.2009.05932.x]

5 **Comba A**, Eren NB, Demir E. Prevalence of celiac disease among school-age children in Çorum, Turkey. *Turk J Gastroenterol* 2018; **29**: 595-600 [PMID: 30260783 DOI: 10.5152/tjg.2018.18020]

6 **Singh P**, Arora S, Singh A, Strand TA, Makharia GK. Prevalence of celiac disease in Asia: A systematic review and meta-analysis. *J Gastroenterol Hepatol* 2016; **31**: 1095-1101 [PMID: 26678020 DOI: 10.1111/jgh.13270]

7 **Makharia GK**, Catassi C. Celiac Disease in Asia. *Gastroenterol Clin North Am* 2019; **48**: 101-113 [PMID: 30711203 DOI: 10.1016/j.gtc.2018.09.007]

8 **Poddighe D**, Rebuffi C, De Silvestri A, Capittini C. Carrier frequency of HLA-DQB1*02 allele in patients affected with celiac disease: A systematic review assessing the potential rationale of a targeted allelic genotyping as a first-line screening. *World J Gastroenterol* 2020; **26**: 1365-1381 [PMID: 32256023 DOI: 10.3748/wjg.v26.i12.1365]

9 **Megiorni F**, Mora B, Bonamico M, Barbato M, Nenna R, Maiella G, Lulli P, Mazzilli MC. HLA-DQ and risk gradient for celiac disease. *Hum Immunol* 2009; **70**: 55-59 [PMID: 19027045 DOI: 10.1016/j.humimm.2008.10.018]

10 **Makharia GK**, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, Bhatia V, Ahuja V, Datta Gupta S, Anand K. Prevalence of celiac disease in the northern part of India: a community based study. *J Gastroenterol Hepatol* 2011; **26**: 894-900 [PMID: 21182543 DOI: 10.1111/j.1440-1746.2010.06606.x]

11 **Dehghani SM**, Haghighat M, Mobayen A, Rezaianzadeh A, Geramizadeh B. Prevalence of celiac disease in healthy Iranian school children. *Ann Saudi Med* 2013; **33**: 159-161 [PMID: 23563005 DOI: 10.5144/0256-4947.2013.159]

12 **Tatar G**, Elsurer R, Simsek H, Balaban YH, Hascelik G, Ozcebe OI, Buyukasik Y, Sokmensuer C. Screening of tissue transglutaminase antibody in healthy blood donors for celiac disease screening in the Turkish population. *Dig Dis Sci* 2004; **49**: 1479-1484 [PMID: 15481323 DOI: 10.1023/b:ddas.0000042250.59327.91]

13 **Wang XQ**, Liu W, Xu CD, Mei H, Gao Y, Peng HM, Yuan L, Xu JJ. Celiac disease in children with diarrhea in 4 cities in China. *J Pediatr Gastroenterol Nutr* 2011; **53**: 368-370 [PMID: 21701402 DOI: 10.1097/MPG.0b013e31822a0128]

14 **Yuan J**, Gao J, Li X, Liu F, Wijmenga C, Chen H, Gilissen LJ. The tip of the "celiac iceberg" in China: a systematic review and meta-analysis. *PLoS One* 2013; **8**: e81151 [PMID: 24324669 DOI: 10.1371/journal.pone.0081151]

15 **Yuan J**, Zhou C, Gao J, Li J, Yu F, Lu J, Li X, Wang X, Tong P, Wu Z, Yang A, Yao Y, Nadif S, Shu H, Jiang X, Wu Y, Gilissen L, Chen H. Prevalence of Celiac Disease Autoimmunity Among Adolescents and Young Adults in China. *Clin Gastroenterol Hepatol* 2017; **15**: 1572-1579. e1 [PMID: 28433781 DOI: 10.1016/j.cgh.2017.04.025]

16 **Chen CY**, Li JN. Insufficient awareness of celiac disease in China: population-based screening is needed. *Chin Med J (Engl)* 2019; **132**: 1513-1515 [PMID: 31188159 DOI: 10.1097/CM9.0000000000000305]

17 **Fukunaga M**, Ishimura N, Fukuyama C, Izumi D, Ishikawa N, Araki A, Oka A, Mishiro T, Ishihara S, Maruyama R, Adachi K, Kinoshita Y. Celiac disease in non-clinical populations of Japan. *J*

*Gastroenterol* 2018; **53**: 208-214 [PMID: 28389733 DOI: 10.1007/s00535-017-1339-9]
18 **Saito S**, Ota S, Yamada E, Inoko H, Ota M. Allele frequencies and haplotypic associations defined by allelic DNA typing at HLA class I and class II loci in the Japanese population. *Tissue Antigens* 2000; **56**: 522-529 [PMID: 11169242 DOI: 10.1034/j.1399-0039.2000.560606.x]
19 **Fukunaga M**, Ishimura N, Abe T, Takeda M, Isomura M, Kinoshita Y, Ishihara S. Serological screening for celiac disease in adults in Japan: Shimane CoHRE study. *JGH Open* 2020; **4**: 558-560 [PMID: 32782937 DOI: 10.1002/jgh3.12334]
20 **Hokari R**, Higashiyama M. Extremely low prevalence of Celiac disease in Japan: Eternal silence or just the calm before the storm? *JGH Open* 2020; **4**: 554-555 [PMID: 32782935 DOI: 10.1002/jgh3.12352]
21 **Poddighe D**, Rakhimzhanova M, Marchenko Y, Catassi C. Pediatric Celiac Disease in Central and East Asia: Current Knowledge and Prevalence. *Medicina (Kaunas)* 2019; **55** [PMID: 30642036 DOI: 10.3390/medicina55010011]
22 **Zanella S**, De Leo L, Nguyen-Ngoc-Quynh L, Nguyen-Duy B, Not T, Tran-Thi-Chi M, Phung-Duc S, Le-Thanh H, Malaventura C, Vatta S, Ziberna F, Mazzocco M, Volpato S, Phung-Tuyet L, Le-Thi-Minh H, Borgna-Pignatti C. Cross-sectional study of coeliac autoimmunity in a population of Vietnamese children. *BMJ Open* 2016; **6**: e011173 [PMID: 27329441 DOI: 10.1136/bmjopen-2016-011173]
23 **Thammarakcharoen T**, Hirankarn N, Sahakitrungruang T, Thongmee T, Kuptawintu P, Kanoonthong S, Chongsrisawat V. Frequency of HLA-DQB1*0201/02 and DQB1*0302 alleles and tissue transglutaminase antibody seropositivity in children with type 1 diabetes mellitus. *Asian Pac J Allergy Immunol* 2017; **35**: 82-85 [PMID: 27543737 DOI: 10.12932/AP0751]
24 **Pham-Short A**, Donaghue KC, Ambler G, Phelan H, Twigg S, Craig ME. Screening for Celiac Disease in Type 1 Diabetes: A Systematic Review. *Pediatrics* 2015; **136**: e170-e176 [PMID: 26077482 DOI: 10.1542/peds.2014-2883]
25 **Yap TW**, Chan WK, Leow AH, Azmi AN, Loke MF, Vadivelu J, Goh KL. Prevalence of serum celiac antibodies in a multiracial Asian population--a first study in the young Asian adult population of Malaysia. *PLoS One* 2015; **10**: e0121908 [PMID: 25799401 DOI: 10.1371/journal.pone.0121908]
26 **Savvateeva LV**, Erdes SI, Antishin AS, Zamyatnin AA Jr. Overview of Celiac Disease in Russia: Regional Data and Estimated Prevalence. *J Immunol Res* 2017; **2017**: 2314813 [PMID: 28316996 DOI: 10.1155/2017/2314813]
27 **Poddighe D**, Turganbekova A, Baymukasheva D, Saduakas Z, Zhanzakova Z, Abdrakhmanova S. Genetic predisposition to celiac disease in Kazakhstan: Potential impact on the clinical practice in Central Asia. *PLoS One* 2020; **15**: e0226546 [PMID: 31895924 DOI: 10.1371/journal.pone.0226546]
28 **Penny HA**, Raju SA, Lau MS, Marks LJ, Baggus EM, Bai JC, Bassotti G, Bontkes HJ, Carroccio A, Danciu M, Derakhshan MH, Ensari A, Ganji A, Green PHR, Johnson MW, Ishaq S, Lebwohl B, Levene A, Maxim R, Mohaghegh Shalmani H, Rostami-Nejad M, Rowlands D, Spiridon IA, Srivastava A, Volta U, Villanacci V, Wild G, Cross SS, Rostami K, Sanders DS. Accuracy of a no-biopsy approach for the diagnosis of coeliac disease across different adult cohorts. *Gut* 2020 [PMID: 33139268 DOI: 10.1136/gutjnl-2020-320913]
29 **Agarwal A**, Chauhan A, Ahuja V, Makharia GK. Opportunities and challenges in the management of celiac disease in Asia. *JGH Open* 2020; **4**: 795-799 [PMID: 33102747 DOI: 10.1002/jgh3.12381]
30 **Dhawan A**, Agarwal A, Mulder CJ, Makharia GK. Celiac disease in the East and the West: Bridging the gaps between the guidelines and their implementation in daily practice is mandatory. *Indian J Gastroenterol* 2019; **38**: 185-189 [PMID: 31313236 DOI: 10.1007/s12664-019-00970-7]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2257-2269

DOI: 10.3748/wjg.v27.i19.2257

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

FRONTIER

# Biomarkers in autoimmune pancreatitis and immunoglobulin G4-related disease

Akane Hara, Tomohiro Watanabe, Kosuke Minaga, Tomoe Yoshikawa, Ken Kamata, Masatoshi Kudo

**ORCID number:** Akane Hara 0000-0002-5251-2011; Tomohiro Watanabe 0000-0001-7781-6305; Kosuke Minaga 0000-0001-5407-7925; Tomoe Yoshikawa 0000-0001-5275-2667; Ken Kamata 0000-0003-1568-0769; Masatoshi Kudo 0000-0002-4102-3474.

**Author contributions:** Hara A, Watanabe T, and Minaga K drafted the manuscript; Yoshikawa T, Kamata K, and Kudo M edited and revised the manuscript; Hara A, Watanabe T, Minaga K, Yoshikawa T, Kamata K and Kudo M have read and approved the final manuscript; all authors therefore meet the criteria for authorship established by the International Committee of Medical Journal Editors.

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest to disclose.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the

**Akane Hara, Tomohiro Watanabe, Kosuke Minaga, Tomoe Yoshikawa, Ken Kamata, Masatoshi Kudo,** Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, Osaka-Sayama 589-8511, Japan

**Corresponding author:** Tomohiro Watanabe, MD, PhD, Associate Professor, Department of Gastroenterology and Hepatology, Kindai University Faculty of Medicine, 377-2 Ohno-Higashi, Osaka-Sayama 589-8511, Japan. tomohiro@med.kindai.ac.jp

## Abstract

Solitary organ autoimmune disorders, formerly known as autoimmune pancreatitis (AIP), autoimmune sialadenitis, and autoimmune sclerosing cholangitis, are now considered organ-specific manifestations of systemic immunoglobulin G4-related disease (IgG4-RD). AIP and IgG4-RD are characterized by elevated serum concentration of IgG4 antibody (Ab), accumulation of IgG4-expressing plasmacytes in the affected organs, and involvement of multiple organs. It is well established that enhanced IgG4 Ab responses are a hallmark of AIP and IgG4-RD for diagnosis and monitoring disease activity. However, a significant fraction of patients with AIP and IgG4-RD who develop chronic fibroinflammatory responses have normal serum concentrations of this IgG subtype. In addition, disease flare-up is sometimes seen even in the presence of normalized serum concentrations of IgG4 Ab after successful induction of remission by prednisolone. Therefore, it is necessary to identify new biomarkers based on the understanding of the pathophysiology of AIP and IgG4-RD. Recently, we found that activation of plasmacytoid dendritic cells producing both interferon-α (IFN-α) and interleukin-33 (IL-33) mediate murine AIP and human IgG4-RD. More importantly, we provided evidence that serum concentrations of IFN-α and IL-33 could be useful biomarkers for the diagnosis and monitoring of AIP and IgG4-RD activity after induction of remission in these autoimmune disorders. In this Frontier article, we have summarized and discussed biomarkers of AIP and IgG4-RD, including Igs, autoAbs, and cytokines to provide useful information not only for clinicians but also for researchers.

**Key Words:** Biomarker; Autoimmune pancreatitis; Immunoglobulin G4-related disease; Plasmacytoid dendritic cells; Cytokine; Chemokine

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Japan

**Peer-review report's scientific quality classification**
Grade A (Excellent): 0
Grade B (Very good): B, B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

**Received:** February 11, 2021
**Peer-review started:** February 11, 2021
**First decision:** March 14, 2021
**Revised:** March 19, 2021
**Accepted:** April 26, 2021
**Article in press:** April 26, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Strainiene S, Tabibian JH
**S-Editor:** Liu M
**L-Editor:** A
**P-Editor:** Ma YJ

**Core Tip:** Autoimmune pancreatitis (AIP) and immunoglobulin G4-related disease (IgG4-RD) are new disease entities characterized by enhanced IgG4 antibody responses. Serum concentration of IgG4 antibody is widely used as a useful biomarker for diagnosis and disease activity monitoring in AIP and IgG4-RD. Recent studies have highlighted the importance of cytokine responses in the immunopathogenesis of these disorders. In this Frontier article, we have summarized our knowledge regarding cytokine responses in AIP and IgG4-RD and then discussed the utility of serum concentrations of cytokines as possible biomarkers.

**Citation:** Hara A, Watanabe T, Minaga K, Yoshikawa T, Kamata K, Kudo M. Biomarkers in autoimmune pancreatitis and immunoglobulin G4-related disease. *World J Gastroenterol* 2021; 27(19): 2257-2269
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2257.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2257

## INTRODUCTION

Autoimmune pancreatitis (AIP) is a unique form of the chronic fibroinflammatory disorder of the pancreas, which is driven by autoimmune responses[1]. AIP is classified into type 1 and type 2, and more than 95% of AIP cases represent the former, which is a pancreatic manifestation of systemic immunoglobulin G4-related disease (IgG4-RD)[2-4]. In this article, type 1 AIP is hereafter referred to as AIP. AIP and IgG4-RD are recently established disease entities proposed by rheumatologists and gastroenterologists[2-4]. As awareness and recognition of these disorders by physicians increase, the number of patients diagnosed with AIP and IgG4-RD is growing. Thus, the clinical manifestations and immunopathogenesis of AIP and IgG4-RD are attracting much attention from physicians and researchers.

IgG4-RD occurs most commonly in elderly men; it is characterized by a marked elevation of serum IgG4 antibody (Ab) and accumulation of plasma cells secreting IgG4 Ab into injured organs[2-4]. Another important feature of IgG4-RD is multiple organ involvement: this disorder preferentially affects the pancreas, bile duct, lung, salivary glands, and kidney. AIP is a pancreatic manifestation of IgG4-RD. The elevated concentration of serum IgG4 Ab is widely used as a diagnostic marker for AIP and IgG4-RD[5,6]. In addition, patients with IgG4-RD exhibiting multiple organ involvement display higher concentrations of serum IgG4 Ab[7,8], suggesting that measurement of serum IgG4 concentration is useful not only for the diagnosis but also for the evaluation of disease activity. It should be noted, however, that the concentration of this IgG subtype is not always regarded as a perfect biomarker for the diagnosis or evaluation of disease activity in AIP and IgG4-RD. In fact, serum concentration of IgG4 Ab is elevated in a significant fraction of patients with pancreatic cancer[9] and about 20% of patients with AIP display normal serum concentration of IgG4 Ab[10]. Furthermore, patients with AIP sometimes relapse even if they have normal serum concentration of IgG4 Ab[11]. Therefore, it is necessary to identify other biomarkers that could be useful for the diagnosis and evaluation of disease activity in AIP and IgG4-RD.

Remarkable progress has been made in understanding the immunopathogenesis of AIP and IgG4-RD. Elucidation of immune networks associated with the development of these autoimmune disorders has led us to identify candidate biomarkers other than IgG4 Ab. In this Frontier article, we summarize recent progress in the biomarkers of AIP and IgG4-RD based on the knowledge of abnormal immune microenvironments.

## IMMUNOPATHOGENESIS OF AUTOIMMUNE PANCREATITIS AND IGG4-RD

### *Adaptive immunity*

AIP and IgG4-RD are characterized by enhanced IgG4 Ab responses; thus, immune microenvironments leading to IgG4 Ab production are likely to be involved in the development of these disorders[2-4]. Various types of differentiated T cell subpopu-

lations are involved in the enhanced IgG4 Ab response (Figure 1). These effector T cells include T helper type 2 (Th2) cells, regulatory T cells (Tregs), follicular helper T (Tfh) cells, and cytotoxic $CD4^+$ T cells ($CD4^+$ CTLs)[4]. Cytokines produced by effector T cell subpopulations promote IgG4 Ab production by B cells.

Interleukin-4 (IL-4), IL-10, and IL-13 secreted by Th2 cells and/or Tregs promoted IgG4 Ab production by healthy control B cells *in vitro*[12]. In fact, expression of IL-10, IL-13, and transforming growth factor-β1 (TGF-β1), also produced by Th2 and/or Tregs, was found to be higher in the livers of patients with IgG4-RD than in the livers of patients with other autoimmune biliary diseases[13]. Moreover, the cytokine responses seen in IgG4-RD were accompanied by the enhanced expression of forkhead box P3 (FOXP3), a critical transcription factor for Tregs[13]. Koyabu *et al*[14] reported that the number of Tregs correlated with that of $IgG4^+$ cells in the livers of IgG4-RD patients. In line with the enhanced expression of Th2 and Treg-associated cytokines in the liver, patients with IgG4-RD displayed higher expression of Th2 cytokines and chemokines such as IL-4, IL-5, IL-10, C-C motif chemokine ligand 17 (CCL17), and CCL22 in the salivary glands as compared with the levels of these molecules in healthy controls and individuals with the Sjogren syndrome[15]. Such enhanced Th2 responses in the salivary glands were accompanied by TGF-β1 production and accumulation of $FOXP3^+$ Tregs[15]. These data support the idea that Th2 cells and Tregs are involved in the development of AIP and IgG4-RD. Given that Tregs are potent negative regulators of autoimmune reactions, it might be possible that activation of Tregs is an epiphenomenon of persistent strong inflammation rather than a component of inflammation in AIP and IgG4-RD.

Ectopic germinal center formation is observed in the salivary glands of IgG4-RD patients[15]. Tfh cells, which express B cell lymphoma 6 (BCL6) and C-X-C chemokine receptor type 5 (CXCR5), and produce IL-21, play critical roles in germinal center reactions[4]. Expression levels of BCL6, CXCR5, and IL-21 in the ectopic germinal centers in the salivary glands were significantly higher in patients with IgG4-RD than in those with Sjogren syndrome[16]. Tfh cells isolated from the salivary glands and peripheral blood of patients with IgG4-RD had a greater capacity to stimulate IgG4 Ab production by B cells than tonsillar Tfh cells[17,18]. The percentage of circulating Tfh2 cells, defined as $CXCR5^+CXCR3^-$ C-C chemokine receptor type $6^-$ cells, positively correlated with serum IgG4 concentration in patients with IgG4-RD[19]. Thus, these data support the idea that Tfh cells are involved in the immunopathogenesis of IgG4-RD through the induction of germinal center reaction. However, the roles played by Tfh cells in AIP have not been clarified.

$CD4^+$ CTLs are a unique population of effector T cells that are often seen in patients with chronic viral infections. $CD4^+$ CTLs are localized in the salivary glands of IgG4-RD patients[20,21]. These cells express T-box-expressed-in-T-cells (T-bet) and produce interferon-g (IFN-γ)[4]. Although both Th1 cells and $CD4^+$ CTLs express T-bet and IFN-γ, they differ in expression levels of myeloid cell markers (CD11b) and in the ability to produce CCL4 and IL-1β[20,21]. In addition, these cells produce several cytotoxic proteins, including perforin and granzymes[20,21]. More importantly, these cells secrete TGF-β1, one of the prototypical pro-fibrogenic factors. Thus, $CD4^+$ CTLs are involved in chronic fibroinflammatory responses associated with IgG4-RD. However, accumulation of $CD4^+$ CTLs has not been verified in the pancreas of patients with AIP.

### *Innate immunity*

Innate immunity is one of the major host defense mechanisms against microbial infections[22,23]. Recognition of microbial components by Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-like receptors (NLRs) induces pro-inflammatory cytokine responses to eradicate microbial infections[22,23]. It is well established that excessive innate immune responses underlie various types of autoimmune disorders[4,24]. Recent studies have highlighted the importance of innate immunity in AIP and IgG4-RD (Figure 1)[4].

We were the first to address the role of innate immunity in the development of AIP and IgG4-RD. We initially examined whether peripheral blood mononuclear cells (PBMCs) isolated from IgG4-RD patients produced pro-inflammatory cytokines upon exposure to TLR ligands and found that they secreted more IgG4 Ab and Th2 cytokines than PBMCs from healthy controls[25]. In the subsequent studies, we utilized a co-culture system composed of peripheral blood $CD14^+$ monocytes, $CD19^+$ B cells, and $CD3^+$ T cells isolated from patients with AIP and IgG4-RD. This co-culture system allowed us to show that B cells produced a large amount of IgG4 Ab in the presence of NLR and TLR ligands upon co-culture with monocytes isolated from patients with AIP and IgG4-RD, but not with monocytes from healthy controls[26].

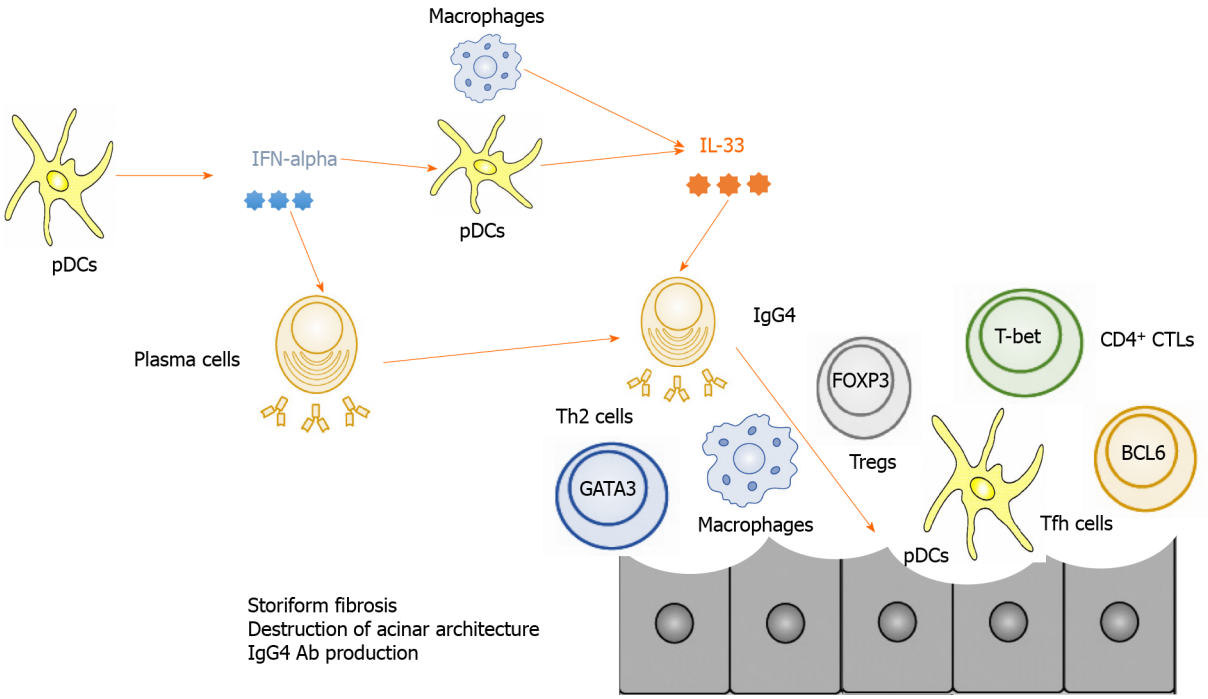



**Figure 1 Immunopathogenesis of autoimmune pancreatitis and immunoglobulin G4-related disease.** Plasmacytoid dendritic cells produce interferon-α and interleukin-33 and thereby mediate chronic fibroinflammatory responses in the pancreas. T helper type 2 cells expressing GATA-binding protein 3, regulatory T cells expressing forkhead box P3, follicular helper T cells expressing B cell lymphoma 6, and CD4$^+$ cytotoxic T cells expressing T-box-expressed-in-T-cells are involved in the development of autoimmune pancreatitis and immunoglobulin G4-related disease. IgG4: Immunoglobulin G4; pDCs: Plasmacytoid dendritic cells; IFN: Interferon; IL: Interleukin; Th2: T helper type 2 cells; Tregs: Regulatory T cells; GATA3: GATA-binding protein 3; FOXP3: Forkhead box P3; Tfh: Follicular helper T; BCL6: B cell lymphoma 6; T-bet: T-box-expressed-in-T-cells.

Interestingly, peripheral blood monocytes isolated from patients with AIP and IgG4-RD efficiently induced IgG4 Ab production by B cells from healthy controls in a T cell-independent manner. Stimulation of TLRs and NLRs led to the production of B cell-activating factor (BAFF), which induced IgG4 Ab responses[26]. In addition to monocytes, peripheral blood basophils isolated from patients with AIP and IgG4-RD also promoted IgG4 Ab production by B cells from healthy controls in a T cell-independent and BAFF-dependent manner[27]. These pioneering studies fully support the concept that excessive innate immune responses are involved in the development of AIP and IgG4-RD. Indeed, the expression of TLRs was verified in the salivary glands and pancreas of patients with AIP and IgG4-RD[28-30].

It remains uncertain whether innate immune responses are shared by peripheral blood and affected organs in AIP and IgG4-RD. To identify innate immune cells responsible for the development of AIP and IgG4-RD, we utilized a murine experimental model of AIP and IgG4-RD. Repeated intraperitoneal injections of polyinosinic-polycytidylic acid [poly (I:C)] into MRL/MpJ mice led to the development of AIP characterized by the destruction of the pancreatic acinar architecture, immune cell infiltration, and fibrosis[31,32]. Thus, this murine experimental AIP model recapitulated pathological findings observed in human AIP. Extensive flow cytometry analyses revealed massive accumulation of plasmacytoid dendritic cells (pDCs), defined as PDCA-1$^+$B220$^{low}$, in the pancreas[31]. pDCs are a specialized DC population that produce type I IFNs (IFN-α) upon recognition of TLR7 and TLR9 ligands[33]. Indeed, activation and accumulation of pDCs in the pancreas mediated experimental AIP through the production of IFN-α, because the depletion of pDCs or neutralization of type I IFN by Abs efficiently prevented the development of AIP[31]. Furthermore, pDCs expressing IFN-α and BAFF were found in the pancreas of patients with AIP and IgG4-RD, and peripheral blood pDCs isolated from these patients promoted IgG4 Ab production by healthy control B cells in a type I IFN dependent manner[31]. Thus, these results strongly suggest that activation of pDCs and type I IFN production are prominent features of murine experimental and human AIP.

Although a unique form of fibrosis, called storiform fibrosis, is one of the characteristic findings in human AIP[2-4], molecular mechanisms accounting for the induction and generation of this fibrogenic response have been poorly understood. We recently discovered that the type I IFN-IL-33 axis plays a pro-inflammatory and pro-

fibrogenic role in chronic alcoholic pancreatitis[34]. Type I IFN production by pancreatic acinar cells acts in concert with TNF-a produced by pancreatic macrophages to induce a robust production of IL-33 by the former cells[34]. Given that type I IFN produced by pDCs mediates experimental AIP, we hypothesized that IL-33 is involved in the generation of chronic fibroinflammatory responses in the pancreas. pDCs, which accumulate in the pancreas after repeated injections of poly (I:C), produced IL-33 in a type I IFN-dependent manner[32]. Importantly, the blockade of IL-33-mediated signaling pathways by an Ab against the IL-33 receptor attenuated chronic fibroinflammatory responses of the pancreas, which was accompanied by a marked reduction in pro-fibrogenic cytokines such as IL-13 and TGF-β1[32]. Immunofluorescence studies of pancreatic specimens from patients with AIP and IgG4-RD confirmed pancreatic localization of pDCs expressing IL-33[32]. Taken together, these results support the idea that activation of pDCs followed by the production of IFN-α and IL-33 mediates both experimental and human AIP. However, it should be noted that pDCs are not the only cellular source of IL-33. For example, M2 macrophages have been shown to co-localize with IL-33 in the salivary glands of IgG4-RD patients [30,35].

## IMMUNOGLOBULINS AS BIOMARKERS IN AUTOIMMUNE PANCREATITIS AND IGG4-RD

The diagnosis of AIP and IgG4-RD relies on the detection of elevated serum concentration of IgG4 Ab as well as on the characteristic pathological findings, including abundant infiltration of IgG4-expressing plasma cells, storiform fibrosis, and obliterative phlebitis[2-4]. Thus, serum level of IgG4 Ab is widely used as an established biomarker for the diagnosis of AIP and IgG4-RD. Moreover, serum concentration of IgG4 declines rapidly after the induction of remission by prednisolone[11]. Indeed, serum concentration of IgG4 Ab was much higher in patients with AIP and IgG4-RD than in individuals with chronic alcoholic pancreatitis and in healthy controls in our previous study[36]. Therefore, there is no doubt that measurement of serum IgG4 Ab concentration in clinical practice is necessary not only for the diagnosis of AIP and IgG4-RD but also for the assessment of disease activity. However, IgG4 Ab level is not always informative for the diagnosis or assessment of disease activity in such patients. Around 20% of patients with AIP have normal serum concentration of IgG4 Ab[10]. Furthermore, a significant fraction of patients with pancreatic cancer also exhibit elevated IgG4 Ab concentration[9]. Moreover, disease flare-up is sometimes seen in patients with AIP and IgG4-RD, even on the background of normalized serum concentrations of IgG4 Ab[11].

IgG4 Ab is unique in that it has a limited ability to activate Fcγ receptors and complements[37]. Thus, IgG4 Ab is considered to play non-pathogenic rather than pathogenic roles in the development of AIP and IgG4-RD. Shiokawa *et al*[38] directly addressed this issue by utilizing a passive transfer of patient IgG subtypes into neonatal mice. They found that pancreatic injury was successfully induced by a passive transfer of total IgG isolated from patients with AIP, but not by total IgG from healthy controls. The degree of pancreatic injury was much greater in neonatal mice treated with the IgG1 Ab from AIP patients than in those that received the IgG4 Ab from the same patients. Moreover, pancreatic injury induced by a passive transfer of IgG1 Ab was efficiently inhibited by a co-transfer of IgG4 Ab[38]. These studies conducted by Shiokawa *et al*[38] strongly suggest that IgG1 Ab rather than IgG4 Ab contributes to the immunopathogenesis of AIP and IgG4-RD. Consistent with this idea, serum concentrations of both IgG1 and IgG4 Abs were significantly higher in patients with AIP and IgG4-RD than in individuals with chronic alcoholic pancreatitis or in healthy controls (Table 1)[36].

As for the other IgG subtypes, no significant differences were observed in serum concentrations of IgG3 Ab in patients with AIP and IgG4-RD in comparison with those in patients with chronic alcoholic pancreatitis or in healthy controls[36]. Serum concentration of IgG2 Ab was significantly lower in patients with AIP and IgG4-RD than in patients with chronic alcoholic pancreatitis (Table 1)[36]. In contrast, another report showed that serum concentration of IgG2 Ab was elevated in patients suffering from IgG4-RD[39]. This discrepancy can be explained by the difference in the organ distribution of IgG4-RD. Serum concentration of this IgG subtype is preferentially elevated in patients with orbital IgG4-RD, but not in those with pancreatic IgG4-RD[36,39].

**Table 1 Possible biomarkers in autoimmune pancreatitis and immunoglobulin G4-related disease**

| Biomarkers | | Ref. |
|---|---|---|
| Immunoglobulins | IgG1 | Minaga *et al*[36] |
| | IgG2 | Chan *et al*[39] |
| | IgE | Minaga *et al*[36], Culver *et al*[41] |
| | IgM | Taguchi *et al*[40] |
| Cytokines | IFN-α | Arai *et al*[31], Minaga *et al*[36], Minaga *et al*[53] |
| | IL-5 | Yamamoto *et al*[49] |
| | IL-6 | Tsukuda *et al*[60] |
| | IL-33 | Furukawa *et al*[35], Minaga *et al*[36], Minaga *et al*[53] |
| | BAFF | Arai *et al*[31], Kiyama *et al*[58] |
| Chemokines | CCL17 | Umeda *et al*[63] |
| Autoantibodies | Laminin 511 | Shiokawa *et al*[42] |
| | Annexin A11 | Hubers *et al*[43] |
| | Galectin-3 | Perugino *et al*[44] |

BAFF: B cell activating factor; CCL17: C-C motif chemokine ligand 17.

Serum total IgG concentration is also elevated in patients with AIP and IgG4-RD[2-4]. Thus, elevations in serum concentrations of both total IgG and IgG4 are prominent features of patients with AIP and IgG4-RD. In contrast to augmented IgG and IgG4 levels, serum concentrations of IgM and IgA are decreased in patients with AIP and IgG4-RD[40]. Furthermore, serum concentration of IgM inversely correlated with those of IgG and IgG4 (Table 1)[40]. The diagnostic value of reduced serum concentrations of IgA and IgM needs to be determined in future studies.

Co-occurrence of AIP/IgG4-RD and allergic disorders is often observed[2-4]. In fact, serum concentration of IgE is significantly higher in patients with AIP and IgG4-RD than in those with chronic alcoholic pancreatitis and in healthy controls[36]. This raises the possibility that serum concentration of IgE can be used as a biomarker for AIP and IgG4-RD. In line with this idea, approximately 50% of patients with AIP and IgG4-RD exhibit elevated serum concentration of IgE[41]. Moreover, changes in serum concentration of IgE are associated with the relapse of these disorders[41]. Therefore, serum concentration of IgE can be used as a biomarker for the diagnosis and prediction of relapse in AIP and IgG4-RD[41].

## AUTOANTIBODIES AS BIOMARKERS IN AUTOIMMUNE PANCREATITIS AND IGG4-RD

Although AIP and IgG4-RD are considered to be caused by autoimmune reactions, autoAbs responsible for the development of autoimmunity have not been identified. Recently, three different types of autoAbs have been identified[42-44]. These autoAbs recognize laminin 511, annexin A11, and galectin-3 (Table 1). Fifty-one percent of AIP patients were positive for autoAb against laminin 511-E8, a truncated variant of the extracellular matrix protein laminin 511. Furthermore, serum IgG1 purified from AIP patients co-localized with laminin 511 in the pancreas of neonatal mice upon passive transfer[42]. Huber *et al*[43] identified annexin A11, a calcium-dependent phospholipid-binding protein, as a candidate autoantigen in AIP. Interestingly, annexin A11-specific IgG4 and IgG1 Abs purified from patients with AIP shared antigenic epitopes and IgG4 autoAbs inhibited pathogenic binding of IgG1 Ab to the shared epitopes[43]. These data suggest that IgG1 autoAbs rather than IgG4 autoAbs play pathogenic roles in the development of AIP and IgG4-RD. Confirmation of these results awaits future studies that should address the diagnostic utility of these autoAbs in a large number of patients with AIP and IgG4-RD. However, identification of autoAbs associated with AIP and IgG4-RD strongly supports the idea that these disorders arise from autoimmune reactions.

## ADAPTIVE IMMUNITY CYTOKINES AS BIOMARKERS IN AUTOIMMUNE PANCREATITIS AND IGG4-RD

Effector $CD4^+$ T cell subpopulations, including Th2 cells, Tregs, Tfh cells, and $CD4^+$ CTLs, are involved in the immunopathogenesis of AIP and IgG4-RD, as shown by the localization of these T cells in the affected organs. Th2 cells, Tregs, Tfh cells, and $CD4^+$ CTLs cells were detected in the peripheral blood of patients with AIP or IgG4-RD[17,19,20,45-48] and found to be markedly decreased after induction of remission[20,46], raising the possibility that serum concentrations of cytokines derived from effector T cells can be useful biomarkers (Table 1 and Figure 2). Yamamoto *et al*[49] found that serum concentration of IL-5 was elevated in patients with IgG4-RD, whereas serum concentrations of IL-10, IL-13, IL-21, and TGF-β1 were comparable in patients and healthy controls. Given that the major cellular source of IL-5 is Th2 cells, these data support the idea that the activation status of Th2 cells might be a surrogate marker for IgG4-RD and AIP. In line with this notion, bile concentrations of Th2 cytokines such as IL-4, IL-5, and IL-13 were significantly higher in the patients with IgG4-related sclerosing cholangitis than in those with primary sclerosing cholangitis[50]. Mechanistically, these Th2 cytokines induce bile leakage due to the impairment of the tight junction-associated biliary epithelial cell barrier, thereby causing chronic biliary inflammation[50]. Moreover, two cases of IgG4-RD successfully treated with dupilumab that neutralizes IL-4 receptor α have been reported[51,52]. Thus, Th2 responses may underlie the immunopathogenesis of AIP and IgG4-RD. Therefore, Th2 cytokines, especially IL-5, can be used as biomarkers of AIP and IgG4-RD.

A positive correlation between serum concentration of IgG4 Ab and circulating numbers of Tfh cells has been demonstrated in patients with IgG4-RD[17,19,46]. However, the utility of serum concentration of IL-21, a prototypical cytokine produced by Tfh cells, for IgG4-RD diagnosis has not been verified. Similarly, the usefulness of serum concentrations of IFN-γ and TGF-β1 produced by $CD4^+$ CTLs as biomarkers of AIP and IgG4-RD has not been reported either.

Therefore, at present, the utility of adaptive immunity cytokines as biomarkers for AIP and IgG4-RD is limited. The reason why previous studies did not successfully identify adaptive immunity cytokines as biomarkers might be partially explained by a broad range of affected organs in AIP and IgG4-RD or by complex effector T cell responses. Thus, the classification of IgG4-RD into subtypes by affected organ distribution might lead to the identification of biomarkers specific for each such subtype.

## INNATE IMMUNITY CYTOKINES AS BIOMARKERS IN AUTOIMMUNE PANCREATITIS AND IGG4-RD

Activation of pDCs and the subsequent robust production of IFN-α and IL-33 are characteristic pathogenic immune responses in experimental AIP and human IgG4-RD[31,32]. These findings led us to examine whether serum concentrations of IFN-α and IL-33 could be useful biomarkers for AIP and IgG4-RD (Table 1 and Figure 2). For this purpose, we measured serum concentrations of these cytokines in patients with AIP and IgG4-RD who met the well-established diagnostic criteria[5,6,36]. In comparison with the patients with chronic alcoholic pancreatitis and healthy controls, the patients with AIP and IgG4-RD displayed markedly elevated serum concentrations of IFN-α and IL-33[36]. In contrast, serum levels of the prototypical proinflammatory cytokines IL-1β and IL-6 were comparable in the patients with AIP/IgG4-RD and those with chronic alcoholic pancreatitis[36]. Serum concentrations of IFN-α and IL-33 positively correlated with those of IgG4 Ab[36]. Thus, measurements of serum concentrations of IFN-α and IL-33 may be very useful for the diagnosis of AIP and IgG4-RD[36].

We then evaluated the diagnostic performance of serum IFN-α and IL-33 concentrations for AIP and IgG4-RD. Surprisingly, the diagnostic performance of serum IFN-α and IL-33 concentrations as diagnostic markers for AIP and IgG4-RD was comparable to that of serum IgG4 Ab, as calculated by the receiver operating characteristic curve analysis[36]. Moreover, the induction of remission by prednisolone markedly reduced serum concentrations of IFN-α and IL-33. Thus, serum IFN-α and IL-33 concentrations can be biomarkers that are useful not only for the diagnosis but also for the assessment of disease activity. Taken together, our data strongly suggested that serum concentrations of IFN-α and IL-33 might serve as novel biomarkers in AIP

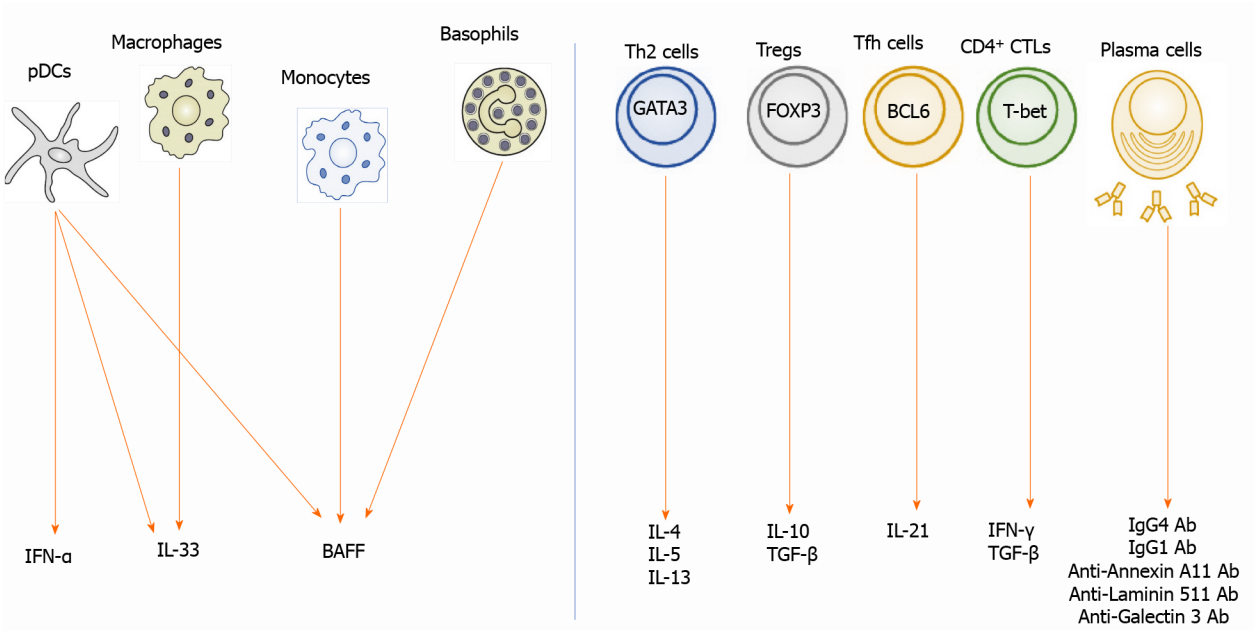



**Figure 2 Biomarkers in autoimmune pancreatitis and immunoglobulin G4-related disease.** Left panel: Interferon-a (IFN-α) is produced by plasmacytoid dendritic cells (pDCs). Interleukin-33 (IL-33) is produced by pDCs and macrophages. B cell-activating factor (BAFF) is produced by pDCs, monocytes, and basophils; Right panel: IL-4, IL-5, and IL-13 are produced by T helper type 2 cells expressing GATA-binding protein 3 (GATA3). IL-10 and transforming growth factor-β1 (TGF-β) are produced by regulatory T cells (Tregs) expressing forkhead box P3. IL-21 is produced by follicular helper T cells expressing B cell lymphoma 6. TGF-β is produced by Tregs and CD4$^+$ cytotoxic T cells (CTLs) expressing T-box-expressed-in-T-cells. IFN-γ is produced by CD4$^+$ CTLs. Plasma cells produce immunoglobulin G1 (IgG1) and IgG4 Ab. These cytokines are possible biomarkers for autoimmune pancreatitis and IgG4-related disease. IgG: Immunoglobulin G; pDCs: Plasmacytoid dendritic cells; IFN: Interferon; IL: Interleukin; BAFF: B cell-activating factor; Th2: T helper type 2 cells; Tregs: Regulatory T cells; GATA3: GATA-binding protein 3; FOXP3: Forkhead box P3; Tfh: Follicular helper T; BCL6: B cell lymphoma 6; T-bet: T-box-expressed-in-T-cells; CTLs: CD4$^+$ cytotoxic T cells.

and IgG4-RD. This idea is fully supported by an observation of an AIP/IgG4-RD case in which serum concentrations of IFN-α and IL-33 were markedly reduced soon after the induction of remission, whereas those of IgG4 remained unchanged even after the successful induction of remission[53]. Identification of the IFN-α-IL-33 axis as a crucial pathogenic pathway as well as a biomarker leads us to speculate that patients with AIP and IgG4-RD can be treated with biologics targeting IFN-α as in the case of systemic lupus erythematosus[54,55].

BAFF and a proliferation-inducing ligand (APRIL) are cytokines produced by antigen-presenting cells[56]. BAFF and APRIL are crucial factors for B cell survival and thus, they promote Ig production[56]. Given that AIP and IgG4-RD are characterized by elevated concentrations of serum total IgG and IgG4, in particular, it is likely that BAFF and APRIL are involved in the immunopathogenesis of these disorders. Indeed, pDCs producing BAFF have been demonstrated in the pancreas of patients with AIP and IgG4-RD[31]. Moreover, T cell-independent class switch recombination of IgG4 Ab requires BAFF production by monocytes[26]. The involvement of B cell survival factors in the development of AIP and IgG4-RD is further supported by the high probability of successful remission induction by rituximab in patients with IgG4-RD[57]. As for the utility of BAFF and APRIL as biomarkers for AIP and IgG4-RD, serum concentrations of BAFF and APRIL were significantly higher in patients with these disorders than in healthy controls[31,58]. In addition, induction of remission by prednisolone markedly reduced serum concentrations of BAFF[58]. Therefore, measurements of serum concentrations of BAFF and APRIL might be very useful not only for the diagnosis but also for monitoring disease activity.

IL-6 is a pleiotropic cytokine associated with autoimmune responses[59]. Although serum concentrations of this cytokine were comparable in patients with chronic pancreatitis, patients with AIP/IgG4-RD, and healthy controls in our study, elevated serum IL-6 level might help to discriminate a specific type of patients with AIP and IgG4-RD. Tsukuda *et al*[60] compared clinical manifestations of patients with AIP and IgG4-RD in relation to serum concentration of IL-6. They found that hepatosplenomegaly and biliary tract involvement tended to be more prevalent in patients with high IL-6 serum level than in those with low IL-6 concentration[60]. However, we need to be cautious regarding the interpretation of these data, because hepatosplenomegaly is often seen in patients with multicentric Castleman disease, an IL-6-driven systemic

autoimmune disorder[61]. Therefore, some patients with AIP and IgG4-RD might exhibit clinical manifestations similar to those of Castleman disease.

These previous studies on innate immunity cytokines have opened up new research vistas that can facilitate identification of novel biomarkers in AIP and IgG4-RD. In particular, serum concentrations of IFN-α and IL-33, which faithfully reflect disease activity, may be informative diagnostic examinations in AIP and IgG4-RD[36].

## CHEMOKINES AS BIOMARKERS IN AUTOIMMUNE PANCREATITIS AND IGG4-RD

AIP and IgG4-RD are characterized by Th2 responses[2-4]. The prototypical Th2 chemokine, thymus and activation-regulated chemokine, also known as CCL17, is a well-established biomarker for atopic dermatitis[62]. Given that atopic dermatitis and IgG4-RD share Th2 responses, it is likely that serum concentration of CCL17 could be a useful biomarker for IgG4-RD and AIP. Umeda *et al*[63] explored the utility of serum concentration of CCL17 as a possible biomarker for IgG4-RD (Table 1). They found that serum concentration of CCL17 was significantly higher in patients with IgG4-RD than in those with Sjogren syndrome or in healthy controls[63]. Although no association between serum concentrations of CCL17 and IgG4 Ab have been observed, those of CCL17 positively correlated with the number of affected organs. However, the utility of this chemokine as a biomarker for AIP has not been examined.

As mentioned above, production of IFN-α by pDCs is a prominent feature of AIP and IgG4-RD. Excessive IFN-α responses result in the robust production of chemokines such as C-X-C motif chemokine ligand 9 (CXCL9) and CXCL10[31]. In fact, the development of experimental AIP is accompanied by the enhanced expression of CXCL9 and CXCL10 in the pancreas[31]. The utility of CXCL9 and CXCL10 as biomarkers for AIP and IgG4-RD awaits future studies with the use of samples from patients with AIP and IgG4-RD.

## CONCLUSION

AIP and IgG4-RD are newly established disease entities[2-4]. Both disorders are characterized by elevated serum concentration of IgG4 Ab and accumulation of IgG4-expressing plasma cells in the affected organs[2-4]. Moreover, the induction of remission by prednisolone is accompanied by a marked decrease in serum concentration of IgG4 Ab in patients with AIP and IgG4-RD[2-4]. Therefore, serum concentration of this IgG subtype is undoubtedly a useful biomarker for both the diagnosis and assessment of disease activity. However, a significant fraction of patients with AIP display active disease even at normal serum IgG4 Ab concentration. Recent elegant studies have shown that IgG1 rather than IgG4 plays the main pathogenic role in the development of AIP and IgG4-RD[38]. Thus, elevated IgG4 Ab responses seen in AIP and IgG4-RD are an epiphenomenon associated with chronic inflammatory reactions. Therefore, novel biomarkers based on the understanding of immunopathogenesis need to be established. We have recently found that pDCs producing IFN-α and IL-33 mediate experimental AIP and human IgG4-RD[31,32]. Interestingly, serum concentrations of IFN-α and IL-33 have been identified as potent biomarkers for the diagnosis and assessment of disease activity in AIP and IgG4-RD[36,53]. Based on recently identified biomarkers of these disorders, we propose diagnostic algorithm for patients with AIP and IgG4-RD exhibiting normal or slightly elevated concentrations of serum IgG4 Ab (Figure 3). As shown in Figure 3, measurement of serum concentrations of cytokines and autoAbs in combination with serum IgG4 Ab might be useful for the diagnosis of AIP and IgG4-RD affluent in diversity.

As our knowledge of the immunopathogenesis of AIP and IgG4-RD increases, many candidate biomarkers will likely be identified in the future. The discovery of such biomarkers will contribute to the clinical practice and advance further our understanding of AIP and IgG4-RD immunopathogenesis.

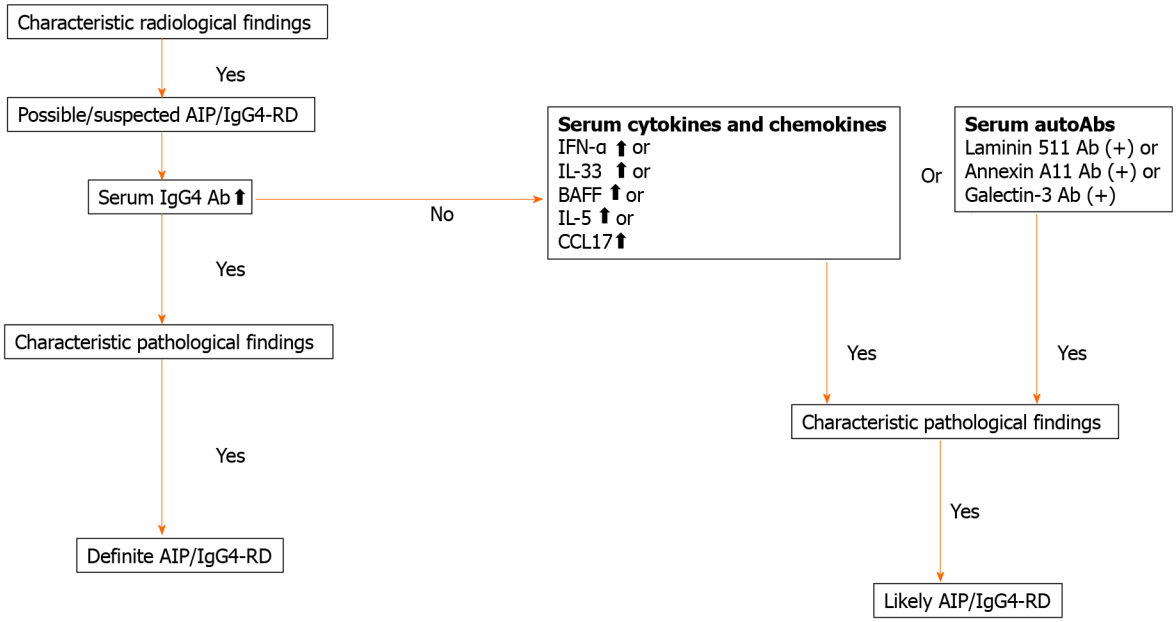



**Figure 3 Diagnostic algorithm for autoimmune pancreatitis and immunoglobulin G4-related disease.** Serum concentration of cytokines and chemokines or the presence of serum auto-antibodies may be useful for diagnosis of autoimmune pancreatitis and immunoglobulin G4 (IgG4)-related disease displaying normal or slightly elevated concentrations of IgG4. AIP: Autoimmune pancreatitis; IgG4-RD: Immunoglobulin G4-related disease; IFN: Interferon; IL: Interleukin; BAFF: B cell-activating factor; CCL17: C-C motif chemokine ligand 17.

## ACKNOWLEDGEMENTS

We would like to thank Ms. Yukiko Ueno for her secretarial assistance.

## REFERENCES

1 **Kamisawa T**, Chari ST, Lerch MM, Kim MH, Gress TM, Shimosegawa T. Recent advances in autoimmune pancreatitis: type 1 and type 2. *Gut* 2013; **62**: 1373-1380 [PMID: 23749606 DOI: 10.1136/gutjnl-2012-304224]
2 **Stone JH**, Zen Y, Deshpande V. IgG4-related disease. *N Engl J Med* 2012; **366**: 539-551 [PMID: 22316447 DOI: 10.1056/NEJMra1104650]
3 **Kamisawa T**, Zen Y, Pillai S, Stone JH. IgG4-related disease. *Lancet* 2015; **385**: 1460-1471 [PMID: 25481618 DOI: 10.1016/S0140-6736(14)60720-0]
4 **Watanabe T**, Minaga K, Kamata K, Kudo M, Strober W. Mechanistic Insights into Autoimmune Pancreatitis and IgG4-Related Disease. *Trends Immunol* 2018; **39**: 874-889 [PMID: 30401468 DOI: 10.1016/j.it.2018.09.005]
5 **Kawa S**, Kamisawa T, Notohara K, Fujinaga Y, Inoue D, Koyama T, Okazaki K. Japanese Clinical Diagnostic Criteria for Autoimmune Pancreatitis, 2018: Revision of Japanese Clinical Diagnostic Criteria for Autoimmune Pancreatitis, 2011. *Pancreas* 2020; **49**: e13-e14 [PMID: 31856100 DOI: 10.1097/MPA.0000000000001443]
6 **Umehara H**, Okazaki K, Nakamura T, Satoh-Nakamura T, Nakajima A, Kawano M, Mimori T, Chiba T. Current approach to the diagnosis of IgG4-related disease - Combination of comprehensive diagnostic and organ-specific criteria. *Mod Rheumatol* 2017; **27**: 381-391 [PMID: 28165852 DOI: 10.1080/14397595.2017.1290911]
7 **Tang J**, Cai S, Ye C, Dong L. Biomarkers in IgG4-related disease: A systematic review. *Semin Arthritis Rheum* 2020; **50**: 354-359 [PMID: 31280934 DOI: 10.1016/j.semarthrit.2019.06.018]
8 **Culver EL**, Sadler R, Simpson D, Cargill T, Makuch M, Bateman AC, Ellis AJ, Collier J, Chapman RW, Klenerman P, Barnes E, Ferry B. Elevated Serum IgG4 Levels in Diagnosis, Treatment Response, Organ Involvement, and Relapse in a Prospective IgG4-Related Disease UK Cohort. *Am J Gastroenterol* 2016; **111**: 733-743 [PMID: 27091321 DOI: 10.1038/ajg.2016.40]
9 **Ghazale A**, Chari ST, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Clain JE, Pearson RK, Pelaez-Luna M, Petersen BT, Vege SS, Farnell MB. Value of serum IgG4 in the diagnosis of autoimmune pancreatitis and in distinguishing it from pancreatic cancer. *Am J Gastroenterol* 2007; **102**: 1646-1653 [PMID: 17555461 DOI: 10.1111/j.1572-0241.2007.01264.x]
10 **Kamisawa T**, Takuma K, Tabata T, Inaba Y, Egawa N, Tsuruta K, Hishima T, Sasaki T, Itoi T. Serum IgG4-negative autoimmune pancreatitis. *J Gastroenterol* 2011; **46**: 108-116 [PMID: 20824290 DOI: 10.1007/s00535-010-0317-2]

11 **Kamisawa T**, Shimosegawa T, Okazaki K, Nishino T, Watanabe H, Kanno A, Okumura F, Nishikawa T, Kobayashi K, Ichiya T, Takatori H, Yamakita K, Kubota K, Hamano H, Okamura K, Hirano K, Ito T, Ko SB, Omata M. Standard steroid treatment for autoimmune pancreatitis. *Gut* 2009; **58**: 1504-1507 [PMID: 19398440 DOI: 10.1136/gut.2008.172908]

12 **Jeannin P**, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. *J Immunol* 1998; **160**: 3555-3561 [PMID: 9531318]

13 **Zen Y**, Fujii T, Harada K, Kawano M, Yamada K, Takahira M, Nakanuma Y. Th2 and regulatory immune reactions are increased in immunoglobin G4-related sclerosing pancreatitis and cholangitis. *Hepatology* 2007; **45**: 1538-1546 [PMID: 17518371 DOI: 10.1002/hep.21697]

14 **Koyabu M**, Uchida K, Miyoshi H, Sakaguchi Y, Fukui T, Ikeda H, Takaoka M, Hirohara J, Nishio A, Uemura Y, Uemoto S, Okazaki K. Analysis of regulatory T cells and IgG4-positive plasma cells among patients of IgG4-related sclerosing cholangitis and autoimmune liver diseases. *J Gastroenterol* 2010; **45**: 732-741 [PMID: 20087609 DOI: 10.1007/s00535-010-0199-3]

15 **Tanaka A**, Moriyama M, Nakashima H, Miyake K, Hayashida JN, Maehara T, Shinozaki S, Kubo Y, Nakamura S. Th2 and regulatory immune reactions contribute to IgG4 production and the initiation of Mikulicz disease. *Arthritis Rheum* 2012; **64**: 254-263 [PMID: 21898360 DOI: 10.1002/art.33320]

16 **Maehara T**, Moriyama M, Nakashima H, Miyake K, Hayashida JN, Tanaka A, Shinozaki S, Kubo Y, Nakamura S. Interleukin-21 contributes to germinal centre formation and immunoglobulin G4 production in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. *Ann Rheum Dis* 2012; **71**: 2011-2019 [PMID: 22753386 DOI: 10.1136/annrheumdis-2012-201477]

17 **Chen Y**, Lin W, Yang H, Wang M, Zhang P, Feng R, Chen H, Peng L, Zhang X, Zhao Y, Zeng X, Zhang F, Zhang W, Lipsky PE. Aberrant Expansion and Function of Follicular Helper T Cell Subsets in IgG4-Related Disease. *Arthritis Rheumatol* 2018; **70**: 1853-1865 [PMID: 29781221 DOI: 10.1002/art.40556]

18 **Kamekura R**, Takano K, Yamamoto M, Kawata K, Shigehara K, Jitsukawa S, Nagaya T, Ito F, Sato A, Ogasawara N, Tsubomatsu C, Takahashi H, Nakase H, Himi T, Ichimiya S. Cutting Edge: A Critical Role of Lesional T Follicular Helper Cells in the Pathogenesis of IgG4-Related Disease. *J Immunol* 2017; **199**: 2624-2629 [PMID: 28916523 DOI: 10.4049/jimmunol.1601507]

19 **Akiyama M**, Suzuki K, Yamaoka K, Yasuoka H, Takeshita M, Kaneko Y, Kondo H, Kassai Y, Miyazaki T, Morita R, Yoshimura A, Takeuchi T. Number of Circulating Follicular Helper 2 T Cells Correlates With IgG4 and Interleukin-4 Levels and Plasmablast Numbers in IgG4-Related Disease. *Arthritis Rheumatol* 2015; **67**: 2476-2481 [PMID: 25989153 DOI: 10.1002/art.39209]

20 **Mattoo H**, Mahajan VS, Maehara T, Deshpande V, Della-Torre E, Wallace ZS, Kulikova M, Drijvers JM, Daccache J, Carruthers MN, Castelino FV, Stone JR, Stone JH, Pillai S. Clonal expansion of CD4(+) cytotoxic T lymphocytes in patients with IgG4-related disease. *J Allergy Clin Immunol* 2016; **138**: 825-838 [PMID: 26971690 DOI: 10.1016/j.jaci.2015.12.1330]

21 **Maehara T**, Mattoo H, Ohta M, Mahajan VS, Moriyama M, Yamauchi M, Drijvers J, Nakamura S, Stone JH, Pillai SS. Lesional CD4+ IFN-γ+ cytotoxic T lymphocytes in IgG4-related dacryoadenitis and sialoadenitis. *Ann Rheum Dis* 2017; **76**: 377-385 [PMID: 27358392 DOI: 10.1136/annrheumdis-2016-209139]

22 **Takeda K**, Akira S. Toll-like receptors in innate immunity. *Int Immunol* 2005; **17**: 1-14 [PMID: 15585605 DOI: 10.1093/intimm/dxh186]

23 **Philpott DJ**, Sorbara MT, Robertson SJ, Croitoru K, Girardin SE. NOD proteins: regulators of inflammation in health and disease. *Nat Rev Immunol* 2014; **14**: 9-23 [PMID: 24336102 DOI: 10.1038/nri3565]

24 **Ganguly D**. Do Type I Interferons Link Systemic Autoimmunities and Metabolic Syndrome in a Pathogenetic Continuum? *Trends Immunol* 2018; **39**: 28-43 [PMID: 28826817 DOI: 10.1016/j.it.2017.07.001]

25 **Akitake R**, Watanabe T, Zaima C, Uza N, Ida H, Tada S, Nishida N, Chiba T. Possible involvement of T helper type 2 responses to Toll-like receptor ligands in IgG4-related sclerosing disease. *Gut* 2010; **59**: 542-545 [PMID: 20332525 DOI: 10.1136/gut.2009.200972]

26 **Watanabe T**, Yamashita K, Fujikawa S, Sakurai T, Kudo M, Shiokawa M, Kodama Y, Uchida K, Okazaki K, Chiba T. Involvement of activation of toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG4 responses in autoimmune pancreatitis. *Arthritis Rheum* 2012; **64**: 914-924 [PMID: 21971969 DOI: 10.1002/art.33386]

27 **Watanabe T**, Yamashita K, Sakurai T, Kudo M, Shiokawa M, Uza N, Kodama Y, Uchida K, Okazaki K, Chiba T. Toll-like receptor activation in basophils contributes to the development of IgG4-related disease. *J Gastroenterol* 2013; **48**: 247-253 [PMID: 22744834 DOI: 10.1007/s00535-012-0626-8]

28 **Fukui Y**, Uchida K, Sakaguchi Y, Fukui T, Nishio A, Shikata N, Sakaida N, Uemura Y, Satoi S, Okazaki K. Possible involvement of Toll-like receptor 7 in the development of type 1 autoimmune pancreatitis. *J Gastroenterol* 2015; **50**: 435-444 [PMID: 25005350 DOI: 10.1007/s00535-014-0977-4]

29 **Yanagawa M**, Uchida K, Ando Y, Tomiyama T, Yamaguchi T, Ikeura T, Fukui T, Nishio A, Uemura Y, Miyara T, Okamoto H, Satoi S, Okazaki K. Basophils activated *via* TLR signaling may contribute to pathophysiology of type 1 autoimmune pancreatitis. *J Gastroenterol* 2018; **53**: 449-460 [PMID: 28921377 DOI: 10.1007/s00535-017-1390-6]

30 **Ishiguro N**, Moriyama M, Furusho K, Furukawa S, Shibata T, Murakami Y, Chinju A, Haque ASMR, Gion Y, Ohta M, Maehara T, Tanaka A, Yamauchi M, Sakamoto M, Mochizuki K, Ono Y, Hayashida JN, Sato Y, Kiyoshima T, Yamamoto H, Miyake K, Nakamura S. Activated M2

Macrophages Contribute to the Pathogenesis of IgG4-Related Disease *via* Toll-like Receptor 7/Interleukin-33 Signaling. *Arthritis Rheumatol* 2020; **72**: 166-178 [PMID: 31339007 DOI: 10.1002/art.41052]

31 **Arai Y**, Yamashita K, Kuriyama K, Shiokawa M, Kodama Y, Sakurai T, Mizugishi K, Uchida K, Kadowaki N, Takaori-Kondo A, Kudo M, Okazaki K, Strober W, Chiba T, Watanabe T. Plasmacytoid Dendritic Cell Activation and IFN-α Production Are Prominent Features of Murine Autoimmune Pancreatitis and Human IgG4-Related Autoimmune Pancreatitis. *J Immunol* 2015; **195**: 3033-3044 [PMID: 26297761 DOI: 10.4049/jimmunol.1500971]

32 **Watanabe T**, Yamashita K, Arai Y, Minaga K, Kamata K, Nagai T, Komeda Y, Takenaka M, Hagiwara S, Ida H, Sakurai T, Nishida N, Strober W, Kudo M. Chronic Fibro-Inflammatory Responses in Autoimmune Pancreatitis Depend on IFN-α and IL-33 Produced by Plasmacytoid Dendritic Cells. *J Immunol* 2017; **198**: 3886-3896 [PMID: 28373582 DOI: 10.4049/jimmunol.1700060]

33 **Swiecki M**, Colonna M. The multifaceted biology of plasmacytoid dendritic cells. *Nat Rev Immunol* 2015; **15**: 471-485 [PMID: 26160613 DOI: 10.1038/nri3865]

34 **Watanabe T**, Sadakane Y, Yagama N, Sakurai T, Ezoe H, Kudo M, Chiba T, Strober W. Nucleotide-binding oligomerization domain 1 acts in concert with the cholecystokinin receptor agonist, cerulein, to induce IL-33-dependent chronic pancreatitis. *Mucosal Immunol* 2016; **9**: 1234-1249 [PMID: 26813347 DOI: 10.1038/mi.2015.144]

35 **Furukawa S**, Moriyama M, Miyake K, Nakashima H, Tanaka A, Maehara T, Iizuka-Koga M, Tsuboi H, Hayashida JN, Ishiguro N, Yamauchi M, Sumida T, Nakamura S. Interleukin-33 produced by M2 macrophages and other immune cells contributes to Th2 immune reaction of IgG4-related disease. *Sci Rep* 2017; **7**: 42413 [PMID: 28205524 DOI: 10.1038/srep42413]

36 **Minaga K**, Watanabe T, Hara A, Kamata K, Omoto S, Nakai A, Otsuka Y, Sekai I, Yoshikawa T, Yamao K, Takenaka M, Chiba Y, Kudo M. Identification of serum IFN-α and IL-33 as novel biomarkers for type 1 autoimmune pancreatitis and IgG4-related disease. *Sci Rep* 2020; **10**: 14879 [PMID: 32938972 DOI: 10.1038/s41598-020-71848-4]

37 **Aalberse RC**, Stapel SO, Schuurman J, Rispens T. Immunoglobulin G4: an odd antibody. *Clin Exp Allergy* 2009; **39**: 469-477 [PMID: 19222496 DOI: 10.1111/j.1365-2222.2009.03207.x]

38 **Shiokawa M**, Kodama Y, Kuriyama K, Yoshimura K, Tomono T, Morita T, Kakiuchi N, Matsumori T, Mima A, Nishikawa Y, Ueda T, Tsuda M, Yamauchi Y, Minami R, Sakuma Y, Ota Y, Maruno T, Kurita A, Sawai Y, Tsuji Y, Uza N, Matsumura K, Watanabe T, Notohara K, Tsuruyama T, Seno H, Chiba T. Pathogenicity of IgG in patients with IgG4-related disease. *Gut* 2016; **65**: 1322-1332 [PMID: 26964842 DOI: 10.1136/gutjnl-2015-310336]

39 **Chan ASY**, Mudhar H, Shen SY, Lang SS, Fernando M, Hilmy MH, Guppy NJ, Rennie I, Dunkley L, Al Jajeh I. Serum IgG2 and tissue IgG2 plasma cell elevation in orbital IgG4-related disease (IgG4-RD): Potential use in IgG4-RD assessment. *Br J Ophthalmol* 2017; **101**: 1576-1582 [PMID: 28351925 DOI: 10.1136/bjophthalmol-2017-310148]

40 **Taguchi M**, Kihara Y, Nagashio Y, Yamamoto M, Otsuki M, Harada M. Decreased production of immunoglobulin M and A in autoimmune pancreatitis. *J Gastroenterol* 2009; **44**: 1133-1139 [PMID: 19626266 DOI: 10.1007/s00535-009-0106-y]

41 **Culver EL**, Sadler R, Bateman AC, Makuch M, Cargill T, Ferry B, Aalberse R, Barnes E, Rispens T. Increases in IgE, Eosinophils, and Mast Cells Can be Used in Diagnosis and to Predict Relapse of IgG4-Related Disease. *Clin Gastroenterol Hepatol* 2017; **15**: 1444-1452. e6 [PMID: 28223204 DOI: 10.1016/j.cgh.2017.02.007]

42 **Shiokawa M**, Kodama Y, Sekiguchi K, Kuwada T, Tomono T, Kuriyama K, Yamazaki H, Morita T, Marui S, Sogabe Y, Kakiuchi N, Matsumori T, Mima A, Nishikawa Y, Ueda T, Tsuda M, Yamauchi Y, Sakuma Y, Maruno T, Uza N, Tsuruyama T, Mimori T, Seno H, Chiba T. Laminin 511 is a target antigen in autoimmune pancreatitis. *Sci Transl Med* 2018; **10**: eaaq0997 [PMID: 30089633 DOI: 10.1126/scitranslmed.aaq0997]

43 **Hubers LM**, Vos H, Schuurman AR, Erken R, Oude Elferink RP, Burgering B, van de Graaf SFJ, Beuers U. Annexin A11 is targeted by IgG4 and IgG1 autoantibodies in IgG4-related disease. *Gut* 2018; **67**: 728-735 [PMID: 28765476 DOI: 10.1136/gutjnl-2017-314548]

44 **Perugino CA**, AlSalem SB, Mattoo H, Della-Torre E, Mahajan V, Ganesh G, Allard-Chamard H, Wallace Z, Montesi SB, Kreuzer J, Haas W, Stone JH, Pillai S. Identification of galectin-3 as an autoantigen in patients with $IgG_4$-related disease. *J Allergy Clin Immunol* 2019; **143**: 736-745. e6 [PMID: 29852256 DOI: 10.1016/j.jaci.2018.05.011]

45 **Yamamoto M**, Takano KI, Kamekura R, Aochi S, Suzuki C, Ichimiya S, Nakase H, Himi T, Takahashi H. Interleukin 5-producing $ST2^+$ memory Th2 cells in IgG4-related dacryoadenitis and sialadenitis. *Mod Rheumatol* 2019; **29**: 856-860 [PMID: 30354922 DOI: 10.1080/14397595.2018.1526357]

46 **Kubo S**, Nakayamada S, Zhao J, Yoshikawa M, Miyazaki Y, Nawata A, Hirata S, Nakano K, Saito K, Tanaka Y. Correlation of T follicular helper cells and plasmablasts with the development of organ involvement in patients with IgG4-related disease. *Rheumatology (Oxford)* 2018; **57**: 514-524 [PMID: 29253269 DOI: 10.1093/rheumatology/kex455]

47 **Miyoshi H**, Uchida K, Taniguchi T, Yazumi S, Matsushita M, Takaoka M, Okazaki K. Circulating naïve and CD4+CD25high regulatory T cells in patients with autoimmune pancreatitis. *Pancreas* 2008; **36**: 133-140 [PMID: 18376303 DOI: 10.1097/MPA.0b013e3181577553]

48 **Mattoo H**, Della-Torre E, Mahajan VS, Stone JH, Pillai S. Circulating Th2 memory cells in IgG4-

related disease are restricted to a defined subset of subjects with atopy. *Allergy* 2014; **69**: 399-402 [PMID: 24382311 DOI: 10.1111/all.12342]

49 **Yamamoto M**, Takano K, Kamekura R, Suzuki C, Ichimiya S, Himi T, Nakase H, Takahashi H. Stage classification of IgG4-related dacryoadenitis and sialadenitis by the serum cytokine environment. *Mod Rheumatol* 2018; **28**: 1004-1008 [PMID: 29385874 DOI: 10.1080/14397595.2018.1436029]

50 **Müller T**, Beutler C, Picó AH, Otten M, Dürr A, Al-Abadi H, Guckelberger O, Meyer Zum Büschenfelde D, Jöhrens K, Volkmann M, Lankisch T, Voigtländer T, Anders M, Shibolet O, Jefferson DM, Podolsky DK, Fischer A, Veltzke-Schlieker W, Adler A, Baumgart DC, Sturm A, Wiedenmann B, Schott E, Berg T. Increased T-helper 2 cytokines in bile from patients with IgG4-related cholangitis disrupt the tight junction-associated biliary epithelial cell barrier. *Gastroenterology* 2013; **144**: 1116-1128 [PMID: 23391819 DOI: 10.1053/j.gastro.2013.01.055]

51 **Ebbo M**, De Sainte-Marie B, Muller R, Piperoglou C, Grados A, Vély F, Schleinitz N. Comment on article: 'Dupilumab as a novel steroid-sparing treatment for $IgG_4$-related disease' by Simpson *et al*. *Ann Rheum Dis* 2020; epub ahead of print [PMID: 31996366 DOI: 10.1136/annrheumdis-2020-217010]

52 **Simpson RS**, Lau SKC, Lee JK. Dupilumab as a novel steroid-sparing treatment for IgG4-related disease. *Ann Rheum Dis* 2020; **79**: 549-550 [PMID: 31857343 DOI: 10.1136/annrheumdis-2019-216368]

53 **Minaga K**, Watanabe T, Kamata K, Takenaka M, Yasukawa S, Kudo M. The IFN-α-IL-33 Axis as Possible Biomarkers in IgG4-Related Disease. *Am J Gastroenterol* 2019; **114**: 1002-1003 [PMID: 31058651 DOI: 10.14309/ajg.0000000000000245]

54 **Furie R**, Khamashta M, Merrill JT, Werth VP, Kalunian K, Brohawn P, Illei GG, Drappa J, Wang L, Yoo S; CD1013 Study Investigators. Anifrolumab, an Anti-Interferon-α Receptor Monoclonal Antibody, in Moderate-to-Severe Systemic Lupus Erythematosus. *Arthritis Rheumatol* 2017; **69**: 376-386 [PMID: 28130918 DOI: 10.1002/art.39962]

55 **Khamashta M**, Merrill JT, Werth VP, Furie R, Kalunian K, Illei GG, Drappa J, Wang L, Greth W; CD1067 study investigators. Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann Rheum Dis* 2016; **75**: 1909-1916 [PMID: 27009916 DOI: 10.1136/annrheumdis-2015-208562]

56 **Mackay F**, Schneider P. Cracking the BAFF code. *Nat Rev Immunol* 2009; **9**: 491-502 [PMID: 19521398 DOI: 10.1038/nri2572]

57 **Carruthers MN**, Topazian MD, Khosroshahi A, Witzig TE, Wallace ZS, Hart PA, Deshpande V, Smyrk TC, Chari S, Stone JH. Rituximab for IgG4-related disease: a prospective, open-label trial. *Ann Rheum Dis* 2015; **74**: 1171-1177 [PMID: 25667206 DOI: 10.1136/annrheumdis-2014-206605]

58 **Kiyama K**, Kawabata D, Hosono Y, Kitagori K, Yukawa N, Yoshifuji H, Omura K, Fujii T, Mimori T. Serum BAFF and APRIL levels in patients with IgG4-related disease and their clinical significance. *Arthritis Res Ther* 2012; **14**: R86 [PMID: 22531553 DOI: 10.1186/ar3810]

59 **Garbers C**, Heink S, Korn T, Rose-John S. Interleukin-6: designing specific therapeutics for a complex cytokine. *Nat Rev Drug Discov* 2018; **17**: 395-412 [PMID: 29725131 DOI: 10.1038/nrd.2018.45]

60 **Tsukuda S**, Ikeura T, Ito T, Nakamaru K, Masuda M, Hori Y, Ikemune M, Yanagawa M, Tanaka T, Tomiyama T, Yamaguchi T, Ando Y, Uchida K, Fukui T, Nishio A, Terasawa R, Tanigawa N, Okazaki K. Clinical implications of elevated serum interleukin-6 in IgG4-related disease. *PLoS One* 2020; **15**: e0227479 [PMID: 31951598 DOI: 10.1371/journal.pone.0227479]

61 **Sato Y**, Kojima M, Takata K, Morito T, Asaoku H, Takeuchi T, Mizobuchi K, Fujihara M, Kuraoka K, Nakai T, Ichimura K, Tanaka T, Tamura M, Nishikawa Y, Yoshino T. Systemic IgG4-related lymphadenopathy: a clinical and pathologic comparison to multicentric Castleman's disease. *Mod Pathol* 2009; **22**: 589-599 [PMID: 19270642 DOI: 10.1038/modpathol.2009.17]

62 **Kakinuma T**, Nakamura K, Wakugawa M, Mitsui H, Tada Y, Saeki H, Torii H, Asahina A, Onai N, Matsushima K, Tamaki K. Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. *J Allergy Clin Immunol* 2001; **107**: 535-541 [PMID: 11240957 DOI: 10.1067/mai.2001.113237]

63 **Umeda M**, Origuchi T, Kawashiri SY, Koga T, Ichinose K, Furukawa K, Sato T, Tsuji S, Endo Y, Takatani A, Shimizu T, Fukui S, Iwamoto N, Igawa T, Tamai M, Nakamura H, Kawakami A. Thymus and Activation-regulated Chemokine as a Biomarker for IgG4-related Disease. *Sci Rep* 2020; **10**: 6010 [PMID: 32265499 DOI: 10.1038/s41598-020-62941-9]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2270-2280

DOI: 10.3748/wjg.v27.i19.2270

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

*REVIEW*

# Receptor for advanced glycation end-products axis and coronavirus disease 2019 in inflammatory bowel diseases: A dangerous liaison?

Armando Rojas, Iván Schneider, Cristian Lindner, Ileana Gonzàlez, Miguel Angel Morales

**ORCID number:** Armando Rojas 0000-0001-9911-7142; Iván Schneider 0000-0001-5294-5995; Cristian Lindner 0000-0002-2642-4288; Ileana Gonzàlez 0000-0002-2488-9380; Miguel Angel Morales 0000-0001-7698-9669.

**Author contributions:** All authors contributed to the original ideas and writing of this paper; Rojas A designed the report and wrote the paper; Schneider I and Lindner C contributed to data collection and draft manuscript preparation and art-work, and both contributed equally; González I and Morales MA contributed to data acquisition and drafting and revising the manuscript.

**Conflict-of-interest statement:** The authors declare that there is no conflict of interest.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: htt

**Armando Rojas, Iván Schneider, Cristian Lindner, Ileana Gonzàlez,** Biomedical Research Labs, Medicine Faculty, Catholic University of Maule, Talca 3634000, Chile

**Miguel Angel Morales,** Department of Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, University of Chile, Santiago 8320000, Chile

**Corresponding author:** Armando Rojas, PhD, Full Professor, Biomedical Research Labs, Medicine Faculty, Catholic University of Maule, 3605 San Miguel Ave, Talca 3634000, Chile. arojasr@ucm.cl

## Abstract

Compelling evidence supports the crucial role of the receptor for advanced glycation end-products (RAGE) axis activation in many clinical entities. Since the beginning of the coronavirus disease 2019 pandemic, there is an increasing concern about the risk and handling of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in inflammatory gastrointestinal disorders, such as inflammatory bowel diseases (IBD). However, clinical data raised during pandemic suggests that IBD patients do not have an increased risk of contracting SARS-CoV-2 infection or develop a more severe course of infection. In the present review, we intend to highlight how two potentially important contributors to the inflammatory response to SARS-CoV-2 infection in IBD patients, the RAGE axis activation as well as the cross-talk with the renin-angiotensin system, are dampened by the high expression of soluble forms of both RAGE and the angiotensin-converting enzyme (ACE) 2. The soluble form of RAGE functions as a decoy for its ligands, and soluble ACE2 seems to be an additionally attenuating contributor to RAGE axis activation, particularly by avoiding the transactivation of the RAGE axis that can be produced by the virus-mediated imbalance of the ACE/angiotensin II/angiotensin II receptor type 1 pathway.

**Key Words:** COVID-19; Inflammatory bowel diseases; Advanced glycation; Angiotensin-converting enzyme 2; Alarmins; Receptor for advanced glycation end-products; Receptor for advanced glycation end-products axis; Inflammation

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

p://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Chile

**Peer-review report's scientific quality classification**

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

**Received:** January 24, 2021
**Peer-review started:** January 24, 2021
**First decision:** February 22, 2021
**Revised:** February 22, 2021
**Accepted:** April 13, 2021
**Article in press:** April 13, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Maric I
**S-Editor:** Fan JR
**L-Editor:** Filipodia
**P-Editor:** Ma YJ

**Core Tip:** Data raised during the pandemic suggest that inflammatory bowel diseases do not have an increased risk of contracting severe acute respiratory syndrome coronavirus 2 infection or develop a more severe course of infection. These findings are in some way unexpected considering that inflammatory bowel disease is a chronic inflammatory state of the gastrointestinal tract. We herein discuss how the receptor for advanced glycation end-products axis activation as well as the cross-talk with the renin-angiotensin system are dampened by the high expression of soluble forms of both receptor for advanced glycation end-products and angiotensin-converting enzyme 2.

**Citation:** Rojas A, Schneider I, Lindner C, Gonzàlez I, Morales MA. Receptor for advanced glycation end-products axis and coronavirus disease 2019 in inflammatory bowel diseases: A dangerous liaison? *World J Gastroenterol* 2021; 27(19): 2270-2280
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2270.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2270

## INTRODUCTION

At the end of 2019, China reported several cases of severe pneumonia of unknown cause; the coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was subsequently identified as the etiological agent[1]. Due to its rapid spread all over the world, the World Health Organization defined coronavirus disease 2019 (COVID-19) as a pandemic on January 30, 2020.

The main symptoms of COVID-19 affect the lower respiratory tract, causing high mortality-rate complications such as acute distress respiratory syndrome[2-6]. However, recent reports reveal that gastrointestinal (GI) manifestations of SARS-CoV-2 infection are common clinical symptoms among patients who develop COVID-19[7-11].

The SARS-CoV-2 uses the cellular transmembrane angiotensin-converting enzyme 2 (ACE2) molecule as the receptor for viral cell entry. Under physiological conditions, epithelial ACE2 is widely expressed in several tissues. However, the expression of epithelial ACE2 in the terminal ileum and colon are amongst the highest in the body, which could explain why COVID-19 patients experience several GI symptoms[12-16].

Consequently, there is an increasing concern about the risk and handling of SARS-CoV-2 infection in inflammatory GI disorders, such as inflammatory bowel disease (IBD). The IBDs are chronic intestinal diseases that comprise Crohn´s disease (CD) and ulcerative colitis, which are characterized by chronic and relapsing intestinal inflammation[17,18]. Thus, since the beginning of the SARS-CoV-2 pandemic, IBD patients were considered a high-risk group for increased severity and adverse outcomes in SARS-CoV-2 infection[19,20].

However, clinical data raised during pandemic suggest that IBD patients do not have an increased risk of contracting SARS-CoV-2 infection or develop a more severe course of infection[21-25]. A compelling body of both clinical and experimental evidence has shed light on the crucial role of the receptor of advanced glycation end-products (RAGE) activation in many chronic inflammatory diseases[26-31]. More recently, the role of RAGE axis activation as a key contributor in the clinical course of SARS-CoV-2 infection has been documented[32].

In the present review, we intend to highlight the role of the RAGE axis activation in the context of SARS-CoV-2 infection and the clinical evolution of the IBD patient.

## RAGE AXIS

Firstly described in 1992, the RAGE is a type I single-pass transmembrane protein that can bind advanced glycation-end products (AGEs). This molecule belongs to the immunoglobulin superfamily of cell surface receptors, which is now considered as a pattern recognition receptor and is regarded as a central mediator in chronic inflammatory and immune responses[33-35].

RAGE is usually expressed at low levels in many cell types and tissues, except for the lungs. However, this expression is noticeably increased under inflammatory conditions[36-38].

Besides the transmembrane form of RAGE, several soluble isoforms of this receptor (sRAGE) are generated either by alternative splicing or by the action of membrane associated-proteases, such matrix metalloproteinase-9 (MMP-9), a disintegrin metalloproteases (ADAM)-10, and ADAM-17[39-42]. These soluble variants may function as a decoy receptor for ligands and thus prevent the interaction with the membrane-anchored full-length RAGE. In consequence, a high bioavailability of sRAGE will decreases the inflammatory responses driven by full-length RAGE activation [35,43,44]. Besides AGEs, RAGE can recognize many other ligands including the alarmin high-mobility group box 1 (HMGB1), members of the S100 protein family, glycosaminoglycans, and amyloid β peptides, among many others[35,45].

As a consequence of RAGE engagement by its ligands, multiple signaling pathways are triggered, including reactive oxygen species, p21ras, extracellular signal-regulated protein kinase 1/2 (p44/p42) mitogen-activated protein (MAP) kinases, p38 and stress-activated protein kinases/c-Jun N-terminal kinase mitogen-activated protein kinases, rhoGTPases, phosphoinositol-3 kinase, and the janus kinase/signal transducer and activator of transcription pathway, having crucial downstream inflammatory consequences such as activation of nuclear factor-kappaB (NF-κB), AP-1, and signal transducer and activator of transcription-3[35].

Indeed, the RAGE axis signaling not only triggers pro-inflammatory gene expression but also a positive feed-forward loop, in which the inflammatory stimuli activate NF-κB, which induces RAGE expression, following an enhanced and sustained inflammatory response[35,46-48].

## RAGE AXIS ACTIVATION IN IBD

Initially, RAGE axis activation was linked to the complications of diabetes such as macro-and microvascular complications[49,50]. However, a growing body of evidence indicates RAGE as a key molecule involved in many chronic inflammatory diseases[28-30,51].

Many underlying molecular mechanisms are involved in the onset and perpetuation of the disease, particularly those fueling the robust pro-inflammatory signals found in IBD patients[26,52]. Noteworthy, some pieces of evidence reveal an increased expression of RAGE and its ligands on intestinal cells in IBD patients, especially in inflamed areas[53-55]. In this context, it is important to highlight that the release of the RAGE ligand HMGB1 and members of the S100 protein family is increased under inflammation conditions[54-57]. Thus, the engagement of RAGE may play an important role in the maintenance of intestinal injury and inflammatory environment [53-57].

Strikingly, increased levels of both MMP-9 and ADAM17 have been reported in IBD patients[58,59], and both metalloproteases are involved in RAGE shedding, thus increasing the levels of sRAGE, which in turn can modulate the inflammatory responses driven by RAGE axis activation in IBD patients[58]. At present, a compelling body of evidence supports the fact that increased sRAGE levels correlate with a decrease in the RAGE activation-mediated inflammatory responses in many clinical entities[60-63]. In this context, it is important to highlight that CD147 significantly contributes to epithelial inflammation in many clinical entities including IBD[64,65], and it has been recently shown to act as a receptor for SARS-CoV-2[66]. Noteworthy, the inhibition of RAGE activation-mediated inflammatory response leads to a reduced expression of CD147[67].

## THE RENIN-ANGIOTENSIN SYSTEM

The renin-angiotensin system (RAS) is a hormonal system regulated by two complementary pathways that mediate opposing effects on inflammation, fibrosis, and cell proliferation[68-70]. Thus, the balance of both pathways determines pro-inflammatory or anti-inflammatory conditions among several systems such as cardiovascular, renal, and respiratory systems[71-74].

The classical pathway mediated *via* ACE, angiotensin II (Ang II) and its receptor Ang II receptor type 1 (AT1R), triggers activation of pro-inflammatory signals such as oxidative and nitrosative stresses, the induction of cytokines and cell adhesion molecules, as well as the activation of transcription factors such NF-κB[75-78]. On the contrary, the alternative pathway predominantly mediated by ACE2, Ang (1-7) and its receptor Mas (MasR), induces the opposite effects of AT1R activation, being an anti-

inflammatory and anti-fibrotic counter regulator of the effects of ACE/Ang II/AT1R[71,75,79,80]. ACE and ACE2 are highly expressed in several tissues such as the lungs, kidneys, and blood vessels. However, the brush border of the ileum and the colon are among the tissues with the highest expression of both enzymes[13-16,81]. Both enzymes can cleave angiotensin, generating different sub-products and regulating the balance between both pathways of the RAS system[79,82,83].

## RAS IMBALANCE IN IBD

Recent studies suggest high expression of the major components of both RAS pathways across the ileum and colon[81]. In this sense, the gut could be an especially susceptible organ for the imbalance of RAS pathways. Thus, the dysregulation of these components could have potential implications for inflammation and fibrosis for IBD patients[84,85]. Strikingly, several studies have revealed that the intestinal expression of ACE2 is inversely correlated with fibrosis in IBD patients[81,86].

Additionally, Ang (1-7) ameliorates colonic myofibroblast collagen secretion *via* MasR[81]. Furthermore, angiotensin receptor blockers and ACE inhibitors are reported to decrease mucosal pro-inflammatory cytokines, ameliorate colitis, and were associated with lower rates of complications, surgery, and hospitalization in patients with IBD[87-89].

Normally, ACE2 breaks down Ang II to Ang 1–7 peptide and thus avoiding the activation of the pro-inflammatory pathways of RAS. However, SARS-CoV-2 can hijack ACE2 and use it to gain entry into host cells[12,90]. Noteworthy, high bioavailability of soluble ACE2 has been reported in IBD patients[81,84], mainly ascribed to the increased level of ADAM17 observed in these patients[58,91-93], which in turn may function as a decoy receptor for SARS-CoV-2 and thus avoiding the hijacking of the counterbalancing enzyme.

This is particularly important considering that a novel ligand-independent mechanism for RAGE transactivation has been recently reported to occur following activation of the AT1R by Ang-II, thus leading to NF-κB dependent expression of pro-inflammatory mediators[48].

## RAGE AXIS ACTIVATION AND RAS IMBALANCE IN IBD PATIENTS INFECTED WITH SARS-COV-2

Contrary to what is expected, considering the pathophysiology of IBD, there is currently no evidence for an increased risk of worse clinical outcomes in patients with IBD in the context of COVID-19[21-25]. The role of the RAGE axis in the pathophysiology of IBD has been suggested by different reports[53-57]. The colonic expression of RAGE and some RAGE ligands, such as HMGB1 and some members of the S100 protein family, are significantly higher in IBD patients[54-56]. Besides, this receptor has been also considered a key contributor to the dysregulated and misdirected COVID-19 inflammatory response[32,94].

However, a counterbalancing element must be added to this scenario: The soluble RAGE. This molecule is generated by alternative splicing or by cleavage of the ectodomain of the membrane-anchored RAGE by the action of both MMP-9 and ADAM17, which are highly expressed in IBD patients[58,59]. Therefore, the high bioavailability of soluble RAGE may dampen RAGE activation, despite the abundance of both receptor and ligands in the inflamed intestinal mucosa of IBD patients.

On the other hand, the high expression of ACE2 in GI tract, especially among IBD patients, makes this tissue a particularly trophic niche for infection with SARS-CoV-2. Furthermore, the ACE2 exhaustion mediated by the entry of SARS-CoV-2 may then induce a robust RAS imbalance in favor of the pro-inflammatory ACE/Ang II/AT1R pathway[95]. These observations suggest that the inflamed gut of IBD patients represents an optimal doorway for SARS-CoV-2 entry, driving poor clinical outcomes in IBD patients who develop COVID-19.

However, this hypothetical scenario also has an important counterbalancing actor, the soluble form of ACE2, which is also increased in patients with IBD due to the shedding of the membrane-anchored ACE2 by ADAM17[58-59]. This is particularly important considering the non-cognate transactivation mechanism described for RAGE because of AT1R activation by Ang II[48], which is dampened by the preservation of membrane-associated ACE2 exhaustion by its soluble form.

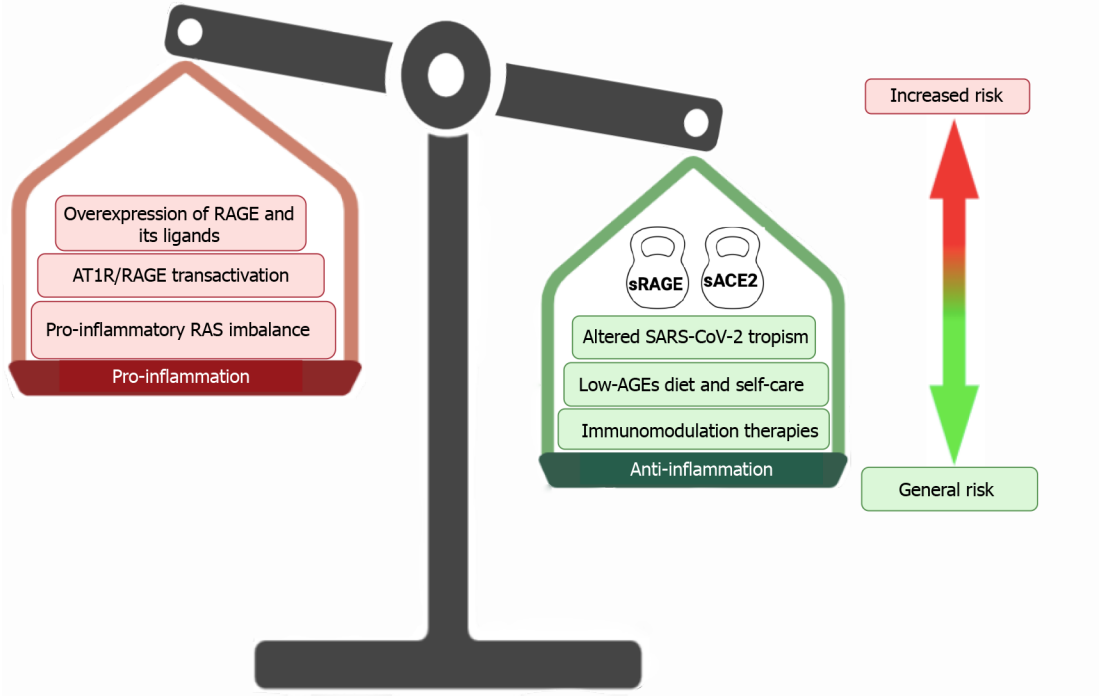



**Figure 1 In inflammatory bowel diseases patients, different inflammation–prone mechanisms are known to be activated.** Among them, the overexpression of receptor for advanced glycation end-products (RAGE) and the abundance of its ligands may produce a sustained activation of the axis, which can be also fueled by a non-cognate mechanism due to the pro-inflammatory rat sarcoma imbalance. These elements seem to be crucial contributors to the worsening course of inflammatory bowel diseases (IBD) patients with coronavirus disease 2019. However, other elements may dampen these inflammatory contributions, such as the high bioavailability of the soluble forms of both RAGE and angiotensin-converting enzyme 2. Soluble angiotensin-converting enzyme 2 may even interfere with severe acute respiratory syndrome coronavirus 2 entry to epithelial cells. Additionally, most if not all IBD patients are under pharmacological treatments directed to control inflammation. IBD patients deserve special attention to their diets, and as consequence, it is likely the ingestion of dietary advanced glycation-end products is also limited. RAGE: Receptor for advanced glycation end-products; RAS: Renin-angiotensin; ACE2: Angiotensin-converting enzyme 2; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; AT1R: Angiotensin II receptor type 1; AGEs: Advanced glycation-end products; sRAGE: Several soluble isoforms of this receptor.

A growing body of evidence demonstrates that in IBD patients the use of systemic immunosuppression is not associated with an increased risk of COVID-19 patients with IBD[96-100]. Furthermore, we must also keep in mind that the main objective of pharmacological treatments in IBD is to reduce inflammation levels. In this sense, in addition to interfering with signaling pathways, many drugs used in the current treatments also decrease the expression of RAGE and the bioavailability of some RAGE ligands, particularly the alarmins HMGB1 and S100 protein family members[96,97]. Indeed, several authors remark the possible protective role of IBD therapy against SARS-CoV-2 infection, especially through interfering with cytokine activity observed in the clinical course of COVID-19[98-100].

Additionally, the IBD patients have a high self-care standard and follow diets that help them to maintain good nutritional levels and the disease under control[101]. Some of these nutritional regimens are associated with a low-AGE diet, which may contribute to reducing the proinflammatory intestinal milieu mediated by RAGE activation[102] (Figure 1).

## CONCLUSION

The COVID-19 pandemics represent the worst challenge for a century for health systems all over the world. Severity and mortality have been highest in people with underlying morbidities. Therefore, special efforts have been done to understand how SARS-CoV-2 may particularly fuel inflammation in many clinical entities where the chronicity of an inflammatory environment is a relevant part of the pathogenesis of diseases. Based on a particularly inflamed landscape depicted in IBD patients, the activation of the RAGE axis as well the RAS imbalance seem to be crucial contributors to worsen inflammation in the gut. However, data raised during the pandemic suggests that IBD patients have neither an increased risk of contracting SARS-CoV-2 infection nor developing a more severe course of infection.

RAGE axis activation seems to be dampened by the high bioavailability of soluble receptors functioning as a decoy for its ligands. Additionally, soluble ACE2 seems to be another attenuating contributor to RAGE axis activation, particularly by avoiding the transactivation of RAGE axis that can be produced by the virus-mediated imbalance of the ACE/Ang II/ AT1R pathway. Thus, RAGE axis activation in COVID-19 IBD patients does not seem to be a dangerous liaison.

## REFERENCES


1 **Zhu N**, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; **382**: 727-733 [PMID: 31978945 DOI: 10.1056/NEJMoa2001017]

2 **Goyal P**, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, Satlin MJ, Campion TR Jr, Nahid M, Ringel JB, Hoffman KL, Alshak MN, Li HA, Wehmeyer GT, Rajan M, Reshetnyak E, Hupert N, Horn EM, Martinez FJ, Gulick RM, Safford MM. Clinical Characteristics of Covid-19 in New York City. *N Engl J Med* 2020; **382**: 2372-2374 [PMID: 32302078 DOI: 10.1056/NEJMc2010419]

3 **Hui KPY**, Cheung MC, Perera RAPM, Ng KC, Bui CHT, Ho JCW, Ng MMT, Kuok DIT, Shih KC, Tsao SW, Poon LLM, Peiris M, Nicholls JM, Chan MCW. Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *Lancet Respir Med* 2020; **8**: 687-695 [PMID: 32386571 DOI: 10.1016/S2213-2600(20)30193-4]

4 **Guan WJ**, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. *N Engl J Med* 2020; **382**: 1708-1720 [PMID: 32109013 DOI: 10.1056/NEJMoa2002032]

5 **Huang C**, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506 [PMID: 31986264 DOI: 10.1016/S0140-6736(20)30183-5]

6 **Lai CC**, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents* 2020; **55**: 105924 [PMID: 32081636 DOI: 10.1016/j.ijantimicag.2020.105924]

7 **Zhou Z**, Zhao N, Shu Y, Han S, Chen B, Shu X. Effect of Gastrointestinal Symptoms in Patients With COVID-19. *Gastroenterology* 2020; **158**: 2294-2297 [PMID: 32199880 DOI: 10.1053/j.gastro.2020.03.020]

8 **Jin X**, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, Hao SR, Jia HY, Cai H, Zhang XL, Yu GD, Xu KJ, Wang XY, Gu JQ, Zhang SY, Ye CY, Jin CL, Lu YF, Yu X, Yu XP, Huang JR, Xu KL, Ni Q, Yu CB, Zhu B, Li YT, Liu J, Zhao H, Zhang X, Yu L, Guo YZ, Su JW, Tao JJ, Lang GJ, Wu XX, Wu WR, Qv TT, Xiang DR, Yi P, Shi D, Chen Y, Ren Y, Qiu YQ, Li LJ, Sheng J, Yang Y. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020; **69**: 1002-1009 [PMID: 32213556 DOI: 10.1136/gutjnl-2020-320926]

9 **Xiao F**, Tang M, Zheng X, Liu Y, Li X, Shan H. Evidence for Gastrointestinal Infection of SARS-CoV-2. *Gastroenterology* 2020; **158**: 1831-1833. e3 [PMID: 32142773 DOI: 10.1053/j.gastro.2020.02.055]

10 **Garg M**, Christensen B, Lubel JS. Gastrointestinal ACE2, COVID-19 and IBD: Opportunity in the Face of Tragedy? *Gastroenterology* 2020; **159**: 1623-1624.e3 [PMID: 32353370 DOI: 10.1053/j.gastro.2020.04.051]

11 **Grassia R**, Testa S, Pan A, Conti CB. SARS-CoV-2 and gastrointestinal tract: The dark side of the pandemic. *Dig Liver Dis* 2020; **52**: 700-701 [PMID: 32423849 DOI: 10.1016/j.dld.2020.04.028]

12 **Hoffmann M**, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 2020; **181**: 271-280. e8 [PMID: 32142651 DOI: 10.1016/j.cell.2020.02.052]

13 **GTEx Consortium**. . The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; **45**: 580-585 [PMID: 23715323 DOI: 10.1038/ng.2653]

14 **Hamming I**, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 2004; **203**: 631-637 [PMID: 15141377 DOI: 10.1002/path.1570]

15 **Harmer D**, Gilbert M, Borman R, Clark KL. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. *FEBS Lett* 2002; **532**: 107-110 [PMID: 12459472 DOI: 10.1016/s0014-5793(02)03640-2]

16 **Sibony M**, Gasc JM, Soubrier F, Alhenc-Gelas F, Corvol P. Gene expression and tissue localization of the two isoforms of angiotensin I converting enzyme. *Hypertension* 1993; **21**: 827-835 [PMID:

8388857 DOI: 10.1161/01.hyp.21.6.827]
17 **Graham DB**, Xavier RJ. Pathway paradigms revealed from the genetics of inflammatory bowel disease. *Nature* 2020; **578**: 527-539 [PMID: 32103191 DOI: 10.1038/s41586-020-2025-2]
18 **Zhang YZ**, Li YY. Inflammatory bowel disease: pathogenesis. *World J Gastroenterol* 2014; **20**: 91-99 [PMID: 24415861 DOI: 10.3748/wjg.v20.i1.91]
19 **An P**, Ji M, Ren H, Su J, Ding NS, Kang J, Yin A, Zhou Q, Shen L, Zhao L, Jiang X, Xiao Y, Tan W, Lv X, Li J, Liu S, Zhou J, Chen H, Xu Y, Liu J, Chen M, Cao J, Zhou Z, Tan S, Yu H, Dong W, Ding Y. Prevention of COVID-19 in patients with inflammatory bowel disease in Wuhan, China. *Lancet Gastroenterol Hepatol* 2020; **5**: 525-527 [PMID: 32311321 DOI: 10.1016/S2468-1253(20)30121-7]
20 **Kennedy NA**, Jones GR, Lamb CA, Appleby R, Arnott I, Beattie RM, Bloom S, Brooks AJ, Cooney R, Dart RJ, Edwards C, Fraser A, Gaya DR, Ghosh S, Greveson K, Hansen R, Hart A, Hawthorne AB, Hayee B, Limdi JK, Murray CD, Parkes GC, Parkes M, Patel K, Pollok RC, Powell N, Probert CS, Raine T, Sebastian S, Selinger C, Smith PJ, Stansfield C, Younge L, Lindsay JO, Irving PM, Lees CW. British Society of Gastroenterology guidance for management of inflammatory bowel disease during the COVID-19 pandemic. *Gut* 2020; **69**: 984-990 [PMID: 32303607 DOI: 10.1136/gutjnl-2020-321244]
21 **Macaluso FS**, Orlando A. COVID-19 in patients with inflammatory bowel disease: A systematic review of clinical data. *Dig Liver Dis* 2020; **52**: 1222-1227 [PMID: 32928672 DOI: 10.1016/j.dld.2020.09.002]
22 **Monteleone G**, Ardizzone S. Are Patients with Inflammatory Bowel Disease at Increased Risk for Covid-19 Infection? *J Crohns Colitis* 2020; **14**: 1334-1336 [PMID: 32215548 DOI: 10.1093/ecco-jcc/jjaa061]
23 **Aziz M**, Fatima R, Haghbin H, Lee-Smith W, Nawras A. The Incidence and Outcomes of COVID-19 in IBD Patients: A Rapid Review and Meta-analysis. *Inflamm Bowel Dis* 2020; **26**: e132-e133 [PMID: 32619003 DOI: 10.1093/ibd/izaa170]
24 **Allocca M**, Fiorino G, Zallot C, Furfaro F, Gilardi D, Radice S, Danese S, Peyrin-Biroulet L. Incidence and Patterns of COVID-19 Among Inflammatory Bowel Disease Patients From the Nancy and Milan Cohorts. *Clin Gastroenterol Hepatol* 2020; **18**: 2134-2135 [PMID: 32360811 DOI: 10.1016/j.cgh.2020.04.071]
25 **Neurath MF**. COVID-19 and immunomodulation in IBD. *Gut* 2020; **69**: 1335-1342 [PMID: 32303609 DOI: 10.1136/gutjnl-2020-321269]
26 **Moura FA**, Goulart MOF, Campos SBG, da Paz Martins AS. The Close Interplay of Nitro-Oxidative Stress, Advanced Glycation end Products and Inflammation in Inflammatory Bowel Diseases. *Curr Med Chem* 2020; **27**: 2059-2076 [PMID: 30182837 DOI: 10.2174/0929867325666180904115633]
27 **Schmidt AM**, Yan SD, Yan SF, Stern DM. The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 2001; **108**: 949-955 [PMID: 11581294 DOI: 10.1172/JCI14002]
28 **Bierhaus A**, Stern DM, Nawroth PP. RAGE in inflammation: a new therapeutic target? *Curr Opin Investig Drugs* 2006; **7**: 985-991 [PMID: 17117586]
29 **Sparvero LJ**, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, Rutledge R, Lin B, Amoscato AA, Zeh HJ, Lotze MT. RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. *J Transl Med* 2009; **7**: 17 [PMID: 19292913 DOI: 10.1186/1479-5876-7-17]
30 **Chuah YK**, Basir R, Talib H, Tie TH, Nordin N. Receptor for advanced glycation end products and its involvement in inflammatory diseases. *Int J Inflam* 2013; **2013**: 403460 [PMID: 24102034 DOI: 10.1155/2013/403460]
31 **Chavakis T**, Bierhaus A, Nawroth PP. RAGE (receptor for advanced glycation end products): a central player in the inflammatory response. *Microbes Infect* 2004; **6**: 1219-1225 [PMID: 15488742 DOI: 10.1016/j.micinf.2004.08.004]
32 **Yalcin Kehribar D**, Cihangiroglu M, Sehmen E, Avci B, Capraz A, Yildirim Bilgin A, Gunaydin C, Ozgen M. The receptor for advanced glycation end product (RAGE) pathway in COVID-19. *Biomarkers* 2021; **26**: 114-118 [PMID: 33284049 DOI: 10.1080/1354750X.2020.1861099]
33 **Schmidt AM**, Vianna M, Gerlach M, Brett J, Ryan J, Kao J, Esposito C, Hegarty H, Hurley W, Clauss M. Isolation and characterization of two binding proteins for advanced glycosylation end products from bovine lung which are present on the endothelial cell surface. *J Biol Chem* 1992; **267**: 14987-14997 [PMID: 1321822]
34 **Yan SF**, Ramasamy R, Schmidt AM. Mechanisms of disease: advanced glycation end-products and their receptor in inflammation and diabetes complications. *Nat Clin Pract Endocrinol Metab* 2008; **4**: 285-293 [PMID: 18332897 DOI: 10.1038/ncpendmet0786]
35 **Rojas A**, Delgado-López F, González I, Pérez-Castro R, Romero J, Rojas I. The receptor for advanced glycation end-products: a complex signaling scenario for a promiscuous receptor. *Cell Signal* 2013; **25**: 609-614 [PMID: 23200851 DOI: 10.1016/j.cellsig.2012.11.022]
36 **Zen K**, Chen CX, Chen YT, Wilton R, Liu Y. Receptor for advanced glycation endproducts mediates neutrophil migration across intestinal epithelium. *J Immunol* 2007; **178**: 2483-2490 [PMID: 17277156 DOI: 10.4049/jimmunol.178.4.2483]
37 **González I**, Romero J, Rodríguez BL, Pérez-Castro R, Rojas A. The immunobiology of the receptor of advanced glycation end-products: trends and challenges. *Immunobiology* 2013; **218**: 790-797

[PMID: 23182709 DOI: 10.1016/j.imbio.2012.09.005]
38 **Oczypok EA**, Perkins TN, Oury TD. All the "RAGE" in lung disease: The receptor for advanced glycation endproducts (RAGE) is a major mediator of pulmonary inflammatory responses. *Paediatr Respir Rev* 2017; **23**: 40-49 [PMID: 28416135 DOI: 10.1016/j.prrv.2017.03.012]
39 **Raucci A**, Cugusi S, Antonelli A, Barabino SM, Monti L, Bierhaus A, Reiss K, Saftig P, Bianchi ME. A soluble form of the receptor for advanced glycation endproducts (RAGE) is produced by proteolytic cleavage of the membrane-bound form by the sheddase a disintegrin and metalloprotease 10 (ADAM10). *FASEB J* 2008; **22**: 3716-3727 [PMID: 18603587 DOI: 10.1096/fj.08-109033]
40 **Zhang L**, Bukulin M, Kojro E, Roth A, Metz VV, Fahrenholz F, Nawroth PP, Bierhaus A, Postina R. Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases. *J Biol Chem* 2008; **283**: 35507-35516 [PMID: 18952609 DOI: 10.1074/jbc.M806948200]
41 **Deuss M**, Reiss K, Hartmann D. Part-time alpha-secretases: the functional biology of ADAM 9, 10 and 17. *Curr Alzheimer Res* 2008; **5**: 187-201 [PMID: 18393804 DOI: 10.2174/156720508783954686]
42 **Metz VV**, Kojro E, Rat D, Postina R. Induction of RAGE shedding by activation of G protein-coupled receptors. *PLoS One* 2012; **7**: e41823 [PMID: 22860017 DOI: 10.1371/journal.pone.0041823]
43 **Grauen Larsen H**, Marinkovic G, Nilsson PM, Nilsson J, Engström G, Melander O, Orho-Melander M, Schiopu A. High Plasma sRAGE (Soluble Receptor for Advanced Glycation End Products) Is Associated With Slower Carotid Intima-Media Thickness Progression and Lower Risk for First-Time Coronary Events and Mortality. *Arterioscler Thromb Vasc Biol* 2019; **39**: 925-933 [PMID: 30917679 DOI: 10.1161/ATVBAHA.118.312319]
44 **Geroldi D**, Falcone C, Emanuele E. Soluble receptor for advanced glycation end products: from disease marker to potential therapeutic target. *Curr Med Chem* 2006; **13**: 1971-1978 [PMID: 16842191 DOI: 10.2174/092986706777585013]
45 **Bucciarelli LG**, Wendt T, Rong L, Lalla E, Hofmann MA, Goova MT, Taguchi A, Yan SF, Yan SD, Stern DM, Schmidt AM. RAGE is a multiligand receptor of the immunoglobulin superfamily: implications for homeostasis and chronic disease. *Cell Mol Life Sci* 2002; **59**: 1117-1128 [PMID: 12222959 DOI: 10.1007/s00018-002-8491-x]
46 **Bierhaus A**, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, Chen J, Hong M, Luther T, Henle T, Klöting I, Morcos M, Hofmann M, Tritschler H, Weigle B, Kasper M, Smith M, Perry G, Schmidt AM, Stern DM, Häring HU, Schleicher E, Nawroth PP. Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. *Diabetes* 2001; **50**: 2792-2808 [PMID: 11723063 DOI: 10.2337/diabetes.50.12.2792]
47 **Hudson BI**, Lippman ME. Targeting RAGE Signaling in Inflammatory Disease. *Annu Rev Med* 2018; **69**: 349-364 [PMID: 29106804 DOI: 10.1146/annurev-med-041316-085215]
48 **Pickering RJ**, Tikellis C, Rosado CJ, Tsorotes D, Dimitropoulos A, Smith M, Huet O, Seeber RM, Abhayawardana R, Johnstone EK, Golledge J, Wang Y, Jandeleit-Dahm KA, Cooper ME, Pfleger KD, Thomas MC. Transactivation of RAGE mediates angiotensin-induced inflammation and atherogenesis. *J Clin Invest* 2019; **129**: 406-421 [PMID: 30530993 DOI: 10.1172/JCI99987]
49 **Ramasamy R**, Yan SF, Schmidt AM. The RAGE axis and endothelial dysfunction: maladaptive roles in the diabetic vasculature and beyond. *Trends Cardiovasc Med* 2005; **15**: 237-243 [PMID: 16226677 DOI: 10.1016/j.tcm.2005.08.003]
50 **Manigrasso MB**, Juranek J, Ramasamy R, Schmidt AM. Unlocking the biology of RAGE in diabetic microvascular complications. *Trends Endocrinol Metab* 2014; **25**: 15-22 [PMID: 24011512 DOI: 10.1016/j.tem.2013.08.002]
51 **Sims GP**, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and cancer. *Annu Rev Immunol* 2010; **28**: 367-388 [PMID: 20192808 DOI: 10.1146/annurev.immunol.021908.132603]
52 **Yadav V**, Varum F, Bravo R, Furrer E, Bojic D, Basit AW. Inflammatory bowel disease: exploring gut pathophysiology for novel therapeutic targets. *Transl Res* 2016; **176**: 38-68 [PMID: 27220087 DOI: 10.1016/j.trsl.2016.04.009]
53 **Ciccocioppo R**, Vanoli A, Klersy C, Imbesi V, Boccaccio V, Manca R, Betti E, Cangemi GC, Strada E, Besio R, Rossi A, Falcone C, Ardizzone S, Fociani P, Danelli P, Corazza GR. Role of the advanced glycation end products receptor in Crohn's disease inflammation. *World J Gastroenterol* 2013; **19**: 8269-8281 [PMID: 24363518 DOI: 10.3748/wjg.v19.i45.8269]
54 **Hu Z**, Wang X, Gong L, Wu G, Peng X, Tang X. Role of high-mobility group box 1 protein in inflammatory bowel disease. *Inflamm Res* 2015; **64**: 557-563 [PMID: 26077468 DOI: 10.1007/s00011-015-0841-x]
55 **Foell D**, Kucharzik T, Kraft M, Vogl T, Sorg C, Domschke W, Roth J. Neutrophil derived human S100A12 (EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease. *Gut* 2003; **52**: 847-853 [PMID: 12740341 DOI: 10.1136/gut.52.6.847]
56 **Yamasaki H**, Mitsuyama K, Masuda J, Kuwaki K, Takedatsu H, Sugiyama G, Yamada S, Sata M. Roles of high-mobility group box 1 in murine experimental colitis. *Mol Med Rep* 2009; **2**: 23-27 [PMID: 21475785 DOI: 10.3892/mmr_00000056]
57 **Manolakis AC**, Kapsoritakis AN, Tiaka EK, Potamianos SP. Calprotectin, calgranulin C, and other members of the s100 protein family in inflammatory bowel disease. *Dig Dis Sci* 2011; **56**: 1601-1611 [PMID: 21203903 DOI: 10.1007/s10620-010-1494-9]

58 **Cesaro A**, Abakar-Mahamat A, Brest P, Lassalle S, Selva E, Filippi J, Hébuterne X, Hugot JP, Doglio A, Galland F, Naquet P, Vouret-Craviari V, Mograbi B, Hofman PM. Differential expression and regulation of ADAM17 and TIMP3 in acute inflamed intestinal epithelia. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1332-G1343 [PMID: 19299578 DOI: 10.1152/ajpgi.90641.2008]

59 **Meijer MJ**, Mieremet-Ooms MA, van der Zon AM, van Duijn W, van Hogezand RA, Sier CF, Hommes DW, Lamers CB, Verspaget HW. Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype. *Dig Liver Dis* 2007; **39**: 733-739 [PMID: 17602907 DOI: 10.1016/j.dld.2007.05.010]

60 **Park L**, Raman KG, Lee KJ, Lu Y, Ferran LJ Jr, Chow WS, Stern D, Schmidt AM. Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. *Nat Med* 1998; **4**: 1025-1031 [PMID: 9734395 DOI: 10.1038/2012]

61 **Lalla E**, Lamster IB, Feit M, Huang L, Spessot A, Qu W, Kislinger T, Lu Y, Stern DM, Schmidt AM. Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice. *J Clin Invest* 2000; **105**: 1117-1124 [PMID: 10772656 DOI: 10.1172/JCI8942]

62 **Wendt T**, Harja E, Bucciarelli L, Qu W, Lu Y, Rong LL, Jenkins DG, Stein G, Schmidt AM, Yan SF. RAGE modulates vascular inflammation and atherosclerosis in a murine model of type 2 diabetes. *Atherosclerosis* 2006; **185**: 70-77 [PMID: 16076470 DOI: 10.1016/j.atherosclerosis.2005.06.013]

63 **Bucciarelli LG**, Wendt T, Qu W, Lu Y, Lalla E, Rong LL, Goova MT, Moser B, Kislinger T, Lee DC, Kashyap Y, Stern DM, Schmidt AM. RAGE blockade stabilizes established atherosclerosis in diabetic apolipoprotein E-null mice. *Circulation* 2002; **106**: 2827-2835 [PMID: 12451010 DOI: 10.1161/01.cir.0000039325.03698.36]

64 **Wang H**, Ye J, Liu R, Chen G, Zhao J, Huang L, Yang F, Li M, Zhang S, Jingxie, Xiong L, Chen H, Xu Y, Su M, Xie Y, Zheng F, Geng L, Xu W, Gong S. Clinical Significance of CD147 in Children with Inflammatory Bowel Disease. *Biomed Res Int* 2020; **2020**: 7647181 [PMID: 33015178 DOI: 10.1155/2020/7647181]

65 **Xu Z**, Liu R, Huang L, Xu Y, Su M, Chen J, Geng L, Xu W, Gong S. CD147 Aggravated Inflammatory Bowel Disease by Triggering NF-kB-Mediated Pyroptosis. *Biomed Res Int* 2020; **2020**: 5341247 [PMID: 32714980 DOI: 10.1155/2020/5341247]

66 **Wang K**, Chen W, Zhang Z, Deng Y, Lian JQ, Du P, Wei D, Zhang Y, Sun XX, Gong L, Yang X, He L, Zhang L, Yang Z, Geng JJ, Chen R, Zhang H, Wang B, Zhu YM, Nan G, Jiang JL, Li L, Wu J, Lin P, Huang W, Xie L, Zheng ZH, Zhang K, Miao JL, Cui HY, Huang M, Zhang J, Fu L, Yang XM, Zhao Z, Sun S, Gu H, Wang Z, Wang CF, Lu Y, Liu YY, Wang QY, Bian H, Zhu P, Chen ZN. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther* 2020; **5**: 283 [PMID: 33277466 DOI: 10.1038/s41392-020-00426-x]

67 **Bao W**, Min D, Twigg SM, Shackel NA, Warner FJ, Yue DK, McLennan SV. Monocyte CD147 is induced by advanced glycation end products and high glucose concentration: possible role in diabetic complications. *Am J Physiol Cell Physiol* 2010; **299**: C1212-C1219 [PMID: 20810913 DOI: 10.1152/ajpcell.00228.2010]

68 **Garg M**, Angus PW, Burrell LM, Herath C, Gibson PR, Lubel JS. Review article: the pathophysiological roles of the renin-angiotensin system in the gastrointestinal tract. *Aliment Pharmacol Ther* 2012; **35**: 414-428 [PMID: 22221317 DOI: 10.1111/j.1365-2036.2011.04971.x]

69 **Khajah MA**, Fateel MM, Ananthalakshmi KV, Luqmani YA. Anti-Inflammatory Action of Angiotensin 1-7 in Experimental Colitis. *PLoS One* 2016; **11**: e0150861 [PMID: 26963721 DOI: 10.1371/journal.pone.0150861]

70 **Santos RAS**, Oudit GY, Verano-Braga T, Canta G, Steckelings UM, Bader M. The renin-angiotensin system: going beyond the classical paradigms. *Am J Physiol Heart Circ Physiol* 2019; **316**: H958-H970 [PMID: 30707614 DOI: 10.1152/ajpheart.00723.2018]

71 **Gaddam RR**, Chambers S, Bhatia M. ACE and ACE2 in inflammation: a tale of two enzymes. *Inflamm Allergy Drug Targets* 2014; **13**: 224-234 [PMID: 25019157 DOI: 10.2174/1871528113666140713164506]

72 **Mehta PK**, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007; **292**: C82-C97 [PMID: 16870827 DOI: 10.1152/ajpcell.00287.2006]

73 **Simões E Silva AC**, Teixeira MM. ACE inhibition, ACE2 and angiotensin-(1-7) axis in kidney and cardiac inflammation and fibrosis. *Pharmacol Res* 2016; **107**: 154-162 [PMID: 26995300 DOI: 10.1016/j.phrs.2016.03.018]

74 **Tan WSD**, Liao W, Zhou S, Mei D, Wong WF. Targeting the renin-angiotensin system as novel therapeutic strategy for pulmonary diseases. *Curr Opin Pharmacol* 2018; **40**: 9-17 [PMID: 29288933 DOI: 10.1016/j.coph.2017.12.002]

75 **Fyhrquist F**, Saijonmaa O. Renin-angiotensin system revisited. *J Intern Med* 2008; **264**: 224-236 [PMID: 18793332 DOI: 10.1111/j.1365-2796.2008.01981.x]

76 **Suzuki Y**, Ruiz-Ortega M, Lorenzo O, Ruperez M, Esteban V, Egido J. Inflammation and angiotensin II. *Int J Biochem Cell Biol* 2003; **35**: 881-900 [PMID: 12676174 DOI: 10.1016/s1357-2725(02)00271-6]

77 **Husain K**, Hernandez W, Ansari RA, Ferder L. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. *World J Biol Chem* 2015; **6**: 209-217 [PMID: 26322175 DOI: 10.4331/wjbc.v6.i3.209]

78 **Capettini LS**, Montecucco F, Mach F, Stergiopulos N, Santos RA, da Silva RF. Role of renin-angiotensin system in inflammation, immunity and aging. *Curr Pharm Des* 2012; **18**: 963-970 [PMID: 22283774 DOI: 10.2174/138161212799436593]

79 **Donoghue M**, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000; **87**: E1-E9 [PMID: 10969042 DOI: 10.1161/01.res.87.5.e1]

80 **Ruiz-Ortega M**, Ruperez M, Esteban V, Rodriguez-Vita J, Sanchez-Lopez E, Egido J. Modulation of angiotensin II effects, A potential novel approach to inflammatory and immune diseases. *Curr Med Chem* 2003; **2**: 379-394 [DOI: 10.2174/1568014033483626]

81 **Garg M**, Royce SG, Tikellis C, Shallue C, Batu D, Velkoska E, Burrell LM, Patel SK, Beswick L, Jackson A, Britto K, Lukies M, Sluka P, Wardan H, Hirokawa Y, Tan CW, Faux M, Burgess AW, Hosking P, Monagle S, Thomas M, Gibson PR, Lubel J. Imbalance of the renin-angiotensin system may contribute to inflammation and fibrosis in IBD: a novel therapeutic target? *Gut* 2020; **69**: 841-851 [PMID: 31409604 DOI: 10.1136/gutjnl-2019-318512]

82 **Santos RAS**, Sampaio WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, Campagnole-Santos MJ. The ACE2/Angiotensin-(1-7)/MAS Axis of the Renin-Angiotensin System: Focus on Angiotensin-(1-7). *Physiol Rev* 2018; **98**: 505-553 [PMID: 29351514 DOI: 10.1152/physrev.00023.2016]

83 **Patel S**, Rauf A, Khan H, Abu-Izneid T. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. *Biomed Pharmacother* 2017; **94**: 317-325 [PMID: 28772209 DOI: 10.1016/j.biopha.2017.07.091]

84 **Garg M**, Burrell LM, Velkoska E, Griggs K, Angus PW, Gibson PR, Lubel JS. Upregulation of circulating components of the alternative renin-angiotensin system in inflammatory bowel disease: A pilot study. *J Renin Angiotensin Aldosterone Syst* 2015; **16**: 559-569 [PMID: 24505094 DOI: 10.1177/1470320314521086]

85 **Hirasawa K**, Sato Y, Hosoda Y, Yamamoto T, Hanai H. Immunohistochemical localization of angiotensin II receptor and local renin-angiotensin system in human colonic mucosa. *J Histochem Cytochem* 2002; **50**: 275-282 [PMID: 11799146 DOI: 10.1177/002215540205000215]

86 **Ferreira-Duarte M**, Estevinho MM, Duarte-Araújo M, Magro F, Morato M. Unraveling the Role of ACE2, the Binding Receptor for SARS-CoV-2, in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2020; **26**: 1787-1795 [PMID: 33064147 DOI: 10.1093/ibd/izaa249]

87 **Jacobs JD**, Wagner T, Gulotta G, Liao C, Li YC, Bissonnette M, Pekow J. Impact of Angiotensin II Signaling Blockade on Clinical Outcomes in Patients with Inflammatory Bowel Disease. *Dig Dis Sci* 2019; **64**: 1938-1944 [PMID: 30725290 DOI: 10.1007/s10620-019-5474-4]

88 **Wengrower D**, Zanninelli G, Pappo O, Latella G, Sestieri M, Villanova A, Faitelson Y, Pines M, Goldin E. Prevention of fibrosis in experimental colitis by captopril: the role of tgf-beta1. *Inflamm Bowel Dis* 2004; **10**: 536-545 [PMID: 15472513 DOI: 10.1097/00054725-200409000-00007]

89 **Mantaka A**, Tsoukali E, Fragkaki M, Karmiris K, Viazis N, Mantzaris GJ, Koutroubakis IE. Is there any role of renin-angiotensin system inhibitors in modulating inflammatory bowel disease outcome? *Eur J Gastroenterol Hepatol* 2021; **33**: 364-371 [PMID: 32925506 DOI: 10.1097/MEG.0000000000001912]

90 **Wang Q**, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY, Wang Q, Zhou H, Yan J, Qi J. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell* 2020; **181**: 894-904. e9 [PMID: 32275855 DOI: 10.1016/j.cell.2020.03.045]

91 **Colón AL**, Menchén LA, Hurtado O, De Cristóbal J, Lizasoain I, Leza JC, Lorenzo P, Moro MA. Implication of TNF-alpha convertase (TACE/ADAM17) in inducible nitric oxide synthase expression and inflammation in an experimental model of colitis. *Cytokine* 2001; **16**: 220-226 [PMID: 11884025 DOI: 10.1006/cyto.2001.0969]

92 **Brynskov J**, Foegh P, Pedersen G, Ellervik C, Kirkegaard T, Bingham A, Saermark T. Tumour necrosis factor alpha converting enzyme (TACE) activity in the colonic mucosa of patients with inflammatory bowel disease. *Gut* 2002; **51**: 37-43 [PMID: 12077089 DOI: 10.1136/gut.51.1.37]

93 **He L**, Du J, Chen Y, Liu C, Zhou M, Adhikari S, Rubin DT, Pekow J, Li YC. Renin-angiotensin system promotes colonic inflammation by inducing $T_H17$ activation *via* JAK2/STAT pathway. *Am J Physiol Gastrointest Liver Physiol* 2019; **316**: G774-G784 [PMID: 30995068 DOI: 10.1152/ajpgi.00053.2019]

94 **De Francesco EM**, Vella V, Belfiore A. COVID-19 and Diabetes: The Importance of Controlling RAGE. *Front Endocrinol (Lausanne)* 2020; **11**: 526 [PMID: 32760352 DOI: 10.3389/fendo.2020.00526]

95 **Rojas A**, Gonzalez I, Morales MA. SARS-CoV-2-mediated inflammatory response in lungs: should we look at RAGE? *Inflamm Res* 2020; **69**: 641-643 [PMID: 32372149 DOI: 10.1007/s00011-020-01353-x]

96 **Bezzio C**, Saibeni S, Variola A, Allocca M, Massari A, Gerardi V, Casini V, Ricci C, Zingone F, Amato A, Caprioli F, Lenti MV, Viganò C, Ascolani M, Bossa F, Castiglione F, Cortelezzi C, Grossi L, Milla M, Morganti D, Pastorelli L, Ribaldone DG, Sartini A, Soriano A, Manes G, Danese S, Fantini MC, Armuzzi A, Daperno M, Fiorino G; Italian Group for the Study of Inflammatory Bowel Disease (IG-IBD). Outcomes of COVID-19 in 79 patients with IBD in Italy: an IG-IBD study. *Gut* 2020; **69**: 1213-1217 [PMID: 32354990 DOI: 10.1136/gutjnl-2020-321411]

97 **Burke KE**, Kochar B, Allegretti JR, Winter RW, Lochhead P, Khalili H, Colizzo FP, Hamilton MJ,

Chan WW, Ananthakrishnan AN. Immunosuppressive Therapy and Risk of COVID-19 Infection in Patients With Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2021; **27**: 155-161 [PMID: 33089863 DOI: 10.1093/ibd/izaa278]

98 **Occhipinti V**, Pastorelli L. Challenges in the Care of IBD Patients During the CoViD-19 Pandemic: Report From a "Red Zone" Area in Northern Italy. *Inflamm Bowel Dis* 2020; **26**: 793-796 [PMID: 32314792 DOI: 10.1093/ibd/izaa084]

99 **Tursi A**, Angarano G, Monno L, Saracino A, Signorile F, Ricciardi A, Papa A. COVID-19 infection in Crohn's disease under treatment with adalimumab. *Gut* 2020; **69**: 1364-1365 [PMID: 32312788 DOI: 10.1136/gutjnl-2020-321240]

100 **Bezzio C**, Pellegrini L, Manes G, Arena I, Picascia D, Della Corte C, Devani M, Schettino M, Saibeni S. Biologic Therapies May Reduce the Risk of COVID-19 in Patients With Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2020; **26**: e107-e109 [PMID: 32869831 DOI: 10.1093/ibd/izaa242]

101 **Sigall-Boneh R**, Levine A, Lomer M, Wierdsma N, Allan P, Fiorino G, Gatti S, Jonkers D, Kierkus J, Katsanos KH, Melgar S, Yuksel ES, Whelan K, Wine E, Gerasimidis K. Research Gaps in Diet and Nutrition in Inflammatory Bowel Disease. A Topical Review by D-ECCO Working Group [Dietitians of ECCO]. *J Crohns Colitis* 2017; **11**: 1407-1419 [PMID: 28961811 DOI: 10.1093/ecco-jcc/jjx109]

102 **Uribarri J**, del Castillo MD, de la Maza MP, Filip R, Gugliucci A, Luevano-Contreras C, Macías-Cervantes MH, Markowicz Bastos DH, Medrano A, Menini T, Portero-Otin M, Rojas A, Sampaio GR, Wrobel K, Garay-Sevilla ME. Dietary advanced glycation end products and their role in health and disease. *Adv Nutr* 2015; **6**: 461-473 [PMID: 26178030 DOI: 10.3945/an.115.008433]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2281-2298

DOI: 10.3748/wjg.v27.i19.2281

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

*REVIEW*

# Individualized treatment options for patients with non-cirrhotic and cirrhotic liver disease

Lukas Hartl, Joshua Elias, Gerhard Prager, Thomas Reiberger, Lukas W Unger

**ORCID number:** Lukas Hartl 0000-0003-3398-6120; Joshua Elias 0000-0003-2137-117X; Gerhard Prager 0000-0003-0257-4844; Thomas Reiberger 0000-0002-4590-3583; Lukas W Unger 0000-0002-8423-2268.

**Author contributions:** Hartl L, Elias J and Unger LW performed literature review, prepared figures and tables and wrote the manuscript; Prager G and Reiberger T gave important intellectual input; all authors contributed intellectually, critically revised the manuscript and approved the final version of the manuscript.

**Supported by** Austrian Science Fund FWF, No. J4396; and Wellcome Trust PhD Fellowship for Clinicians, No. UNS59491.

**Conflict-of-interest statement:** LH, JE, GP and LWU declare no conflicts of interest related to this manuscript. TR received grant support from Abbvie, Boehringer-Ingelheim, Gilead, MSD, Philips Healthcare, Gore; speaking honoraria from Abbvie, Gilead, Gore, Intercept, Roche, MSD; consulting/advisory board fee from Abbvie, Bayer, Boehringer-Ingelheim, Gilead, Intercept, MSD, Siemens; and travel support from Abbvie, Boehringer-Ingelheim, Gilead and Roche.

**Lukas Hartl, Thomas Reiberger,** Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna A-1090, Austria

**Lukas Hartl, Thomas Reiberger,** Vienna Hepatic Hemodynamic Lab, Medical University of Vienna, Vienna A-1090, Austria

**Joshua Elias, Lukas W Unger,** Cambridge Institute of Therapeutic Immunology and Infectious Disease, Jeffrey Cheah Biomedical Centre, University of Cambridge, Cambridge CB2 0AW, United Kingdom

**Joshua Elias,** Division of Gastroenterology and Hepatology, Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge CB2 0QQ, United Kingdom

**Gerhard Prager, Lukas W Unger,** Division of Visceral Surgery, Department of General Surgery, Medical University of Vienna, Vienna A-1090, Austria

**Corresponding author:** Lukas W Unger, MD, PhD, Doctor, Division of Visceral Surgery, Department of General Surgery, Medical University of Vienna, Währinger Gürtel 18-20, Vienna A-1090, Austria. lukas.unger@meduniwien.ac.at

## Abstract

The obesity pandemic has led to a significant increase in patients with metabolic dysfunction-associated fatty liver disease (MAFLD). While dyslipidemia, type 2 diabetes mellitus and cardiovascular diseases guide treatment in patients without signs of liver fibrosis, liver related morbidity and mortality becomes relevant for MAFLD's progressive form, non-alcoholic steatohepatitis (NASH), and upon development of liver fibrosis. Statins should be prescribed in patients without significant fibrosis despite concomitant liver diseases but are underutilized in the real-world setting. Bariatric surgery, especially Y-Roux bypass, has been proven to be superior to conservative and/or medical treatment for weight loss and resolution of obesity-associated diseases, but comes at a low but existent risk of surgical complications, reoperations and very rarely, paradoxical progression of NASH. Once end-stage liver disease develops, obese patients benefit from liver transplantation (LT), but may be at increased risk of perioperative infectious complications. After LT, metabolic comorbidities are commonly observed, irrespective of the underlying liver disease, but MAFLD/NASH patients are at even higher risk of disease recurrence. Few studies with low patient numbers evaluated if, and when, bariatric surgery may be an option to avoid disease recurrence but more high-quality studies are needed to establish clear recom-

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** Austria

**Peer-review report's scientific quality classification**

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

**Received:** January 24, 2021
**Peer-review started:** January 24, 2021
**First decision:** March 7, 2021
**Revised:** March 19, 2021
**Accepted:** April 25, 2021
**Article in press:** April 25, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Kwan H
**S-Editor:** Fan JR
**L-Editor:** A
**P-Editor:** Ma YJ

mendations. In this review, we summarize the most recent literature on treatment options for MAFLD and NASH and highlight important considerations to tailor therapy to individual patient's needs in light of their risk profile.

**Key Words:** Metabolic dysfunction-associated fatty liver disease; Non-alcoholic fatty liver disease; Portal hypertension; Cirrhosis; Bariatric surgery; Metabolism

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** No single therapy fits all needs, sometimes resulting in complex clinical decision making. While some etiologies can distinctly be characterized, a multifactorial disease such as metabolic dysfunction-associated fatty liver disease requires thorough assessment of comorbidities and severity of concomitant fibrosis to assess a patient's overall risk. While (guided) physical exercise is usually safe and well tolerated and strict treatment of diabetes and dyslipidemia is warranted, patients often fail to change their lifestyle, resulting in life-long drug dependency for comorbidities. Bariatric surgery has therefore become a valid option for obese patients and should be offered in eligible patients before liver fibrosis develops.

**Citation:** Hartl L, Elias J, Prager G, Reiberger T, Unger LW. Individualized treatment options for patients with non-cirrhotic and cirrhotic liver disease. *World J Gastroenterol* 2021; 27(19): 2281-2298
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2281.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2281

## INTRODUCTION

In recent decades, the fractional contribution of different etiologies to the total burden of chronic liver disease (CLD) has shifted. On the one hand these changes are driven by a decrease in hepatitis C virus (HCV) related morbidity which has decreased by 40% in the United States[1] and led to HCV becoming a less common indication for liver transplantation (LT) in Europe[2], a trend that will likely be seen globally in the near future. On the other hand there is a steady and significant increase in non-alcoholic fatty liver disease (NAFLD), overall resulting in a relative shift of CLD etiologies, and an even further absolute increase in NAFLD related morbidity. While HCV related liver disease is a domain of hepatologists and transplant units, NAFLD, recently proposed to be re-named metabolic dysfunction-associated fatty liver disease (MAFLD)[3,4], is associated with extrahepatic diseases, such as central obesity[5], sleep apnea, type 2 diabetes mellitus (T2DM), cardiovascular diseases, and bone and joint disorders, all contributing to relevant morbidity and affecting different specialties[6].

Mirroring the obesity pandemic and in line with CLD etiology shifts, the number of LTs due to non-alcoholic steatohepatitis (NASH)-related cirrhosis, which results from progression of MAFLD, has markedly increased[1,2,7,8], with NASH already representing the second most frequent cause for LT in the United States[1,9,10]. In addition, the prevalence of hepatocellular carcinoma due to NASH is also rapidly increasing [2,8,11,12], probably resulting in an even higher need for LT due to MAFLD/NASH in the future. Thus, this review summarizes current treatment options in MAFLD, tailored to individual patient's disease stage in light of the most recent evidence. We provide a short overview of the core messages in Figure 1 and highlight several studies on the most important topics, which are discussed in further detail below, in Table 1.

## NAFLD/MAFLD AND THE METABOLIC SYNDROME

MAFLD is commonly considered a hepatic manifestation of the metabolic syndrome (MS)[13,14]. It is defined as excessive hepatic fat accumulation with insulin resistance, steatosis in > 5% of hepatocytes in histological analysis (or > 5.6% by quantitative fat/water-selective magnetic resonance imaging or proton magnetic resonance

**Table 1 Overview of important studies concerning the management of metabolic dysfunction-associated fatty liver disease/non-alcoholic steatohepatitis patients**

| | Ref. | Study design | No. of patients | Liver disease | Main findings |
|---|---|---|---|---|---|
| Diet/physical exercise | Berzigotti *et al*[57], 2017 | Prospective, uncontrolled | 60 (50 completed the study) | Cirrhosis, BMI ≥ 26 kg/m$^2$, portal hypertension | Moderate exercise was safe in patients with compensated cirrhosis<br>Diet and moderate exercise reduced body weight and portal pressure<br>Weight loss ≥ 10% is associated with more pronounced portal pressure reduction |
| | Wong *et al*[55], 2018 | Randomized controlled trial | 154 | NAFLD | Regular exercise associated with significantly more frequent remission of NAFLD (assessed by proton-magnetic MR-spectroscopy)<br>NAFLD remission in 67% of non-overweight patients (baseline BMI < 25 kg/m$^2$) with lifestyle intervention |
| Dyslipidemia | Unger *et al*[150], 2019 | Retrospective | 1265 | CLD | 34.2% of non-advanced and 48.2% of advanced CLD patients did not receive guideline-conform statin therapy<br>Guideline-conform statin use was associated with improved overall survival in compensated, but not in decompensated CLD patients |
| | Abraldes *et al*[69], 2009 | Randomized controlled trial | 59 | Cirrhosis and portal hypertension | Simvastatin reduced portal pressure (-8.3) in both patients, who did and did not also receive beta-blockers<br>Simvastatin improved liver perfusion<br>The effects of simvastatin were additive to beta-adrenergic blockade |
| | Nelson *et al*[72], 2009 | Randomized controlled trial | 16 | NASH | Simvastatin reduced low-density lipoprotein by 26%<br>Simvastatin was well-tolerated<br>Simvastatin did not histologically improve NASH (but small sample size, only $n$ = 10 follow-up biopsies) |
| T2DM | Lavine *et al*[108], 2011 | Randomized controlled trial | 173 | NAFLD | Sustained ALT level reduction was similar in the metformin and placebo group<br>Metformin did not change the NAFLD activity score |
| | Cusi *et al*[102], 2016 | Randomized controlled trial | 101 | NASH and prediabetes/T2DM | Significantly more patients receiving pioglitazone (59%) resolved NASH compared to placebo (23%)<br>Pioglitazone improved fibrosis score (-0.9 *vs* placebo 0.0)<br>Pioglitazone improved insulin sensitivity in liver, muscle and adipose tissue |
| | Armstrong *et al*[104], 2016 | Randomized controlled trial | 52 | NASH | Significantly more patients receiving liraglutide (39%) resolved NASH compared to placebo (9%)<br>Significantly less patients receiving liraglutide (9%) exhibited fibrosis progression compared to placebo (36%)<br>Liraglutide was safe and well-tolerated |
| Bariatric surgery | Lassailly *et al*[114], 2015 | Prospective | 109 | NASH | NASH was resolved in 85% of patients one year after surgery and even in 94% with mild NASH before surgery (assessed *via* biopsy)<br>NASH persistence was higher in patients after gastric banding (30.4%) compared to gastric bypass (7.6%) |

| | | | | | |
|---|---|---|---|---|---|
| | Goossens *et al*[117], 2016 | Retrospective | 59 | NASH | NASH is an independent predictor of overall mortality after bariatric surgery<br><br>NASH may reduce the overall survival benefit of bariatric surgery |
| | Eilenberg *et al*[118], 2018 | Retrospective | 10 | NAFLD/NASH | Liver dysfunction, liver steatosis/fibrosis and cirrhosis may occur after bariatric surgery<br><br>Lengthening of the alimentary or common limb may lead to a clinical improvement in these patients |
| Post-LT | Krasnoff *et al*[151], 2006 | Randomized controlled trial | 151 | Post-LT | Exercise and dietary counseling intervention improved exercise capacity and self-reported general health<br><br>Adherence to the intervention was associated with positive trends in exercise capacity and body composition (% body fat) |
| | Zamora-Valdes *et al*[152], 2018 | Prospective | 29 | NAFLD/NASH/obese ACLD | Patients, who received sleeve gastrectomy at the time of LT had more pronounced and sustained weight loss<br><br>They also had a lower prevalences of hepatic steatosis, hypertension and insulin resistance 3 yr after LT |
| | Patel *et al*[139], 2019 | Retrospective | 495 | Post-LT | Statins were underused after LT (54.3% of patients with known coronary artery disease did not receive statin therapy)<br><br>Statin use was well-tolerated<br><br>Statin therapy was associated with improved overall survival |

BMI: Body mass index; NAFLD: Non-alcoholic fatty liver disease; CLD: Chronic liver disease; NASH: Non-alcoholic steatohepatitis; ALT: Alanine aminotransferase; T2DM: Type 2 diabetes mellitus; LT: Liver transplantation; ACLD: Advanced chronic liver disease.

spectroscopy), and exclusion of secondary causes as well as alcoholic fatty liver disease, *e.g.* daily alcohol consumption of < 30 g for men and < 20 g for women, commonly resulting in difficulties to differentiate between alcoholic fatty liver disease and MAFLD in retrospective studies[15]. The severity of MAFLD can vary, ranging from simple steatosis[16] to NASH with chronic inflammation and fibrosis to liver cirrhosis[17,18]. Unfortunately, NASH diagnosis can, to date, only be made histologically by presence of macrovesicular steatosis, ballooning degeneration of hepatocytes, scattered inflammation, and Mallory-Denk bodies[19]. This limitation has led to the search for alternative non-invasive diagnostic procedures that avoid the need for liver biopsy, reviewed by *e.g.* Paternostro *et al*[20], to identify patients that are most likely to suffer from liver-related complications[21].

Before significant fibrosis develops, however, several factors contribute to the development of MAFLD, such as nutrition[22-24], insulin resistance[25,26], adipokines [27], gut microbiota[28,29], and genetic as well as epigenetic factors[30,31]. The close association of energy metabolism and fatty liver disease is illustrated by the fact that MAFLD patients suffer from increased risk for cardiovascular disease[32,33], T2DM[34-36], as well as chronic kidney disease[37]. According to a meta-analysis by Younossi *et al*[38], 51.3% of NAFLD and 81.8% of NASH patients are obese, 22.5% and 43.6% suffer from T2DM, and 69.2% and 72.1% from dyslipidemia, respectively[38]. This indicates that neither of the diseases should be addressed in an isolated fashion as they impact each other and contribute to disease progression. Thus, MAFLD patients must be seen as metabolically multimorbid, which is reflected by increased cardiovascular mortality compared to liver-related mortality in individuals without significant liver fibrosis[38]. Once liver fibrosis develops, however, liver-related mortality becomes more relevant. Recent evidence from high quality studies suggests that concomitant fibrosis, and especially cirrhosis, rather than NASH *per se* significantly increase liver-related morbidity and mortality[39-41]. Thus, well-established tools such as transient elastography with adapted cutoff values may allow risk stratification, and identification of significant fibrosis should result in state-of-the-art therapy with a liver-centered approach[20].

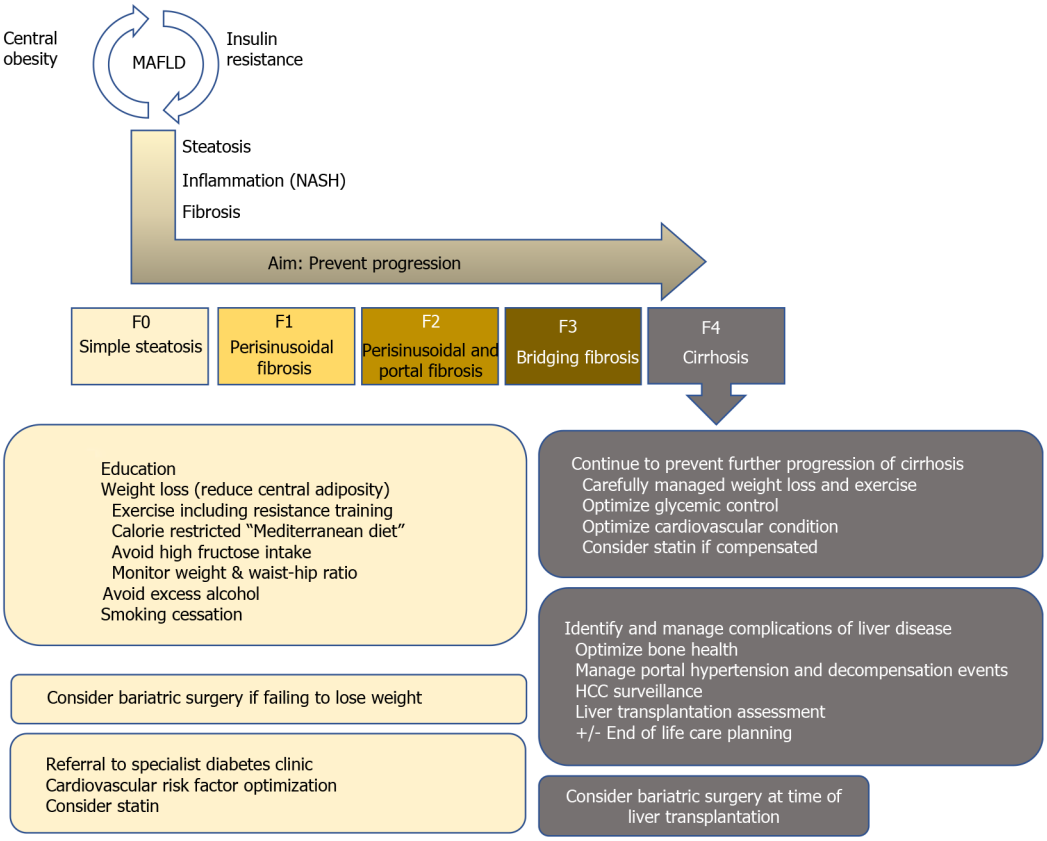



**Figure 1 Treatment recommendations based on liver fibrosis severity in metabolic dysfunction-associated fatty liver disease patients.**
HCC: Hepatocellular carcinoma; MAFLD: Metabolic dysfunction-associated fatty liver disease; NASH: Non-alcoholic steatohepatitis.

## THERAPEUTIC OPTIONS IN MAFLD/NASH

As mentioned above, the first step in risk stratification for individual patients should be assessment of presence/absence of liver fibrosis. In case of absence of liver fibrosis, regardless of the underlying etiology, removal of the damaging agent is vital to prevent development of fibrosis and subsequent portal-hypertensive decompensation events. In MAFLD, lifestyle modifications should be seen as the cornerstone of causative treatment, as obesity, high-fat diet and physical inactivity are strongly associated with development as well as progression of the disease[42]. Unfortunately, to date, no pharmacological treatment has specifically been approved for MAFLD, and current trials on drugs for MAFLD or NASH target mostly metabolic pathways to improve insulin resistance or dyslipidemia. As of 2018, more than 300 substances were in clinical trials for MAFLD/NASH[43,44]. However, the majority of trials have fallen short of proving efficacy and the most effective, to date, are repurposed drugs such as statins[45]. In terms of newly developed compounds, a recent prospective, placebo-controlled study of obeticholic acid (OCA), which is a farnesoid X receptor agonist that was shown to decrease hepatic fibrosis and reduce inflammation in preclinical studies, found that OCA improved fibrosis severity in patients with NASH[46]. Of note, however, complete NASH resolution was not more common in patients treated with either OCA dosing intensity (placebo: 8%; OCA 10 mg daily: 11%; OCA 25 mg daily, 12%), and overall fibrosis improvement was still only achieved in approximately 1/4 of patients (fibrosis improvement of ≥ 1 stage: Placebo: 12%, OCA 10 mg daily: 18%, and OCA 25 mg daily: 23%), highlighting the complexity of NASH treatment. Nevertheless, with this first successful trial, a broader repertoire of pharmacological agents will hopefully be available in the near future.

## OBESITY MANAGEMENT, DIET AND EXERCISE

Adequate therapy for obesity is of utmost relevance, as obesity *per se* independently increases the risk for cardiovascular disease[47] and independently predicted clinical decompensation in a subgroup-analysis of a placebo-controlled trial assessing beta

blockers for the prevention of esophageal varices, irrespective of the underlying etiology[48]. Furthermore, morbidly obese patients, defined as patients with a body mass index (BMI) ≥ 40 kg/m², have a significantly higher LT waiting list mortality, and benefit more from LT according to Schlansky *et al*[49], although the cause of death was not available from this United Network for Organ Sharing registry based study[49].

Lifestyle interventions are crucial, as a weight loss of 7%-10% of initial body weight is already associated with histological improvement in MAFLD with a reduction of steatosis, ballooning and lobular inflammation[50,51]. Even lower rates of sustained weight loss (about 5%) can decrease steatosis[52], liver enzymes[53] and the risk of developing T2DM[54]. Remission of MAFLD due to lifestyle interventions has also been demonstrated in non-obese patients with MAFLD[55] despite the fact that the underlying causes of lean MAFLD are unclear[56]. Guidelines suggest that the lifestyle modifications recommended to patients with MAFLD should be structured and include prescribed physical activity including resistance training, a calory restricted "Mediterranean" diet, avoidance of high fructose foods and avoidance of excess alcohol consumption. In addition, smoking cessation is important to improve the cardiovascular risk profile.

Both diet and exercise are safe in patients with compensated cirrhosis[57], have been shown to be highly effective for treatment of risk factors (cardiovascular disease and T2DM, respectively)[50,51,58], and lower portal pressure in overweight CLD patients regardless of etiology[57]. Importantly, however, recommendations for weight loss in obese NAFLD/NASH patients with cirrhosis are more cautious, as uncontrolled weight loss in decompensated patients may worsen sarcopenia and frailty[47]. Thus, diligent planning of diet and exercise is required to ensure weight loss with an adequate intake of nutrients, especially proteins. It should also be considered to be mandatory to investigate, whether patients have an indication for non-selective beta-blocker (NSBB) prophylaxis against variceal hemorrhage before enrollment into an exercise program, as NSBB counteract exercise-mediated increases of hepatic venous pressure gradient (HVPG)[59,60].

Importantly, evidence from a recently published randomized controlled trial suggests that once-weekly subcutaneous semaglutide leads to sustained and clinically relevant weight reduction (mean weight loss -14.9% in semaglutide-treated patients compared to -2.4% in the placebo group, respectively), with a more pronounced amelioration of cardiometabolic risk factors and patient-reported physical functioning in non-diabetic obese individuals[61]. Thus, these first encouraging results suggest that more effective pharmacological therapies may become available in the future.

## DYSLIPIDEMIA

Dyslipidemia is a major risk factor for the development and progression of atherosclerotic cardiovascular disease[62] and often presents as a comorbidity in patients with CLD[63]. Lipid profiles can be altered by liver diseases due to impaired cholesterol synthesis, leading to a seemingly improved lipid profile with CLD disease progression[63]. Nevertheless, pharmacologically, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibition *via* statins is by far the most important treatment option for dyslipidemia, leading to a decrease of systemic levels of low-density lipoprotein (LDL) cholesterol, as well as other pleiotropic effects[64]. Generally, statins are well-tolerated, however, 10%-15% of patients experience adverse events such as myalgia with or without increase of creatin kinase[64,65]. From a liver perspective, the long-standing dogma that statin therapy is contraindicated in patients with CLD has been proven to be outdated[63]. We and others could show that in real-life settings, statins are underutilized in CLD patients[63,66]. Despite clear indications for statin utilization to reduce cardiovascular morbidity and mortality, outlined in the American College of Cardiology/American Heart Association guidelines, we found that 34.2% of patients with non-advanced CLD and 48.2% patients with advanced CLD did not receive statins despite having a clear indication, and we found that guideline-conformed statin use translated to improved overall survival of compensated CLD, but not decompensated CLD patients[63]. Others have found that statins directly influence liver-specific outcome by lowering the risk of hepatic decompensation [67,68], potentially by reducing HVPG, improving hepatocyte function[69] and ameliorating sinusoidal endothelial dysfunction[70,71], overall indicating that statins should at least be prescribed in patients with non-cirrhotic CLD with cardiovascular risk profiles. In a small pilot trial, simvastatin did improve lipid profiles, but did not affect steatosis levels and necroinflammation in 16 NASH patients. However, it also

did not do any harm although results have to be interpreted with caution due to the small sample size[72].

Overall, most studies have found that, if adhering to available guidelines for statin initiation in patients without decompensated liver disease, adverse events rates are low, and the majority of studies reported beneficial effects of statins in compensated CLD, irrespective of CLD etiology[73-79].

## T2DM

An association between MAFLD and T2DM is well-established[80]. MAFLD and T2DM commonly coexist[81,82] and even in T2DM patients with normal serum alanine aminotransferase levels, the prevalence of liver steatosis is high[83]. Conversely, many studies demonstrated high rates of NASH in T2DM patients[84-86], and it has also been shown that T2DM is strongly associated with liver fibrosis[87-90]. Two studies based on liver histology found that MAFLD patients with T2DM commonly develop severe fibrosis, namely 40.3% and 41.0%, respectively[85,86]. Other studies, assessing liver stiffness by transient elastography, showed that 17.7% and 5.6% of diabetic patients suffer from advanced fibrosis[91,92]. This is of high importance, as liver fibrosis is the crucial factor associated with long-term outcome in MAFLD patients[93,94] and indeed, MAFLD and T2DM synergistically lead to an increased rate of adverse outcomes[95] including increased liver-related and overall mortality[96,97].

Thus, regulation of insulin sensitivity is essential in patients with MAFLD and there is growing evidence for pharmacological treatments that are effective for treating both T2DM and MAFLD[93]. Pioglitazone, an insulin sensitizer that stimulates adipocyte differentiation by peroxisome proliferator-activated receptor *g* agonism[98], has, for example, shown beneficial effects on NAFLD. Pioglitazone reduced biopsy-assessed NAFLD severity and liver fat content in patients with[99], but also without T2DM[100] upon short-term treatment. Moreover, a randomized controlled trial showed significantly more frequent resolution of NASH in patients treated with pioglitazone (34%) than with placebo (19%)[101]. However, fibrosis was not ameliorated and also insulin resistance only partially decreased, which may be attributable to the low administered pioglitazone dose of 30 mg *per* day[93]. In another randomized controlled trial in NASH patients with T2DM or prediabetes, 45 mg pioglitazone *per* day improved histological NAFLD activity score, fibrosis and insulin sensitivity[102]. Importantly, side effects of pioglitazone include weight gain, fluid retention with increased risk of congestive heart failure, as well as decrease of bone mineral density, resulting in atypical fractures[98], which has to be actively screened for when prescribing pioglitazone in MAFLD patients.

Glucagon-like peptide-1 (GLP-1) receptor agonists also represent a valuable treatment option for patients with MAFLD, as they improve glucose-dependent insulin secretion, but also promote weight loss and lower liver transaminase levels[103]. In a pilot trial, subcutaneous liraglutide decreased liver fat content and was associated with more frequent NASH resolution, as compared to placebo (39% *vs* 9%)[104]. In contrast, metformin, the first-line T2DM medication, does not consistently improve hepatic steatosis or inflammation in patients with NASH[105-109]. Overall, however, antidiabetic drugs show great promise for treatment of MAFLD/NASH (and weight loss) but more adequately designed randomized controlled trials, are needed.

## BARIATRIC SURGERY AND MAFLD/NASH REGRESSION

As mentioned above, the co-existence of several metabolic diseases, summarized as metabolic syndrome, has led to the development of invasive/surgical treatment options. While bariatric surgery was a niche phenomenon for several years, its benefit with regards to weight loss and subsequent improvement of insulin resistance/T2DM is established by now[110]. Moreover, due to improved success rates with regards to weight loss compared to conservative approaches, bariatric surgery patients have a significantly better 10-year[111] and 20-year overall survival than comparable patients that were treated conservatively, although, despite this improvement, their life expectancy is still lower than the general population's[112]. Recent evidence suggests that the major benefit results from weight loss itself and is not attributed to any other metabolic effects of bypass surgery. These assumptions come from a study that compared patients with Y-Roux bypass to patients who lost the same amount of

weight by dietary/lifestyle changes and observed similar effects, indicating that bypass surgery *per se* does not alter metabolism more than weight loss itself[113]. In terms of liver-specific outcomes, bariatric surgery has not been taken into account as treatment option in several meta-analyses on NASH resolution, despite available properly designed studies. In general, bariatric surgery results in resolution of NASH in the majority of patients (85% in a study by Lassailly *et al*[114], with 64.2% of patients undergoing bypass surgery and 5.5% of sleeve gastrectomiy) and regression of fibrosis[114]. However, not all procedures are equal, and Y-Roux bypass is considered to be the most effective strategy for sustainable weight loss to date[115]. A recent hierarchical network meta-analysis included 48 high-quality trials and found that pioglitazone and Y-Roux gastric bypass had the best effect on improvement of NAFLD Activity Score[116], suggesting a causative connection between glucose metabolism and fatty liver development. While bariatric surgery impacts on NASH, NASH and liver fibrosis, expectedly, also impact on postoperative outcome after bariatric surgery[117]. This, again, highlights that metabolic diseases do not exist as isolated diseases but must be treated together. Importantly, bariatric surgery is only offered to severely obese patients, while the general population is often overweight, but not obese, and thus not eligible for surgery, warranting further basic research studies disentangling the mechanisms of MAFLD/NASH development. Noteworthy, a very small fraction of patients develops NASH or suffers from NASH/fibrosis aggravation after bariatric surgery, requiring adequate post-operative care for early detection of complications and further emphasizing the need for ongoing research[118]. Considering that bariatric surgery is increasingly utilized, prospective studies answering the remaining questions on the connection of insulin resistance, fatty liver, and fibrosis progression should become available in the near future.

## OBESITY AND MAFLD/NASH BEFORE AND AFTER LT

In general, patients with cirrhosis/end-stage liver disease should be managed according to available guidelines for the treatment of portal hypertension, as liver-related mortality is the main cause of death in end-stage liver disease, with special regard to the above-mentioned pitfalls in obese patients[119]. According to the 2018 Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients LT report, 36.9% of adult patients undergoing LT were obese [BMI (30 kg/m$^2$)] including 14.8% with a BMI of more than 35 (kg/m$^2$)[120]. Despite the caveat that BMI is not an ideal parameter in patients with end-stage liver disease due to ascites, these data still highlight obesity as an important comorbidity in LT. Due to increasing experience in treatment of these patients, morbid obesity [BMI (40 kg/m$^2$)] is no longer seen as a contraindication for LT[47], as morbidly obese patients clearly profit from LT[49,121]. However, specific challenges include technical difficulties during surgery, as well as higher morbidity in the postoperative course, especially due to an increased risk of infections[122-125]. Ultimately, these challenges translate to an increased 30 d mortality[126]. However, outcomes seem to be gradually improving, as Schlansky *et al*[49] could detect impaired post-OP survival before but not after 2007[49]. In terms of long-term outcomes of NASH LT recipients, survival rates are comparable to other etiologies despite the fact that Malik *et al*[127] found an alarming 50% 1-year mortality rate among obese NASH patients ≥ 60 years old with T2DM and arterial hypertension[127]. Thus, pre-transplant work-up warrants extensive risk-benefit evaluation on a case-to-case basis before listing for LT to avoid unexpected complications[128].

Following LT, weight gain is common irrespective of the underlying CLD and type of transplanted organ. In general, approximately one in three LT recipients becomes overweight or obese within 3 years[129] and decreased physical activity, excess energy intake and older age favor development of sarcopenic obesity with increased risk of cardiovascular and metabolic comorbidities[130,131]. Although a clear research agenda has been set out in 2014 by the American Society for Transplantation[132], outcome measures are heterogeneous, and liver transplant recipients are underrepresented in these studies. A recent review of 2 observational and 3 randomized controlled trials by Dunn *et al*[133] reported that exercise intervention groups generally performed better at strength testing, energy expenditure in metabolic equivalents, and peak or maximal oxygen uptake[133]. An even more recently published prospective study reported that financial incentives resulted in more patients achieving their target of > 7000 steps *per* day, which, however, did not translate into less weight gain[134]. Another study using a smartphone app found that

35% of participants significantly increased their physical performance, but did not report whether this translated into an outcome benefit[135]. Thus, despite positive impacts on surrogate parameters, little to no high-quality evidence is available on whether exercise directly affects overall survival or liver related outcome after transplantation.

Similar to a lack of high-quality data on exercise programs, more prospective studies are needed to evaluate the effect of bariatric surgery at the time of LT. Recently, a meta-analysis of available studies on bariatric surgery during or after LT found that sleeve gastrectomy is the most commonly performed procedure and that bariatric surgery-related morbidity and mortality rates were 37% and 0.6%, respectively. Regarding outcome parameters, BMI was significantly lower in bariatric surgery patients 2 years after LT, with significantly lower rates of arterial hypertension and diabetes mellitus[136]. Of note, however, prospective randomized studies are needed to compare whether the benefits outweigh the risks in terms of overall outcome, which poses several difficulties in this setting.

In addition to weight gain, prevalence of dyslipidemia is high in the post-LT setting and affects approximately 40%-70%[137]. Partly, dyslipidemia and impaired glucose tolerance are metabolic adverse effects of immunosuppressants such as calcineurin inhibitors, mammalian target of rapamycin inhibitors and corticosteroids[8,138]. Thus, statins are commonly used after LT, however, data regarding statin therapy and potential effects on portal pressure and hepatocyte function in the post-transplant setting are scarce and a clear guideline for post-transplant statin use is not available[138]. Nevertheless, it has been shown that dyslipidemia is linked to increased morbidity and mortality in LT recipients and recently, a study by Patel *et al*[139] demonstrated good tolerance of statins and a survival benefit of statin-treated patients after LT, favoring statin use also in this setting[139]. Moreover, experimental studies in rats have demonstrated a graft-protecting effect of statins, when added to the cold storage solution[140,141]. Overall, prospective high-quality studies defining cut-offs are lacking, but available evidence suggests beneficial effects of statins in the post-LT setting.

Despite ameliorated glycogen synthesis, only few patients exhibit improved insulin sensitivity after LT[8]. Contrarily, 10% to 30% of patients suffer from new onset T2DM after LT, which is linked to the use of corticosteroids and tacrolimus[142,143]. In the immediate post-transplant period, insulin is considered the safest and most effective choice for anti-hyperglycemic therapy[144-147]. For the management of persistent T2DM after LT, however, evidence is scarce. A recent meta-analysis concluded that safety and efficacy cannot be concluded for various anti-hyperglycemic agents in the post-transplant setting, as the available studies are not of high enough quality[148]. Thus, anti-hyperglycemic therapy after the first-line metformin should be selected according to patient preference, as well as clinical characteristics such as presence of chronic kidney disease, heart failure or obesity[147,149].

## AUTHOR'S PERSPECTIVE

MAFLD/NASH is a complex disease entity that poses challenges for clinical practice and requires interdisciplinary management for optimal patient care. In recent years, several novel concepts have been established, and bariatric surgery has been proven to be an effective treatment option. Additionally, recent trial results suggest that novel therapeutics, or repurposed drugs, may be effective to improve MAFLD or achieve sustainable weight loss and potentially secondary improvement of MAFLD/NASH. Thus, the multifactorial nature of the disease and the interconnectedness of different aspects require up-to-date knowledge, especially as more therapeutics will likely become available. These developments require an individualized treatment plan and should be based on patients' preferences, as compliance is of utmost importance.

In patients with advanced CLD or end-stage NASH, eligibility assessment for LT should be conducted in due time. Once patients undergo orthotopic LT, metabolic comorbidities should be closely monitored and adequately treated. In the future, the special metabolic vulnerability of LT patients will become even more relevant, as NASH as indication for LT is rapidly increasing, emphasizing the importance of future trials in this special patient population.

## CONCLUSION

With the growing obesity epidemic and the rising prevalence of MAFLD/NASH, management of patients with CLD has become quite complex. MAFLD/NASH patients are often multimorbid, exhibiting various features of the metabolic syndrome, which altogether increase the risk of cardiovascular morbidity and mortality. In the early stages of liver disease without signs of liver fibrosis (MAFLD), management of comorbidities guides the therapy, while in patients who develop NASH and liver fibrosis, liver-related complications and mortality become relevant.

Unfortunately, there is a general lack of high-quality studies reporting important end points, such as fibrosis severity, which impedes comparability of the available results. Lifestyle interventions such as specific diets and exercise represent an etiological treatment for MAFLD/NASH patients and have been proven to be safe even for patients with cirrhosis and portal hypertension. Moreover, it has been shown that even moderate weight loss can lead to histological improvement, making lifestyle intervention an essential part of MAFLD/NASH management. Bariatric surgery is superior for weight loss of morbidly obese patients compared to conservative weight loss regimen, however, the risk of bariatric surgery is higher in patients with CLD and in some patients, severe liver dysfunction after bariatric surgery does occur.

Statins should be prescribed for all compensated patients with dyslipidemia or other risk factors like cardiovascular disease, but are heavily underutilized. While there is evidence that statin therapy is safe and also effective in MAFLD/NASH patients, large randomized controlled trials are still lacking. Concerning T2DM therapy, new anti-hyperglycemic agents such as pioglitazone or GLP-1 agonists are promising, but specific side effects may be detrimental and have to be considered. Metformin remains the first-line antihyperglycemic therapy.

Once end-stage liver disease has developed, obese patients benefit from LT, but also have increased perioperative risk, especially due to infections. After LT, metabolic complications are common. However, to date, there is little high-quality data concerning management of post-LT dyslipidemia and T2DM. Randomized controlled trials are needed to ensure the best possible care for these patient groups.

## REFERENCES


1 **Cotter TG**, Charlton M. Nonalcoholic Steatohepatitis After Liver Transplantation. *Liver Transpl* 2020; **26**: 141-159 [PMID: 31610081 DOI: 10.1002/lt.25657]

2 **Adam R**, Karam V, Cailliez V, O Grady JG, Mirza D, Cherqui D, Klempnauer J, Salizzoni M, Pratschke J, Jamieson N, Hidalgo E, Paul A, Andujar RL, Lerut J, Fisher L, Boudjema K, Fondevila C, Soubrane O, Bachellier P, Pinna AD, Berlakovich G, Bennet W, Pinzani M, Schemmer P, Zieniewicz K, Romero CJ, De Simone P, Ericzon BG, Schneeberger S, Wigmore SJ, Prous JF, Colledan M, Porte RJ, Yilmaz S, Azoulay D, Pirenne J, Line PD, Trunecka P, Navarro F, Lopez AV, De Carlis L, Pena SR, Kochs E, Duvoux C; all the other 126 contributing centers (www. eltr.org) and the European Liver and Intestine Transplant Association (ELITA). 2018 Annual Report of the European Liver Transplant Registry (ELTR) - 50-year evolution of liver transplantation. *Transpl Int* 2018; **31**: 1293-1317 [PMID: 30259574 DOI: 10.1111/tri.13358]

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

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

5 **Pang Q**, Zhang JY, Song SD, Qu K, Xu XS, Liu SS, Liu C. Central obesity and nonalcoholic fatty liver disease risk after adjusting for body mass index. *World J Gastroenterol* 2015; **21**: 1650-1662 [PMID: 25663786 DOI: 10.3748/wjg.v21.i5.1650]

6 **Younossi Z**, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, Wai-Sun Wong V, Yilmaz Y, George J, Fan J, Vos MB. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. *Hepatology* 2019; **69**: 2672-2682 [PMID: 30179269 DOI: 10.1002/hep.30251]

7 **Mathurin P**, Lucey MR. Liver transplantation in patients with alcohol-related liver disease: current status and future directions. *Lancet Gastroenterol Hepatol* 2020; **5**: 507-514 [PMID: 32277903 DOI: 10.1016/S2468-1253(19)30451-0]

8 **Pais R**, Barritt AS 4th, Calmus Y, Scatton O, Runge T, Lebray P, Poynard T, Ratziu V, Conti F.

NAFLD and liver transplantation: Current burden and expected challenges. *J Hepatol* 2016; **65**: 1245-1257 [PMID: 27486010 DOI: 10.1016/j.jhep.2016.07.033]

9 **Burra P**, Becchetti C, Germani G. NAFLD and liver transplantation: Disease burden, current management and future challenges. *JHEP Rep* 2020; **2**: 100192 [PMID: 33163950 DOI: 10.1016/j.jhepr.2020.100192]

10 **Wong RJ**, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology* 2015; **148**: 547-555 [PMID: 25461851 DOI: 10.1053/j.gastro.2014.11.039]

11 **Younossi Z**, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, Eguchi Y, Wong VW, Negro F, Yilmaz Y, Romero-Gomez M, George J, Ahmed A, Wong R, Younossi I, Ziayee M, Afendy A; Global Nonalcoholic Steatohepatitis Council. Nonalcoholic Steatohepatitis Is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. *Clin Gastroenterol Hepatol* 2019; **17**: 748-755. e3 [PMID: 29908364 DOI: 10.1016/j.cgh.2018.05.057]

12 **Rinella ME**. Nonalcoholic fatty liver disease: a systematic review. *JAMA* 2015; **313**: 2263-2273 [PMID: 26057287 DOI: 10.1001/jama.2015.5370]

13 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]

14 **Tilg H**, Effenberger M. From NAFLD to MAFLD: when pathophysiology succeeds. *Nat Rev Gastroenterol Hepatol* 2020; **17**: 387-388 [PMID: 32461575 DOI: 10.1038/s41575-020-0316-6]

15 **European Association for the Study of the Liver (EASL)**. European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

16 **Papatheodoridi M**, Cholongitas E. Diagnosis of Non-alcoholic Fatty Liver Disease (NAFLD): Current Concepts. *Curr Pharm Des* 2018; **24**: 4574-4586 [PMID: 30652642 DOI: 10.2174/1381612825666190117102111]

17 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]

18 **Fleming KA**, Morton JA, Barbatis C, Burns J, Canning S, McGee JO. Mallory bodies in alcoholic and non-alcoholic liver disease contain a common antigenic determinant. *Gut* 1981; **22**: 341-344 [PMID: 6166516 DOI: 10.1136/gut.22.5.341]

19 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ; Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

20 **Paternostro R**, Reiberger T, Bucsics T. Elastography-based screening for esophageal varices in patients with advanced chronic liver disease. *World J Gastroenterol* 2019; **25**: 308-329 [PMID: 30686900 DOI: 10.3748/wjg.v25.i3.308]

21 **Vilar-Gomez E**, Chalasani N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. *J Hepatol* 2018; **68**: 305-315 [PMID: 29154965 DOI: 10.1016/j.jhep.2017.11.013]

22 **Kechagias S**, Ernersson A, Dahlqvist O, Lundberg P, Lindström T, Nystrom FH; Fast Food Study Group. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. *Gut* 2008; **57**: 649-654 [PMID: 18276725 DOI: 10.1136/gut.2007.131797]

23 **Bergheim I**, Weber S, Vos M, Krämer S, Volynets V, Kaserouni S, McClain CJ, Bischoff SC. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. *J Hepatol* 2008; **48**: 983-992 [PMID: 18395289 DOI: 10.1016/j.jhep.2008.01.035]

24 **Moore JB**, Gunn PJ, Fielding BA. The role of dietary sugars and de novo lipogenesis in non-alcoholic fatty liver disease. *Nutrients* 2014; **6**: 5679-5703 [PMID: 25514388 DOI: 10.3390/nu6125679]

25 **Bugianesi E**, Moscatiello S, Ciaravella MF, Marchesini G. Insulin resistance in nonalcoholic fatty liver disease. *Curr Pharm Des* 2010; **16**: 1941-1951 [PMID: 20370677 DOI: 10.2174/138161210791208875]

26 **Guilherme A**, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008; **9**: 367-377 [PMID: 18401346 DOI: 10.1038/nrm2391]

27 **Kucukoglu O**, Sowa JP, Mazzolini GD, Syn WK, Canbay A. Hepatokines and adipokines in NASH-related hepatocellular carcinoma. *J Hepatol* 2021; **74**: 442-457 [PMID: 33161047 DOI: 10.1016/j.jhep.2020.10.030]

28 **Kolodziejczyk AA**, Zheng D, Shibolet O, Elinav E. The role of the microbiome in NAFLD and NASH. *EMBO Mol Med* 2019; **11** [PMID: 30591521 DOI: 10.15252/emmm.201809302]

29 **Safari Z**, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci* 2019; **76**: 1541-1558 [PMID: 30683985 DOI: 10.1007/s00018-019-03011-w]

30 **Eslam M**, Valenti L, Romeo S. Genetics and epigenetics of NAFLD and NASH: Clinical impact. *J Hepatol* 2018; **68**: 268-279 [PMID: 29122391 DOI: 10.1016/j.jhep.2017.09.003]

31 **Carlsson B**, Lindén D, Brolén G, Liljeblad M, Bjursell M, Romeo S, Loomba R. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2020; **51**: 1305-1320 [PMID: 32383295 DOI: 10.1111/apt.15738]

32 **Targher G**, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. *N Engl J Med* 2010; **363**: 1341-1350 [PMID: 20879883 DOI: 10.1056/NEJMra0912063]

33 **Targher G**, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: A meta-analysis. *J Hepatol* 2016; **65**: 589-600 [PMID: 27212244 DOI: 10.1016/j.jhep.2016.05.013]

34 **Yamada T**, Fukatsu M, Suzuki S, Wada T, Yoshida T, Joh T. Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup. *J Gastroenterol Hepatol* 2010; **25**: 352-356 [PMID: 19817963 DOI: 10.1111/j.1440-1746.2009.05998.x]

35 **Fan JG**, Li F, Cai XB, Peng YD, Ao QH, Gao Y. Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. *J Gastroenterol Hepatol* 2007; **22**: 1086-1091 [PMID: 17608855 DOI: 10.1111/j.1440-1746.2006.04781.x]

36 **Adams LA**, Waters OR, Knuiman MW, Elliott RR, Olynyk JK. NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: an eleven-year follow-up study. *Am J Gastroenterol* 2009; **104**: 861-867 [PMID: 19293782 DOI: 10.1038/ajg.2009.67]

37 **Mantovani A**, Petracca G, Beatrice G, Csermely A, Lonardo A, Schattenberg JM, Tilg H, Byrne CD, Targher G. Non-alcoholic fatty liver disease and risk of incident chronic kidney disease: an updated meta-analysis. *Gut* 2020 [PMID: 33303564 DOI: 10.1136/gutjnl-2020-323082]

38 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]

39 **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]

40 **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]

41 **Mann JP**, Carter P, Armstrong MJ, Abdelaziz HK, Uppal H, Patel B, Chandran S, More R, Newsome PN, Potluri R. Hospital admission with non-alcoholic fatty liver disease is associated with increased all-cause mortality independent of cardiovascular risk factors. *PLoS One* 2020; **15**: e0241357 [PMID: 33108366 DOI: 10.1371/journal.pone.0241357]

42 **Stavropoulos K**, Imprialos K, Pittaras A, Faselis C, Narayan P, Kokkinos P. Lifestyle Modifications in Non-Alcoholic Fatty Liver Disease and Non- Alcoholic Steatohepatitis. *Curr Vasc Pharmacol* 2018; **16**: 239-245 [PMID: 28637408 DOI: 10.2174/1570161115666170621080835]

43 **Drew L**. Drug development: Sprint finish. *Nature* 2017; **551**: S86-S89 [PMID: 32080575 DOI: 10.1038/d41586-017-06926-1]

44 **Eslam M**, Alvani R, Shiha G. Obeticholic acid: towards first approval for NASH. *Lancet* 2019; **394**: 2131-2133 [PMID: 31813639 DOI: 10.1016/S0140-6736(19)32963-0]

45 **Jalili R**, Somi MH, Hosseinifard H, Salehnia F, Ghojazadeh M, Makhdami N, Shirmohammadi M. The Evaluation of Effective Drugs for the Treatment of Non-Alcoholic Fatty Liver Disease: A Systematic Review and Network Meta-Analysis. *Adv Pharm Bull* 2020; **10**: 542-555 [PMID: 33072533 DOI: 10.34172/apb.2020.065]

46 **Younossi ZM**, Ratziu V, Loomba R, Rinella M, Anstee QM, Goodman Z, Bedossa P, Geier A, Beckebaum S, Newsome PN, Sheridan D, Sheikh MY, Trotter J, Knapple W, Lawitz E, Abdelmalek MF, Kowdley KV, Montano-Loza AJ, Boursier J, Mathurin P, Bugianesi E, Mazzella G, Olveira A, Cortez-Pinto H, Graupera I, Orr D, Gluud LL, Dufour JF, Shapiro D, Campagna J, Zaru L, MacConell L, Shringarpure R, Harrison S, Sanyal AJ; REGENERATE Study Investigators. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2019; **394**: 2184-2196 [PMID: 31813633 DOI: 10.1016/S0140-6736(19)33041-7]

47 **Moctezuma-Velazquez C**, Márquez-Guillén E, Torre A. Obesity in the Liver Transplant Setting. *Nutrients* 2019; **11** [PMID: 31652761 DOI: 10.3390/nu11112552]

48 **Berzigotti A**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Morillas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Groszmann RJ; Portal Hypertension Collaborative Group. Obesity is an independent risk factor for clinical decompensation in patients with cirrhosis. *Hepatology* 2011; **54**: 555-561 [PMID: 21567436 DOI: 10.1002/hep.24418]

49 **Schlansky B**, Naugler WE, Orloff SL, Enestvedt CK. Higher Mortality and Survival Benefit in Obese Patients Awaiting Liver Transplantation. *Transplantation* 2016; **100**: 2648-2655 [PMID: 27575690 DOI: 10.1097/TP.0000000000001461]

50 **Uusitupa M**. Lifestyle changes and cardiovascular risk reduction in diabetes. *Lancet Diabetes Endocrinol* 2016; **4**: 877-878 [PMID: 27595919 DOI: 10.1016/S2213-8587(16)30185-1]

51 **Peng L**, Wang J, Li F. Weight reduction for non-alcoholic fatty liver disease. *Cochrane Database Syst Rev* 2011; **6**: CD003619 [PMID: 21678341 DOI: 10.1002/14651858.CD003619.pub3]

52 **Lazo M**, Solga SF, Horska A, Bonekamp S, Diehl AM, Brancati FL, Wagenknecht LE, Pi-Sunyer FX, Kahn SE, Clark JM; Fatty Liver Subgroup of the Look AHEAD Research Group. Effect of a

12-month intensive lifestyle intervention on hepatic steatosis in adults with type 2 diabetes. *Diabetes Care* 2010; **33**: 2156-2163 [PMID: 20664019 DOI: 10.2337/dc10-0856]

53 **Suzuki A**, Lindor K, St Saver J, Lymp J, Mendes F, Muto A, Okada T, Angulo P. Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J Hepatol* 2005; **43**: 1060-1066 [PMID: 16140415 DOI: 10.1016/j.jhep.2005.06.008]

54 **Jensen MD**, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, Hu FB, Hubbard VS, Jakicic JM, Kushner RF, Loria CM, Millen BE, Nonas CA, Pi-Sunyer FX, Stevens J, Stevens VJ, Wadden TA, Wolfe BM, Yanovski SZ; American College of Cardiology/American Heart Association Task Force on Practice Guidelines; Obesity Society. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. *J Am Coll Cardiol* 2014; **63**: 2985-3023 [PMID: 24239920 DOI: 10.1016/j.jacc.2013.11.004]

55 **Wong VW**, Wong GL, Chan RS, Shu SS, Cheung BH, Li LS, Chim AM, Chan CK, Leung JK, Chu WC, Woo J, Chan HL. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. *J Hepatol* 2018; **69**: 1349-1356 [PMID: 30142427 DOI: 10.1016/j.jhep.2018.08.011]

56 **Unger LW**, Forstner B, Muckenhuber M, Scheuba K, Eigenbauer E, Scheiner B, Pfisterer N, Paternostro R, Trauner M, Mandorfer M, Reiberger T. Hepatic Steatosis in Lean Patients: Risk Factors and Impact on Mortality. *Dig Dis Sci* 2020; **65**: 2712-2718 [PMID: 31875288 DOI: 10.1007/s10620-019-06000-y]

57 **Berzigotti A**, Albillos A, Villanueva C, Genescá J, Ardevol A, Augustín S, Calleja JL, Bañares R, García-Pagán JC, Mesonero F, Bosch J; Ciberehd SportDiet Collaborative Group. Effects of an intensive lifestyle intervention program on portal hypertension in patients with cirrhosis and obesity: The SportDiet study. *Hepatology* 2017; **65**: 1293-1305 [PMID: 27997989 DOI: 10.1002/hep.28992]

58 **Romero-Gómez M**, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. *J Hepatol* 2017; **67**: 829-846 [PMID: 28545937 DOI: 10.1016/j.jhep.2017.05.016]

59 **Bandi JC**, García-Pagán JC, Escorsell A, François E, Moitinho E, Rodés J, Bosch J. Effects of propranolol on the hepatic hemodynamic response to physical exercise in patients with cirrhosis. *Hepatology* 1998; **28**: 677-682 [PMID: 9731558 DOI: 10.1002/hep.510280312]

60 **Albillos A**, Zamora J, Martínez J, Arroyo D, Ahmad I, De-la-Peña J, Garcia-Pagán JC, Lo GH, Sarin S, Sharma B, Abraldes JG, Bosch J, Garcia-Tsao G; Baveno Cooperation. Stratifying risk in the prevention of recurrent variceal hemorrhage: Results of an individual patient meta-analysis. *Hepatology* 2017; **66**: 1219-1231 [PMID: 28543862 DOI: 10.1002/hep.29267]

61 **Wilding JPH**, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, McGowan BM, Rosenstock J, Tran MTD, Wadden TA, Wharton S, Yokote K, Zeuthen N, Kushner RF; STEP 1 Study Group. Once-Weekly Semaglutide in Adults with Overweight or Obesity. *N Engl J Med* 2021; **384**: 989 [PMID: 33567185 DOI: 10.1056/NEJMoa2032183]

62 **Benjamin EJ**, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* 2017; **135**: e146-e603 [PMID: 28122885 DOI: 10.1161/CIR.0000000000000485]

63 **Unger LW**, Forstner B, Schneglberger S, Muckenhuber M, Eigenbauer E, Scheiner B, Mandorfer M, Trauner M, Reiberger T. Patterns and prevalence of dyslipidemia in patients with different etiologies of chronic liver disease. *Wien Klin Wochenschr* 2019; **131**: 395-403 [PMID: 31493100 DOI: 10.1007/s00508-019-01544-5]

64 **Cholesterol Treatment Trialists' (CTT) Collaboration**, Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; **376**: 1670-1681 [PMID: 21067804 DOI: 10.1016/S0140-6736(10)61350-5]

65 **Abd TT**, Jacobson TA. Statin-induced myopathy: a review and update. *Expert Opin Drug Saf* 2011; **10**: 373-387 [PMID: 21342078 DOI: 10.1517/14740338.2011.540568]

66 **Blais P**, Lin M, Kramer JR, El-Serag HB, Kanwal F. Statins Are Underutilized in Patients with Nonalcoholic Fatty Liver Disease and Dyslipidemia. *Dig Dis Sci* 2016; **61**: 1714-1720 [PMID: 26707137 DOI: 10.1007/s10620-015-4000-6]

67 **Wong JC**, Chan HL, Tse YK, Yip TC, Wong VW, Wong GL. Statins reduce the risk of liver decompensation and death in chronic viral hepatitis: a propensity score weighted landmark analysis. *Aliment Pharmacol Ther* 2017; **46**: 1001-1010 [PMID: 28940673 DOI: 10.1111/apt.14341]

68 **Kim RG**, Loomba R, Prokop LJ, Singh S. Statin Use and Risk of Cirrhosis and Related Complications in Patients With Chronic Liver Diseases: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2017; **15**: 1521-1530. e8 [PMID: 28479502 DOI: 10.1016/j.cgh.2017.04.039]

69 **Abraldes JG**, Albillos A, Bañares R, Turnes J, González R, García-Pagán JC, Bosch J. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled

trial. *Gastroenterology* 2009; **136**: 1651-1658 [PMID: 19208350 DOI: 10.1053/j.gastro.2009.01.043]

70 **La Mura V**, Pasarín M, Meireles CZ, Miquel R, Rodríguez-Vilarrupla A, Hide D, Gracia-Sancho J, García-Pagán JC, Bosch J, Abraldes JG. Effects of simvastatin administration on rodents with lipopolysaccharide-induced liver microvascular dysfunction. *Hepatology* 2013; **57**: 1172-1181 [PMID: 23184571 DOI: 10.1002/hep.26127]

71 **Marrone G**, Russo L, Rosado E, Hide D, García-Cardeña G, García-Pagán JC, Bosch J, Gracia-Sancho J. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. *J Hepatol* 2013; **58**: 98-103 [PMID: 22989565 DOI: 10.1016/j.jhep.2012.08.026]

72 **Nelson A**, Torres DM, Morgan AE, Fincke C, Harrison SA. A pilot study using simvastatin in the treatment of nonalcoholic steatohepatitis: A randomized placebo-controlled trial. *J Clin Gastroenterol* 2009; **43**: 990-994 [PMID: 19448566 DOI: 10.1097/MCG.0b013e31819c392e]

73 **Yang YH**, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, Chen PC. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. *J Hepatol* 2015; **63**: 1111-1117 [PMID: 26196278 DOI: 10.1016/j.jhep.2015.07.006]

74 **Mohanty A**, Tate JP, Garcia-Tsao G. Statins Are Associated With a Decreased Risk of Decompensation and Death in Veterans With Hepatitis C-Related Compensated Cirrhosis. *Gastroenterology* 2016; **150**: 430-40. e1 [PMID: 26484707 DOI: 10.1053/j.gastro.2015.10.007]

75 **Tsan YT**, Lee CH, Ho WC, Lin MH, Wang JD, Chen PC. Statins and the risk of hepatocellular carcinoma in patients with hepatitis C virus infection. *J Clin Oncol* 2013; **31**: 1514-1521 [PMID: 23509319 DOI: 10.1200/JCO.2012.44.6831]

76 **Simon TG**, Bonilla H, Yan P, Chung RT, Butt AA. Atorvastatin and fluvastatin are associated with dose-dependent reductions in cirrhosis and hepatocellular carcinoma, among patients with hepatitis C virus: Results from ERCHIVES. *Hepatology* 2016; **64**: 47-57 [PMID: 26891205 DOI: 10.1002/hep.28506]

77 **Huang YW**, Lee CL, Yang SS, Fu SC, Chen YY, Wang TC, Hu JT, Chen DS. Statins Reduce the Risk of Cirrhosis and Its Decompensation in Chronic Hepatitis B Patients: A Nationwide Cohort Study. *Am J Gastroenterol* 2016; **111**: 976-985 [PMID: 27166128 DOI: 10.1038/ajg.2016.179]

78 **Stojakovic T**, Claudel T, Putz-Bankuti C, Fauler G, Scharnagl H, Wagner M, Sourij H, Stauber RE, Winkler K, März W, Wascher TC, Trauner M. Low-dose atorvastatin improves dyslipidemia and vascular function in patients with primary biliary cirrhosis after one year of treatment. *Atherosclerosis* 2010; **209**: 178-183 [PMID: 19782361 DOI: 10.1016/j.atherosclerosis.2009.08.052]

79 **Cash WJ**, O'Neill S, O'Donnell ME, McCance DR, Young IS, McEneny J, McDougall NI, Callender ME. Randomized controlled trial assessing the effect of simvastatin in primary biliary cirrhosis. *Liver Int* 2013; **33**: 1166-1174 [PMID: 23672463 DOI: 10.1111/liv.12191]

80 **Marchesini G**, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; **107**: 450-455 [PMID: 10569299 DOI: 10.1016/s0002-9343(99)00271-5]

81 **Williamson RM**, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, Frier BM, Van Look LA, Johnston GI, Reynolds RM, Strachan MW; Edinburgh Type 2 Diabetes Study Investigators. Prevalence of and risk factors for hepatic steatosis and nonalcoholic Fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* 2011; **34**: 1139-1144 [PMID: 21478462 DOI: 10.2337/dc10-2229]

82 **Portillo-Sanchez P**, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, Subbarayan S, Webb A, Hecht J, Cusi K. High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. *J Clin Endocrinol Metab* 2015; **100**: 2231-2238 [PMID: 25885947 DOI: 10.1210/jc.2015-1966]

83 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798 [PMID: 18752331 DOI: 10.1002/hep.22429]

84 **Abrams GA**, Kunde SS, Lazenby AJ, Clements RH. Portal fibrosis and hepatic steatosis in morbidly obese subjects: A spectrum of nonalcoholic fatty liver disease. *Hepatology* 2004; **40**: 475-483 [PMID: 15368453 DOI: 10.1002/hep.20323]

85 **Goh GB**, Pagadala MR, Dasarathy J, Unalp-Arida A, Sargent R, Hawkins C, Sourianarayanane A, Khiyami A, Yerian L, Pai RK, Dasarathy S, McCullough AJ. Clinical spectrum of non-alcoholic fatty liver disease in diabetic and non-diabetic patients. *BBA Clin* 2015; **3**: 141-145 [PMID: 26675585 DOI: 10.1016/j.bbacli.2014.09.001]

86 **Bazick J**, Donithan M, Neuschwander-Tetri BA, Kleiner D, Brunt EM, Wilson L, Doo E, Lavine J, Tonascia J, Loomba R. Clinical Model for NASH and Advanced Fibrosis in Adult Patients With Diabetes and NAFLD: Guidelines for Referral in NAFLD. *Diabetes Care* 2015; **38**: 1347-1355 [PMID: 25887357 DOI: 10.2337/dc14-1239]

87 **Hossain N**, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, Goodman Z, Younossi Z. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1224-1229, 1229.e1-1229. e2 [PMID: 19559819 DOI: 10.1016/j.cgh.2009.06.007]

88 **Doycheva I**, Cui J, Nguyen P, Costa EA, Hooker J, Hofflich H, Bettencourt R, Brouha S, Sirlin CB, Loomba R. Non-invasive screening of diabetics in primary care for NAFLD and advanced fibrosis

by MRI and MRE. *Aliment Pharmacol Ther* 2016; **43**: 83-95 [PMID: 26369383 DOI: 10.1111/apt.13405]

89 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

90 **Loomba R**, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, Bass NM; Nonalcoholic Steatohepatitis Clinical Research Network. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. *Hepatology* 2012; **56**: 943-951 [PMID: 22505194 DOI: 10.1002/hep.25772]

91 **Kwok R**, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, Shu SS, Chan AW, Yeung MW, Chan JC, Kong AP, Wong VW. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. *Gut* 2016; **65**: 1359-1368 [PMID: 25873639 DOI: 10.1136/gutjnl-2015-309265]

92 **Koehler EM**, Plompen EP, Schouten JN, Hansen BE, Darwish Murad S, Taimr P, Leebeek FW, Hofman A, Stricker BH, Castera L, Janssen HL. Presence of diabetes mellitus and steatosis is associated with liver stiffness in a general population: The Rotterdam study. *Hepatology* 2016; **63**: 138-147 [PMID: 26171685 DOI: 10.1002/hep.27981]

93 **Tilg H**, Moschen AR, Roden M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 32-42 [PMID: 27729660 DOI: 10.1038/nrgastro.2016.147]

94 **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**: 389-97. e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]

95 **Hazlehurst JM**, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism* 2016; **65**: 1096-1108 [PMID: 26856933 DOI: 10.1016/j.metabol.2016.01.001]

96 **de Marco R**, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M. Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. *Diabetes Care* 1999; **22**: 756-761 [PMID: 10332677 DOI: 10.2337/diacare.22.5.756]

97 **Younossi ZM**, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. *Clin Gastroenterol Hepatol* 2004; **2**: 262-265 [PMID: 15017611 DOI: 10.1016/s1542-3565(04)00014-x]

98 **Phielix E**, Szendroedi J, Roden M. The role of metformin and thiazolidinediones in the regulation of hepatic glucose metabolism and its clinical impact. *Trends Pharmacol Sci* 2011; **32**: 607-616 [PMID: 21824668 DOI: 10.1016/j.tips.2011.06.006]

99 **Belfort R**, Harrison SA, Brown K, Darland C, Finch J, Hardies J, Balas B, Gastaldelli A, Tio F, Pulcini J, Berria R, Ma JZ, Dwivedi S, Havranek R, Fincke C, DeFronzo R, Bannayan GA, Schenker S, Cusi K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006; **355**: 2297-2307 [PMID: 17135584 DOI: 10.1056/NEJMoa060326]

100 **Aithal GP**, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, Austin AS, Freeman JG, Morgan L, Webber J. Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1176-1184 [PMID: 18718471 DOI: 10.1053/j.gastro.2008.06.047]

101 **Sanyal AJ**, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M, Clark J, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010; **362**: 1675-1685 [PMID: 20427778 DOI: 10.1056/NEJMoa0907929]

102 **Cusi K**, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, Tio F, Hardies J, Darland C, Musi N, Webb A, Portillo-Sanchez P. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. *Ann Intern Med* 2016; **165**: 305-315 [PMID: 27322798 DOI: 10.7326/M15-1774]

103 **Fruci B**, Giuliano S, Mazza A, Malaguarnera R, Belfiore A. Nonalcoholic Fatty liver: a possible new target for type 2 diabetes prevention and treatment. *Int J Mol Sci* 2013; **14**: 22933-22966 [PMID: 24264040 DOI: 10.3390/ijms141122933]

104 **Armstrong MJ**, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016; **387**: 679-690 [PMID: 26608256 DOI: 10.1016/S0140-6736(15)00803-X]

105 **Bugianesi E**, Gentilcore E, Manini R, Natale S, Vanni E, Villanova N, David E, Rizzetto M, Marchesini G. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2005; **100**: 1082-1090 [PMID: 15842582 DOI: 10.1111/j.1572-0241.2005.41583.x]

106 **Haukeland JW**, Konopski Z, Eggesbø HB, von Volkmann HL, Raschpichler G, Bjøro K, Haaland T, Løberg EM, Birkeland K. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol* 2009; **44**: 853-860 [PMID: 19811343 DOI: 10.1080/00365520902845268]

107 **Shields WW**, Thompson KE, Grice GA, Harrison SA, Coyle WJ. The Effect of Metformin and Standard Therapy versus Standard Therapy alone in Nondiabetic Patients with Insulin Resistance and Nonalcoholic Steatohepatitis (NASH): A Pilot Trial. *Therap Adv Gastroenterol* 2009; **2**: 157-163 [PMID: 21180541 DOI: 10.1177/1756283X09105462]

108 **Lavine JE**, Schwimmer JB, Van Natta ML, Molleston JP, Murray KF, Rosenthal P, Abrams SH, Scheimann AO, Sanyal AJ, Chalasani N, Tonascia J, Ünalp A, Clark JM, Brunt EM, Kleiner DE, Hoofnagle JH, Robuck PR; Nonalcoholic Steatohepatitis Clinical Research Network. Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the TONIC randomized controlled trial. *JAMA* 2011; **305**: 1659-1668 [PMID: 21521847 DOI: 10.1001/jama.2011.520]

109 **Torres DM**, Jones FJ, Shaw JC, Williams CD, Ward JA, Harrison SA. Rosiglitazone versus rosiglitazone and metformin versus rosiglitazone and losartan in the treatment of nonalcoholic steatohepatitis in humans: a 12-month randomized, prospective, open- label trial. *Hepatology* 2011; **54**: 1631-1639 [PMID: 21748770 DOI: 10.1002/hep.24558]

110 **Sjöström L**, Lindroos AK, Peltonen M, Torgerson J, Bouchard C, Carlsson B, Dahlgren S, Larsson B, Narbro K, Sjöström CD, Sullivan M, Wedel H; Swedish Obese Subjects Study Scientific Group. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* 2004; **351**: 2683-2693 [PMID: 15616203 DOI: 10.1056/NEJMoa035622]

111 **Sjöström L**, Narbro K, Sjöström CD, Karason K, Larsson B, Wedel H, Lystig T, Sullivan M, Bouchard C, Carlsson B, Bengtsson C, Dahlgren S, Gummesson A, Jacobson P, Karlsson J, Lindroos AK, Lönroth H, Näslund I, Olbers T, Stenlöf K, Torgerson J, Agren G, Carlsson LM; Swedish Obese Subjects Study. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med* 2007; **357**: 741-752 [PMID: 17715408 DOI: 10.1056/NEJMoa066254]

112 **Carlsson LMS**, Sjöholm K, Jacobson P, Andersson-Assarsson JC, Svensson PA, Taube M, Carlsson B, Peltonen M. Life Expectancy after Bariatric Surgery in the Swedish Obese Subjects Study. *N Engl J Med* 2020; **383**: 1535-1543 [PMID: 33053284 DOI: 10.1056/NEJMoa2002449]

113 **Yoshino M**, Kayser BD, Yoshino J, Stein RI, Reeds D, Eagon JC, Eckhouse SR, Watrous JD, Jain M, Knight R, Schechtman K, Patterson BW, Klein S. Effects of Diet versus Gastric Bypass on Metabolic Function in Diabetes. *N Engl J Med* 2020; **383**: 721-732 [PMID: 32813948 DOI: 10.1056/NEJMoa2003697]

114 **Lassailly G**, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, Raverdy V, Leteurtre E, Dharancy S, Louvet A, Romon M, Duhamel A, Pattou F, Mathurin P. Bariatric Surgery Reduces Features of Nonalcoholic Steatohepatitis in Morbidly Obese Patients. *Gastroenterology* 2015; **149**: 379-88; quiz e15 [PMID: 25917783 DOI: 10.1053/j.gastro.2015.04.014]

115 **Adams TD**, Davidson LE, Litwin SE, Kim J, Kolotkin RL, Nanjee MN, Gutierrez JM, Frogley SJ, Ibele AR, Brinton EA, Hopkins PN, McKinlay R, Simper SC, Hunt SC. Weight and Metabolic Outcomes 12 Years after Gastric Bypass. *N Engl J Med* 2017; **377**: 1143-1155 [PMID: 28930514 DOI: 10.1056/NEJMoa1700459]

116 **Panunzi S**, Maltese S, Verrastro O, Labbate L, De Gaetano A, Pompili M, Capristo E, Bornstein SR, Mingrone G. Pioglitazone and bariatric surgery are the most effective treatments for non-alcoholic steatohepatitis: A hierarchical network meta-analysis. *Diabetes Obes Metab* 2021; **23**: 980-990 [PMID: 33368954 DOI: 10.1111/dom.14304]

117 **Goossens N**, Hoshida Y, Song WM, Jung M, Morel P, Nakagawa S, Zhang B, Frossard JL, Spahr L, Friedman SL, Negro F, Rubbia-Brandt L, Giostra E. Nonalcoholic Steatohepatitis Is Associated With Increased Mortality in Obese Patients Undergoing Bariatric Surgery. *Clin Gastroenterol Hepatol* 2016; **14**: 1619-1628 [PMID: 26492845 DOI: 10.1016/j.cgh.2015.10.010]

118 **Eilenberg M**, Langer FB, Beer A, Trauner M, Prager G, Staufer K. Significant Liver-Related Morbidity After Bariatric Surgery and Its Reversal-a Case Series. *Obes Surg* 2018; **28**: 812-819 [PMID: 28965313 DOI: 10.1007/s11695-017-2925-x]

119 **Reiberger T**, Püspök A, Schoder M, Baumann-Durchschein F, Bucsics T, Datz C, Dolak W, Ferlitsch A, Finkenstedt A, Graziadei I, Hametner S, Karnel F, Krones E, Maieron A, Mandorfer M, Peck-Radosavljevic M, Rainer F, Schwabl P, Stadlbauer V, Stauber R, Tilg H, Trauner M, Zoller H, Schöfl R, Fickert P. Austrian consensus guidelines on the management and treatment of portal hypertension (Billroth III). *Wien Klin Wochenschr* 2017; **129**: 135-158 [PMID: 29063233 DOI: 10.1007/s00508-017-1262-3]

120 **Kwong A**, Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, Noreen SM, Foutz J, Miller E, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2018 Annual Data Report: Liver. *Am J Transplant* 2020; **20** Suppl s1: 193-299 [PMID: 31898413 DOI: 10.1111/ajt.15674]

121 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Liver transplantation. *J Hepatol* 2016; **64**: 433-485 [PMID: 26597456 DOI: 10.1016/j.jhep.2015.10.006]

122 **Hakeem AR**, Cockbain AJ, Raza SS, Pollard SG, Toogood GJ, Attia MA, Ahmad N, Hidalgo EL, Prasad KR, Menon KV. Increased morbidity in overweight and obese liver transplant recipients: a single-center experience of 1325 patients from the United Kingdom. *Liver Transpl* 2013; **19**: 551-562 [PMID: 23408499 DOI: 10.1002/lt.23618]

123 **LaMattina JC**, Foley DP, Fernandez LA, Pirsch JD, Musat AI, D'Alessandro AM, Mezrich JD. Complications associated with liver transplantation in the obese recipient. *Clin Transplant* 2012; **26**: 910-918 [PMID: 22694047 DOI: 10.1111/j.1399-0012.2012.01669.x]

124 **Sawyer RG**, Pelletier SJ, Pruett TL. Increased early morbidity and mortality with acceptable long-term function in severely obese patients undergoing liver transplantation. *Clin Transplant* 1999; **13**:

126-130 [PMID: 10081649 DOI: 10.1034/j.1399-0012.1999.130111.x]
125 **Sundaram V**, Kaung A, Rajaram A, Lu SC, Tran TT, Nissen NN, Klein AS, Jalan R, Charlton MR, Jeon CY. Obesity is independently associated with infection in hospitalised patients with end-stage liver disease. *Aliment Pharmacol Ther* 2015; **42**: 1271-1280 [PMID: 26510540 DOI: 10.1111/apt.13426]
126 **Barone M**, Viggiani MT, Losurdo G, Principi M, Leandro G, Di Leo A. Systematic review with meta-analysis: post-operative complications and mortality risk in liver transplant candidates with obesity. *Aliment Pharmacol Ther* 2017; **46**: 236-245 [PMID: 28488418 DOI: 10.1111/apt.14139]
127 **Malik SM**, deVera ME, Fontes P, Shaikh O, Ahmad J. Outcome after liver transplantation for NASH cirrhosis. *Am J Transplant* 2009; **9**: 782-793 [PMID: 19344467 DOI: 10.1111/j.1600-6143.2009.02590.x]
128 **Unger LW**, Mandorfer M, Reiberger T. Portal Hypertension after Liver Transplantation—Causes and Management. *Curr Hepatol Rep* 2019; **18**: 59-66 [DOI: 10.1007/s11901-019-00450-8]
129 **Richards J**, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. *Transpl Int* 2005; **18**: 461-466 [PMID: 15773968 DOI: 10.1111/j.1432-2277.2004.00067.x]
130 **Choudhary NS**, Saigal S, Saraf N, Mohanka R, Rastogi A, Goja S, Menon PB, Mishra S, Mittal A, Soin AS. Sarcopenic obesity with metabolic syndrome: a newly recognized entity following living donor liver transplantation. *Clin Transplant* 2015; **29**: 211-215 [PMID: 25594826 DOI: 10.1111/ctr.12505]
131 **Hong HC**, Hwang SY, Choi HY, Yoo HJ, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Relationship between sarcopenia and nonalcoholic fatty liver disease: the Korean Sarcopenic Obesity Study. *Hepatology* 2014; **59**: 1772-1778 [PMID: 23996808 DOI: 10.1002/hep.26716]
132 **Mathur S**, Janaudis-Ferreira T, Wickerson L, Singer LG, Patcai J, Rozenberg D, Blydt-Hansen T, Hartmann EL, Haykowsky M, Helm D, High K, Howes N, Kamath BM, Lands L, Marzolini S, Sonnenday C. Meeting report: consensus recommendations for a research agenda in exercise in solid organ transplantation. *Am J Transplant* 2014; **14**: 2235-2245 [PMID: 25135579 DOI: 10.1111/ajt.12874]
133 **Dunn MA**, Rogal SS, Duarte-Rojo A, Lai JC. Physical Function, Physical Activity, and Quality of Life After Liver Transplantation. *Liver Transpl* 2020; **26**: 702-708 [PMID: 32128971 DOI: 10.1002/lt.25742]
134 **Serper M**, Barankay I, Chadha S, Shults J, Jones LS, Olthoff KM, Reese PP. A randomized, controlled, behavioral intervention to promote walking after abdominal organ transplantation: results from the LIFT study. *Transpl Int* 2020; **33**: 632-643 [PMID: 31925833 DOI: 10.1111/tri.13570]
135 **Duarte-Rojo A**, Bloomer PM, Rogers RJ, Hassan MA, Dunn MA, Tevar AD, Vivis SL, Bataller R, Hughes CB, Ferrando AA, Jakicic JM, Kim WR. Introducing EL-FIT (Exercise and Liver FITness): A Smartphone App to Prehabilitate and Monitor Liver Transplant Candidates. *Liver Transpl* 2021; **27**: 502-512 [PMID: 33232547 DOI: 10.1002/lt.25950]
136 **Lopez-Lopez V**, Ruiz-Manzanera JJ, Eshmuminov D, Lehmann K, Schneider M, von der Groeben M, de Angulo DR, Gajownik U, Pons JA, Sanchez-Bueno F, Robles-Campos R, Ramirez-Romero P. Are We Ready for Bariatric Surgery in a Liver Transplant Program? *Obes Surg* 2020; **31**: 1214-1222 [PMID: 33225408 DOI: 10.1007/s11695-020-05118-7]
137 **Johnston SD**, Morris JK, Cramb R, Gunson BK, Neuberger J. Cardiovascular morbidity and mortality after orthotopic liver transplantation. *Transplantation* 2002; **73**: 901-906 [PMID: 11923689 DOI: 10.1097/00007890-200203270-00012]
138 **Unger LW**, Berlakovich GA, Trauner M, Reiberger T. Management of portal hypertension before and after liver transplantation. *Liver Transpl* 2018; **24**: 112-121 [PMID: 28752925 DOI: 10.1002/lt.24830]
139 **Patel SS**, Rodriguez VA, Siddiqui MB, Faridnia M, Lin FP, Chandrakumaran A, Laurenzano J, Clinton J, Kowlgi GN, Kirkman D, Sima AP, Liptrap E, Bhati C, Siddiqui MS. The Impact of Coronary Artery Disease and Statins on Survival After Liver Transplantation. *Liver Transpl* 2019; **25**: 1514-1523 [PMID: 31344758 DOI: 10.1002/lt.25613]
140 **Gracia-Sancho J**, García-Calderó H, Hide D, Marrone G, Guixé-Muntet S, Peralta C, García-Pagán JC, Abraldes JG, Bosch J. Simvastatin maintains function and viability of steatotic rat livers procured for transplantation. *J Hepatol* 2013; **58**: 1140-1146 [PMID: 23428876 DOI: 10.1016/j.jhep.2013.02.005]
141 **Russo L**, Gracia-Sancho J, García-Calderó H, Marrone G, García-Pagán JC, García-Cardeña G, Bosch J. Addition of simvastatin to cold storage solution prevents endothelial dysfunction in explanted rat livers. *Hepatology* 2012; **55**: 921-930 [PMID: 22031447 DOI: 10.1002/hep.24755]
142 **Bianchi G**, Marchesini G, Marzocchi R, Pinna AD, Zoli M. Metabolic syndrome in liver transplantation: relation to etiology and immunosuppression. *Liver Transpl* 2008; **14**: 1648-1654 [PMID: 18975273 DOI: 10.1002/lt.21588]
143 **Heisel O**, Heisel R, Balshaw R, Keown P. New onset diabetes mellitus in patients receiving calcineurin inhibitors: a systematic review and meta-analysis. *Am J Transplant* 2004; **4**: 583-595 [PMID: 15023151 DOI: 10.1046/j.1600-6143.2003.00372.x]
144 **Wallia A**, Parikh ND, O'Shea-Mahler E, Schmidt K, DeSantis AJ, Tian L, Levitsky J, Molitch ME. Glycemic control by a glucose management service and infection rates after liver transplantation. *Endocr Pract* 2011; **17**: 546-551 [PMID: 21324822 DOI: 10.4158/EP10343.OR]
145 **Park C**, Hsu C, Neelakanta G, Nourmand H, Braunfeld M, Wray C, Steadman RH, Hu KQ, Cheng RT, Xia VW. Severe intraoperative hyperglycemia is independently associated with surgical site

infection after liver transplantation. *Transplantation* 2009; **87**: 1031-1036 [PMID: 19352123 DOI: 10.1097/TP.0b013e31819cc3e6]

146 **Wallia A**, Parikh ND, Molitch ME, Mahler E, Tian L, Huang JJ, Levitsky J. Posttransplant hyperglycemia is associated with increased risk of liver allograft rejection. *Transplantation* 2010; **89**: 222-226 [PMID: 20098286 DOI: 10.1097/TP.0b013e3181c3c2ff]

147 **Grancini V**, Resi V, Palmieri E, Pugliese G, Orsi E. Management of diabetes mellitus in patients undergoing liver transplantation. *Pharmacol Res* 2019; **141**: 556-573 [PMID: 30690071 DOI: 10.1016/j.phrs.2019.01.042]

148 **Lo C**, Jun M, Badve SV, Pilmore H, White SL, Hawley C, Cass A, Perkovic V, Zoungas S. Glucose-lowering agents for treating pre-existing and new-onset diabetes in kidney transplant recipients. *Cochrane Database Syst Rev* 2017; **2**: CD009966 [PMID: 28238223 DOI: 10.1002/14651858.CD009966.pub2]

149 **Davies MJ**, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, Rossing P, Tsapas A, Wexler DJ, Buse JB. Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2018; **41**: 2669-2701 [PMID: 30291106 DOI: 10.2337/dci18-0033]

150 **Unger LW**, Forstner B, Schneglberger S, Muckenhuber M, Eigenbauer E, Bauer D, Scheiner B, Mandorfer M, Trauner M, Reiberger T. Guideline-conform statin use reduces overall mortality in patients with compensated liver disease. *Sci Rep* 2019; **9**: 11674 [PMID: 31406146 DOI: 10.1038/s41598-019-47943-6]

151 **Krasnoff JB**, Vintro AQ, Ascher NL, Bass NM, Paul SM, Dodd MJ, Painter PL. A randomized trial of exercise and dietary counseling after liver transplantation. *Am J Transplant* 2006; **6**: 1896-1905 [PMID: 16889545 DOI: 10.1111/j.1600-6143.2006.01391.x]

152 **Zamora-Valdes D**, Watt KD, Kellogg TA, Poterucha JJ, Di Cecco SR, Francisco-Ziller NM, Taner T, Rosen CB, Heimbach JK. Long-term outcomes of patients undergoing simultaneous liver transplantation and sleeve gastrectomy. *Hepatology* 2018; **68**: 485-495 [PMID: 29457842 DOI: 10.1002/hep.29848]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2299-2311

DOI: 10.3748/wjg.v27.i19.2299

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

*MINIREVIEWS*

# Clinical characteristics and outcomes of patients with hepatic angiomyolipoma: A literature review

Paul Calame, Gaëlle Tyrode, Delphine Weil Verhoeven, Sophie Félix, Anne Julia Klompenhouwer, Vincent Di Martino, Eric Delabrousse, Thierry Thévenot

**ORCID number:** Paul Calame 0000-0002-9832-9837; Gaëlle Tyrode 0000-0002-4714-2124; Delphine Weil Verhoeven 0000-0002-9648-813X; Sophie Félix 0000-0001-6330-7788; Anne Julia Klompenhouwer 0000-0002-6740-3862; Vincent Di Martino 0000-0002-2022-690X; Eric Delabrousse 0000-0003-1498-6248; Thierry Thévenot 0000-0003-3974-2784.

**Author contributions:** Thévenot T designed the research; Thévenot T, Calame P, and Félix S analyzed the data; Thévenot T, Calame P and Tyrode G wrote the paper; Thé venot T and Calame P made critical revisions related to important content of the revised manuscript; Weil Verhoeven D, Klompenhouwer AJ, Di Martino V, Delabrousse E and Thévenot T provided the final approval of the version to be published.

**Conflict-of-interest statement:** No conflicts of interest exist.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build

**Paul Calame, Eric Delabrousse,** Department of Radiology, Jean Minjoz University Hospital, Besançon 25030, France

**Gaëlle Tyrode, Delphine Weil Verhoeven, Vincent Di Martino, Thierry Thévenot,** Department of Hepatology, Jean Minjoz University Hospital, Besançon 25030, France

**Sophie Félix,** Department of Pathology, Jean Minjoz University Hospital, Besançon 25000, France

**Anne Julia Klompenhouwer,** Department of Surgery, Erasmus University Medical Center, Rotterdam PO Box 2040, The Netherlands

**Corresponding author:** Thierry Thévenot, PhD, Professor, Department of Hepatology, Jean Minjoz University Hospital, 3 Boulevard A. Fleming, Besançon 25030, France.
tthevenot@chu-besancon.fr

## Abstract

First reported in 1976, hepatic angiomyolipoma (HAML) is a rare mesenchymal liver tumor occurring mostly in middle-aged women. Diagnosis of the liver mass is often incidental on abdominal imaging due to the frequent absence of specific symptoms. Nearly 10% of HAMLs are associated with tuberous sclerosis complex. HAML contains variable proportions of blood vessels, smooth muscle cells and adipose tissue, which renders radiological diagnosis hazardous. Cells express positivity for HMB-45 and actin, thus these tumors are integrated into the group of perivascular epithelioid cell tumors. Typically, a HAML appears on magnetic resonance imaging (or computed tomography scan) as a hypervascular solid tumor with fatty areas and with washout, and can easily be misdiagnosed as other liver tumors, particularly hepatocellular carcinoma. The therapeutic strategy is not clearly defined, but surgical resection is indicated for symptomatic patients, for tumors showing an aggressive pattern (*i.e.*, changes in size on imaging or high proliferation activity and atypical epithelioid pattern on liver biopsy), for large (> 5 cm) biopsy-proven HAML, and if doubts remain on imaging or histology. Conservative management may be justified in other conditions, since most cases follow a benign clinical course. In summary, the correct diagnosis of HAML is challenging on imaging and relies mainly on pathological findings.

**Key Words:** Angiomyolipoma; Liver; Tuberous sclerosis complex; Imaging; Pathology;

upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** France

**Peer-review report's scientific quality classification**

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

**Received:** October 27, 2020
**Peer-review started:** October 27, 2020
**First decision:** December 18, 2020
**Revised:** December 31, 2020
**Accepted:** March 18, 2021
**Article in press:** March 18, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Alshami A, Lan C
**S-Editor:** Gao CC
**L-Editor:** Webster JR
**P-Editor:** Liu JH

Potentially malignant

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** Hepatic angiomyolipoma (HAML) is a rare, but not exceptional, mesenchymal liver tumor. HAML contains variable proportions of blood vessels, smooth muscle cells and adipose tissue, which renders its radiological diagnosis challenging. In most cases, this tumor follows a benign clinical course but more aggressive behavior may complicate management, which remains poorly codified. This review presents the main demographic and histological characteristics of HAML, summarizes reported cases of HAML with spontaneous rupture and aggressive behavior, and finally proposes a pragmatic algorithm for the management of HAML based on the most recent knowledge.

**Citation:** Calame P, Tyrode G, Weil Verhoeven D, Félix S, Klompenhouwer AJ, Di Martino V, Delabrousse E, Thévenot T. Clinical characteristics and outcomes of patients with hepatic angiomyolipoma: A literature review. *World J Gastroenterol* 2021; 27(19): 2299-2311
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2299.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2299

## INTRODUCTION

Angiomyolipoma (AML) is a solid mesenchymal tumor, mainly described in the kidney, and belongs to the group of perivascular epithelioid cell tumors (PEComas)[1]. Hepatic localization of AML, described for the first time in 1976[2], is rare, since only around 600 cases were reported after an exhaustive search of the literature up to the year 2017[3]. Hepatic AML (HAML) poses a veritable diagnostic challenge in radiological terms, especially when fat content is low, because this type of tumor may appear as a hypervascular tumor associated with a washout phase that mimics other, more common hypervascular hepatic tumors, such as hepatocellular carcinoma[4-7]. The natural course of HAML is mostly benign, although several cases have been reported to exhibit aggressive behavior with metastasis or recurrence after surgery[8-24], or spontaneous rupture[8,25-33]. These rare but dramatic observations unavoidably compound the complexity of managing patients with HAML. Due to its difficult radiological diagnosis, its potentially aggressive behavior, and its poorly codified management, we aimed to analyze the recent literature regarding this rare, but not exceptional liver tumor, and to provide a pragmatic algorithm for its management according to the most recent knowledge.

## PATIENT CHARACTERISTICS

HAML is a tumor usually occurring in a non-cirrhotic liver, and mainly affects middle-aged women. A retrospective analysis of the literature carried out up to 2016 identified 292 patients with one or more HAML, and most of them (nearly 74%) were women, with a median age ranging between 24 years and 53 years across studies[3]. HAML mainly locates in the right liver (60% of cases[7]), is unique in 84% of cases, and median size ranges from 2 cm to 12.7 cm[3,34]. This type of tumor is often detected incidentally during medical check-ups (42% to 72% of cases) since most subjects are asymptomatic[3,34,35]. Symptoms revealing HAML may include abdominal pain or discomfort, bloating, weight loss or, more rarely, discovery of an abdominal mass on palpation[35]. A few cases of spontaneous rupture of HAML have been reported (Table 1)[8,25-33]. Tumor size (≥ 4 cm) and pregnancy are two recognized conditions favoring rupture of renal AML[36,37], but the small number of reported cases of HAML rupture precludes identification of predictors of rupture in the liver. In the ten cases reported in Table 1, tumor size was usually large, between 5 cm and 12 cm (except for one case due to an inflammatory variant of HAML with a subcapsular location[32]), and there was no age preference (mean age: 48 years; range: 22 years to 77 years). All patients underwent emergent or delayed resection of the tumor,

**Table 1 Cases of spontaneous rupture of hepatic angiomyolipoma**

| Ref. | Sex | Age, yr | Symptoms | Abdominal radiological findings | Treatment | Outcomes |
|---|---|---|---|---|---|---|
| Huber *et al*[25] 1996 | F | 22 | Hemorrhagic shock with clinical symptoms of acute abdomen | CT scan: multiple tumors of the liver (the largest in segment III measured 8 cm) and both kidneys and a splenic lesion with a diameter of 4 cm | Surgical resection of segments II and III | Postoperative course was uneventful. Discharge from hospital 12 d later |
| Guidi *et al*[26] 1997 | M | 74 | Sudden onset of upper-quadrant pain | CT scan: liver tumor of 10 cm × 8 cm in the segments I and V and another small mass of 4 cm × 3 cm in segment IV. Fluid was present in the upper abdominal compartments | Surgical resection of the hemorrhagic hepatic mass | Postoperative course was uneventful. Discharge from hospital 8 d later |
| Tsui *et al*[27] 1999 | F | 41 | Acute rupture of a subcapsular tumor | 9 cm | Surgical resection | Patient in healthy condition 4 yr after surgery |
| Zhou *et al*[28] 2008 | ND | ND | Hemorrhagic shock | Ultrasonography showed a 5-cm "cavernous hemangioma" in the right hepatic lobe | Emergency laparotomy for hemostasis | No tumor recurrence or metastasis was found during follow-up of 2-3 yr |
| Ding *et al*[8] 2011 | F | 56 | ND | A rupture of the tumor measuring 6 cm × 6 cm in segment VI was confirmed by emergent laparotomy | Liver suture followed by segmentectomy | No serious morbidity in the postoperative course |
| Occhionorelli *et al*[29] 2013 | F | 25 | Sudden onset of abdominal upper-quadrant pain and hypotension, after two recent syncopal episodes | CT scan showed a hepatic tumor in the left lobe (8.6 cm × 7.2 cm) with suspected peritoneal blood leakage | Hemorrhage initially managed by manual compression, followed by deep and pro-coagulant tissue adhesives. After 48 hours, the patient underwent left-liver lobectomy | Postoperative course was uneventful. Discharge from hospital 9 d later |
| Aoki *et al*[30] 2014 | F | 70 | Sudden onset of back pain on the right side | CT scan: hepatic tumor in segment VII measuring 7 cm in diameter accompanied by subcapsular hematoma with extravasation | Transcatheter arterial embolization. Right hepatic lobectomy was carried out 39 d later | Five days after surgery, she had thrombi in the left popliteal vein and the left pulmonary artery. Insertion of an IVC filter which was removed due to sepsis. She was discharged 24 d after surgery. There was no recurrence 42 mo following surgery |
| Tajima *et al*[31] 2014 | M | 38 | Upper abdominal pain | CT scan showed a tumor measuring 10.5 cm × 9.5 cm × 7 cm in the posterior segment of the right hepatic lobe that had ruptured into the space between the liver and the diaphragm | Transcatheter arterial embolization was performed. The patient developed fever and the hematoma surrounding the liver was drained. No infection was confirmed but right lobectomy was performed | ND |
| Kai *et al*[32] 2015 | F | 77 | Sudden abdominal pain and transient loss of consciousness | CT scan: hemoperitoneum with subcapsular hematoma at the left lobe and a hepatic nodule measuring 2.3 cm in diameter in segment II | Conservative initial treatment with periodic imaging studies. Transcatheter arterial chemoembolization was performed because a diagnosis of HCC was suggested. Surgical resection (laparoscopic left lateral segmentectomy) was performed 4 mo later | Postoperative course was uneventful. Discharge from hospital 7 d later No signs of recurrence at 3.5 yr after surgery |
| Kim *et al*[33] 2017 | M | 31 | Sudden onset severe abdominal pain in the right upper quadrant area | CT scan: Mass of approximately 12 cm in the right hepatic lobe with hemorrhage along the perihepatic space | Emergent angiography with embolization.Hepatic resection was performed 15 d later | Postoperative course was uneventful |

F: Female; M: Male. ND: Not determined; CT: Computed tomography; HCC: Hepatocellular carcinoma; IVC: Inferior vena cava.

sometimes preceded by preoperative embolization. Routine laboratory tests (including liver tests) are usually normal, as are serum tumor markers (alpha-fetoprotein, carcinoembryonic antigen and carbohydrate antigen 19-9[38].

The association between tuberous sclerosis complex (TSC) and renal AML, first described in 1911[39], is observed in 50% of cases, while the association between TSC and HAML is only observed in 5% to 15% of cases[3,40]. TSC is an autosomal dominant genetic disorder with a birth incidence of 1:6000[41], although sporadic cases due to *de novo* mutation are the most frequent presentation in the absence of a family history. TSC results from a mutation of *TSC1* or *TSC2*, which code for hamartin and tuberin, respectively[42]. These proteins are critical regulators of cell growth and proliferation, potentially through their upstream modulator, mammalian target of rapamycin (mTOR). Loss of function or dysfunction of either protein results in the development of hamartomas in numerous organ systems, including the brain, kidneys, heart and liver[43]. In patients with TSC, HAML is frequently associated with renal AML. A recent retrospective study showed that among 25 patients with HAML, 88% also had renal AML, and *TSC2* patients had a higher frequency of HAML compared to *TSC1* patients (18% *vs* 5%; $P = 0.037$)[42]. In contrast to previous reports, the predominance of female gender observed in patients with HAML but no TSC was not observed in patients with the TSC-HAML association[42,44].

## IMMUNOHISTOLOGICAL CHARACTERISTICS OF HAML

Histological examination is the gold standard for HAML diagnosis, since diagnosis by imaging is difficult. Of note, even histological analysis of liver biopsy was shown to misdiagnose HAML in about 15% of cases in a recent multicenter study[34]. The World Health Organization defines PEComas as "mesenchymal tumors containing distinctive perivascular epithelioid cells". AML, which belongs to the PEComa group, is composed of adipose tissue, smooth muscle and vessels with dystrophic walls (Figure 1). The histological and immunohistochemical characteristics class HAML in the group of PEComas, an entity that brings together tumors of different histology, but with a common immunohistochemical signature, namely co-expression of melanocytic and muscle markers (see below)[1,40,45]. The PEComa family includes AML, clear cell "sugar" tumor of the lung, lymphangioleiomyomatosis (LAM), and a variety of unusual visceral, intra-abdominal, and soft tissue/bone tumors, described under the term "clear cell myomelanocytic tumor of the falciform ligament/Ligamentum teres"[46]. There is a strong association between AML and TSC, and between LAM and TSC[47], although the link is less marked for other members of the PEComa family[46].

Perivascular epithelioid cells are characterized by their perivascular location, often with a radial arrangement of cells around vessels. Typically, these cells are mostly epithelioid when they are just around the vessels, whereas spindle cells resembling smooth muscle are seen further away from the vessels. In the liver, the aspect is most often only epithelioid without spindle cells. Adipose cells are usually found distant from the blood vessels. Wide variation is seen in the relative proportion of epithelioid, spindle, and lipid-distended cells. Depending on the relative proportion of these different tissues, there are, on the one hand, conventional AMLs with a predominance of lipomatous or myomatous cells or vessels, and, on the other hand, epithelioid AMLs containing at least 10% epithelioid cells[48]. Mixed and myomatous AMLs are the most frequent (respectively 36% and 42% of the 151 HAML cases with informative data)[3]. Another subtype of HAML, namely inflammatory HAML, has also been recognized, although only 14 cases have been reported in the English-language literature[49]. Overall, this tumor is characterized by inflammatory infiltration exceeding 50% of tumor area, and the main types of inflammatory cells are lymphocytes (100%), plasma cells (93%) and histiocytes (71%)[49].

Macroscopically, HAML is well circumscribed, unencapsulated, smooth and brownish in color; however, the existence of hemorrhage or intra-tumor necrosis can change its appearance. On microscopic examination, cells typically have clear or slightly eosinophilic cytoplasms, small, central, round or oval nuclei, with a small nucleolus. "Atypical" AML presents cytological atypia, a multinucleated nucleus, focal necrosis and an increase in the number of mitoses[48].

By immunohistochemistry, these tumors are positive for both melanocytic markers (HBM-45 and melan-A are the more sensitive markers) and smooth muscle markers (actin and/or desmin) with variable extent of staining[46,50]. HBM-45 is the most specific marker of AML[38]. Actin non-immunoreactivity does not exclude a tumor from the PEComa group[51]. Classically, AML does not express epithelial markers (like cytokeratin), S100 protein or alpha-fetoprotein[52]. Estrogen and progesterone receptors are frequently positive in classic renal AML but are only rarely positive in

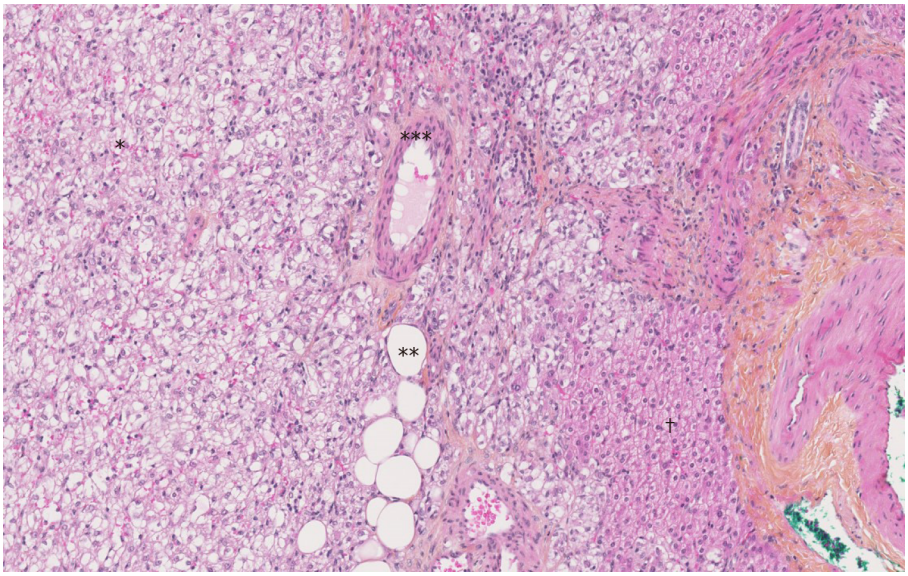



**Figure 1 The Hematoxylin-Eosin-Saffron staining image of hepatic angiomyolipoma.** There are three components of hepatic angiomyolipoma: vessel (*), adipocytes (**) and numerous epithelioid cells (***). There are fewer hepatocytes (†) (magnification × 10).

extrarenal PEComas, including HAML, suggesting the absence of the role of sex hormones in the pathogenesis and growth of HAML, despite a clear predominance in women[45,53].

There are numerous possible differential diagnoses of PEComas depending on the location and the predominant tissue composing the tumor. Given their uniform expression of melanocytic markers, PEComas may be confused with both conventional melanoma and clear cell sarcoma, but these latter typically have strong expression of S100 protein, and do not stain with smooth muscle actin. Due to its preferential abdominal location, the presence of epithelioid and spindle cells, and the occasional positive KIT (CD117) staining in HAML, the diagnosis of gastrointestinal stromal tumor is sometimes discussed. Depending on the size of the contingent of epithelioid cells, spindle-shaped cells or adipocytes, AML can also be confused with carcinoma, smooth muscle neoplasm or adipocytic tumor[45,54].

## PROGNOSIS

The scarcity of PEComas precludes the identification of robust criteria to discriminate benign AML from other tumors with more aggressive behavior. The first description of a likely "malignant" HAML is recent[55], and although the authors do not clearly indicate the malignant nature of this tumor, the reported characteristics (*i.e.*, large size, cytological atypia and presence of necrosis) and the tumor-related death of the patient are robust arguments in favor of a "malignant" case. From a series of 24 PEComas of the soft tissue and gynecologic tract (not including AML) with a median follow-up of 30 mo (range: 10-84), Folpe *et al*[46] observed 3 local recurrences and 5 distant metastases (8/24, 33% of cases), 2 deaths (8%), 4 patients (17%) alive with metastatic or unresectable local disease, and 18 patients (75%) alive with no evidence of disease. A combined analysis of these 24 cases plus 45 other reported cases in the literature with sufficient available follow-up information identified the following variables associated with an increased risk of recurrence or metastasis: tumor size greater than 5 cm, infiltrative growth pattern, high nuclear grade, necrosis, and mitotic activity > 1/50 high power field. Consequently, these authors developed a provisional classification of PEComas with increasing aggressive potential (Table 2). Cases of HAML with aggressive behavior are reported in Table 3[8-24]. Regarding HAML, it is mainly the epithelioid type that confers a risk of aggressive behavior[34]. In the review published in 2017, the mortality rate associated with HAML was 0.8%[3].

### *Imaging findings*

The imaging features of HAML vary greatly depending on the highly variable proportion of fat, smooth muscle and vascular elements. Diagnosis can be challenging, and depends mainly on the amount of fat present, which is the key to HAML

**Table 2 Classification of perivascular epithelioid cell tumors according to their malignant potential[1,27]**

| Classification | Criteria |
|---|---|
| Benign | No worrisome features: (1) Tumor size < 5 cm; (2) No infiltration; (3) Non-high nuclear grade and cellularity; (4) Mitotic activity ≤ 1/50 HPF; (5) No necrosis; and (6) No vascular invasion |
| Uncertain malignant potential | Tumor with: (1) Pleomorphism/multinucleated giant cells only; or (2) Size > 5 cm only |
| Aggressive behavior | Two or more worrisome features: (1) Size > 5 cm; (2) Peripheral infiltration; (3) High nuclear grade and cellularity; (4) Mitotic activity > 1/50 HPF; (5) Ischemic tumor necrosis for large tumor; and (6) Vascular invasion |
| According to the WHO classification of tumors[1] | As with GISTs, the main predictors of a risk of metastatic behavior are marked nuclear atypia, diffuse pleomorphism and mitotic activity of more than 1 mitosis per 1 $mm^2$ |

GIST: Gastrointestinal stromal tumor; HPF: High-power field.

diagnosis. On ultrasound, the lesion is usually well circumscribed, hyperechoic or mixed echoic and after injection of ultrasound contrast, presents rapid enhancement in the arterial phase compared to the adjacent liver. In the portal and delayed phase, HAML can display either hypo, iso or hyperenhancement[4]. Computed tomography (CT) shows a hypodense tumor with fatty areas within the lesion (density around -50 HU). Classically, this solid tumor is hypervascular (Figure 2) with wash-out in the portal and late portal phase [CT or magnetic resonance imaging (MRI)]. HAML with few or no vessels on histologic examination show persistent portal and late-phase enhancement, whereas HAML with richly vascularized tissue is more likely to show wash-out[5]. The tumor signal on MRI is hyperintense in T2 weighted sequences and variable in T1 weighted sequences. MRI is the most sensitive imaging technique to detect liver fat using in-phase and opposed-phase T1 gradient echo sequences. The drop-out signal within a liver lesion on the opposed-phase sequences indicates the presence of fat within the lesion (Figure 3A and B). The imaging features on MRI after injection of contrast medium are similar to those observed on CT scan. When using a hepatocyte specific agent (gadoxetic acid or gadobenate dimeglumine), the lesion shows a hyposignal in the hepatobiliary phase (Figure 4).

Data regarding HAML evaluation using fluorine-18-fluorodeoxyglucose positron emission tomography ($^{18}$F-FDG-PET) are limited. FDG uptake in HAML is variable and the value of $^{18}$F-FDG-PET for diagnosing or managing this type of tumor is unclear[56].

Given the imaging characteristics of HAML (hypervascular lesion with a fat component in a healthy liver and with frequent wash-out), differential diagnoses are benign hepatocytic tumors (steatotic or telangiectasia adenoma, fat focal nodular hyperplasia) and malignant hepatocytic tumors (mainly hepatocellular carcinoma). When the diagnosis is challenging, especially with hepatocellular carcinoma, the absence of a capsule and the visualization of a drainage vein are two useful radiological features that can be helpful for HAML diagnosis when they are present[6].

## MANAGEMENT OF HAML

Due to its rarity, the diagnosis of HAML on imaging (and even histological examination[34]) is difficult. Consequently, the clinical management of HAML patients should take place in expert centers for a multidisciplinary work-up involving radiologists, pathologists and hepatologists. Obtaining a liver biopsy is strongly advised to better balance the risk of surgery (resection of centrohepatic tumor will be at higher risk, for instance) against the risk of tumor-related complications. Thus, in the presence of asymptomatic HAML, without cytological atypia on biopsy, but at high risk of complicated resection, regular radiological monitoring will be preferred[34].

Analysis of the literature shows that the majority of patients are treated with surgical resection (84% and 76% of patients in two large case series[3,34]). For other patients, regular radiological monitoring is justified by the uncertainty surrounding the risk of HAML progression. Although the risk of tumor recurrence after resection or metastases has rarely been described, the identification of radiological and especially histological factors predicting an unfavorable course (Table 1) is essential for a collegial therapeutic decision. Furthermore, patient compliance with regular radiological monitoring will also be an important argument in decision-making[3,35].

**Table 3 Reported cases of hepatic angiomyolipoma with aggressive behavior**

| Ref. | Sex | Age, yr | Size | Types | Treatment | Duration of follow-up | Outcome |
|---|---|---|---|---|---|---|---|
| Croquet *et al*[9] 2000 | F | 16 | 19 cm × 12 cm × 8 cm | Epithelioid | SR | 6 yr | Recurrence in the liver, associated with renal angiomyolipoma |
| Dalle *et al* [10] 2000 | F | 70 | 15 cm | Epithelioid | SR | 5 mo | Recurrence in the liver with a lesion measuring 15 cm and presence of multiple metastases in the liver |
| Flemming *et al*[11] 2000 | F | 51 | 2 nodules: 0.5 cm and 15 cm | Epithelioid | SR | 3 yr | Recurrence in the right hepatic lobe and presence of multiple metastases |
| McKinney *et al*[12] 2005 | F | 14 | 11 cm × 7 cm × 8 cm | NS | SR, interferon α | 1 yr | Recurrence with a hepatic lesion measuring 9 cm × 6 cm × 14 cm, appearance of lymph nodes and hepatic metastases. Death after disease progression |
| Parfitt *et al* [13] 2007 | F | 60 | 14 cm × 11 cm | Epithelioid | SR | 9 yr | Recurrence in the liver and appearance of metastases in the trapezius muscle, the left lung and the tail of the pancreas |
| Yang *et al* [14] 2007 | F | 37 | 13 cm × 9 cm × 9 cm | Classic | SR | 14 mo | Recurrence in the right hepatic lobe 6 months after SR, appearance of pulmonary metastases 11 mo after SR and death occurred at 14 mo |
| Deng *et al* [15] 2008 | M | 30 | 18 cm × 14 cm | Classic | SR, Chemotherapy | 3 yr and 4 mo | Recurrence with a hepatic lesion measuring 11 cm and metastases in pancreatic tail and portal vein thrombosis 3 yr after SR. Chemotherapy was initiated but 4 mo later pulmonary metastases appeared. Death occurred after disease progression |
| Nguyen *et al*[16] 2008 | F | 43 | 11 cm × 7.5 cm × 7.5 cm | Classic | SR | 6 mo | Recurrence in the liver 6 mo after SR, together with metastases in the peritoneum, omentum, stomach and spleen. Death after disease progression |
| Xu *et al*[17] 2009 | F | 33 | 2 nodules: 1 cm and 6 cm | Epithelioid | SR | 1 yr | Recurrence in the left hepatic lobe |
| Zeng *et al* [18] 2010 | NS | NS | 6 cm | NS | SR | 9 yr | Recurrence in the right hepatic lobe with a lesion measuring 6 cm |
| Butte *et al* [19] 2011 | F;M | 54; 41 | NS; 9 cm | NS; Epithelioid | SR; SR | 53 mo; 41 mo | Recurrence in the liver 53 mo after SR; Occurrence of pulmonary and retroperitoneal metastases 41 mo after SR |
| Hu *et al*[20] 2011 | F | NS | NS | NS | SR | 14 mo | Appearance of local and distant metastases 6 mo after SR. Death occurred 14 mo after SR |
| Ding *et al* [8] 2011 | F | 31 | 8 cm × 8 cm | NS | SR | 7 yr | Recurrence in the right hepatic lobe 6 yr after SR and death occurred one year later |
| Wang *et al* [21] 2015 | F | 37 | 7 cm × 9 cm | Classic | SR | 3 yr | Recurrence of two hepatic nodules in the right lobe (13 cm × 12 cm and 2.3 cm × 1.8 cm) 3 yr after SR. Arterial chemoembolization was performed, followed by liver transplantation |
| Fukuda *et al*[22] 2016 | M | 58 | 6.3 cm | Epithelioid | SR | 9 yr | Metastases occurred in the right lung 7 yr after SR and were treated by pneumonectomy. No recurrence was observed after 2 yr of follow-up |
| Marcuzzi *et al*[23] 2018 | F | 47 | 3.8 cm × 4.6 cm × 4.7 cm+ 2 hepatic lesions measuring 6 mm and 5 mm | Epithelioid | SR | 8 yr and 8 mo | CT scan was performed 6 yr and 4 mo after the initial presentation: the hepatic lesion had grown in size to an estimated 10.9 cm × 9.7 cm × 11.2 cm and the adjacent lesions had grown to 1.9 cm and 2.4 cm with a new lesion on the kidney of 4.6 cm × 5.1 cm. 16 mo later, MRI showed an increase in size of the hepatic lesion (12 cm × 11 cm), and kidney lesion (6.2 cm × 5.6 cm). SR performed 2 mo later. 6 mo after SR, recurrence in the resection line and in the hepatic segment II |
| Yan *et al* [24] 2018 | NS | NS | 15 cm | Epithelioid | SR | 9 yr | Recurrence in the liver 9 yr after SR with invasion of the inferior vena cava and diaphragm, and appearance of pulmonary metastases |

F: Female; M: Male; NS: Not specified; SR: Surgical resection; CT: Computed tomography; MRI: Magnetic resonance imaging.

The optimal radiological follow-up is not well defined, but the first radiological evaluation may take place at one year, since HAMLs were described to increase by only 0.77 cm per year in a series of 29 patients followed radiologically[3], and radiological progression affected only 6 of the 29 patients (20%). Later, radiological monitoring could be performed twice a year[3], but the frequency will depend on the

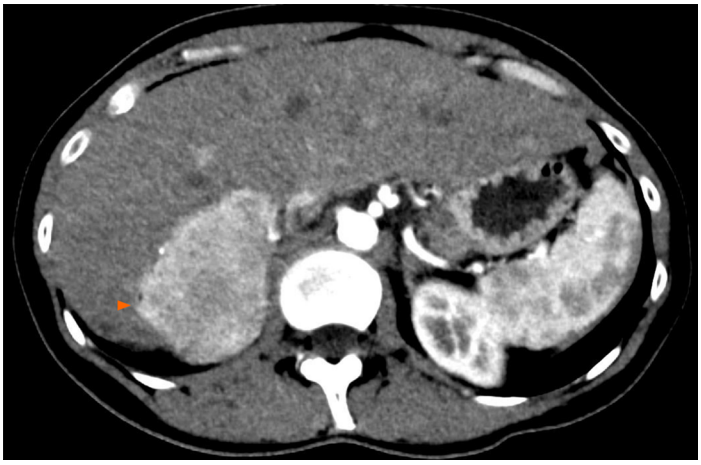


**Figure 2 Angiomyolipoma in a healthy 33-year-old woman.** Abdominal computed tomography on arterial phase showed a hypervascular solid tumor localized in the right posterior segment (arrowheads).

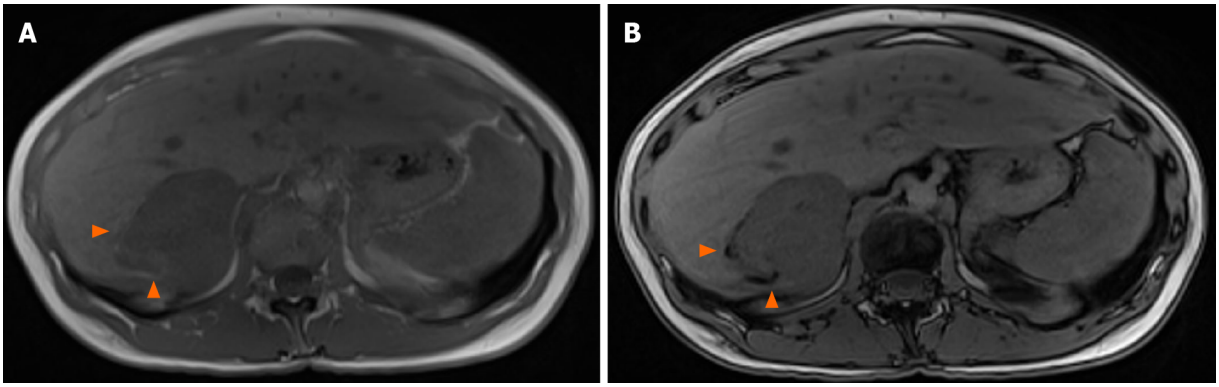



**Figure 3 T1 weighted magnetic resonance images.** Signal dropout at the periphery of the lesion due to fat contingents (arrowhead). A: In-phase; B: Opposed-phase.

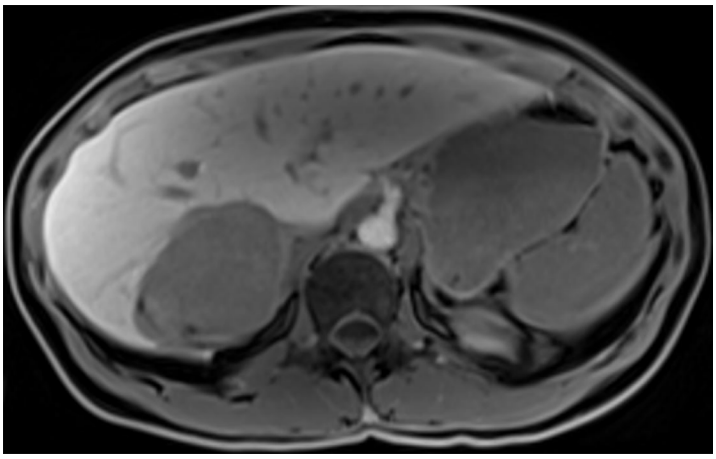


**Figure 4 T1 weighted images one hour after hepatocyte-specific agent injection (gadobenate dimeglumine).** Hyposignal of the lesion indicates that this is not a hepatocytic tumor.

magnitude of tumor progression during the first years of monitoring. Evidently, persistent tumor progression on successive imaging will require a surgical approach. Surgical resection is therefore recommended when there is uncertainty regarding the histological nature of the lesion after liver biopsy, tumor progression on imaging, tumor-related symptoms, and when the tumor exceeds 5 cm[34,38]. The recurrence rate after surgical resection was 2.4% (6 of 246 patients in the series reported by

Klompenhouwer *et al*[3]). The local or distant post-resection recurrence rate is 10% in the case of epithelioid-type HAML[52].

Liver transplantation (LT) has sometimes been erroneously performed for a suspected diagnosis of cholangiocarcinoma or hemangiosarcoma mimicking a hepatocellular carcinoma[3,34]. These flawed diagnoses further underline the interest of systematic liver biopsy as well as radiological and anatomopathological expertise. Since the first reported case of LT for HAML in 2010[57], other exceptional cases have been added[21,58]. LT was performed as a last resort treatment for unresectable HAML due to excessive size or a significant number of hepatic tumors.

Other therapeutic alternatives have been reported, such as radiofrequency ablation, arterial embolization or the use of sirolimus[35,42]. mTOR inhibitors, which include sirolimus and everolimus, are immunosuppressive molecules used in transplantation, and which also have antiproliferative properties. In a multicenter, double-blind, placebo-controlled, phase 3 trial (EXIST-2), 118 patients with at least one renal AML larger than 3 cm associated with a definite TSC diagnosis or sporadic LAM were randomized to receive oral everolimus 10 mg/d ($n$ = 79, mean dosage: 8.6 mg/d, median duration: 38 wk) or placebo ($n$ = 39). The trial showed a beneficial effect of everolimus in reducing the size of AML (response rate: 42% *vs* 0% in the placebo group; $P$ < 0.0001). Response was evaluated as a composite endpoint including a reduction ≥ 50% of the AML volume[59].

The favorable outcomes reported in the EXIST-2 trial led to an open label extension undertaken by the same authors. This study demonstrated a pronounced benefit of everolimus for the patients who continued on this drug. The response rate improved from 42% in the primary analysis (median exposure 8.7 mo)[59] to 54% (median exposure 28.9 mo), and the long-term use of everolimus appeared safe[60].

The pooled analysis of two randomized trials[59,61] comparing 109 and 53 patients with renal AML treated respectively with everolimus and placebo for 6 mo confirmed the efficacy of everolimus in reducing tumor volume by 50% or more (risk ratio = 24.69; $P$ = 0.001)[62]. Everolimus is currently indicated for the treatment of adult patients with renal AML and TSC not requiring immediate surgery. Some patients with HAML associated with TSC have been treated with sirolimus, which also proved efficacious in reducing tumor volume[54]. The role of mTOR inhibitors for patients with HAML remains undefined, but these molecules could be used, as for the kidney, in a palliative context. The long-term safety profile is consistent with that previously reported and no new safety issues have raised concern[60].

In a retrospective Chinese series (2009-2016) of 92 patients diagnosed with histologically proven HAML measuring between 2 cm and 5 cm, ultrasonography-guided radiofrequency ablation after liver biopsy was used in 22/92 patients. No tumor recurrence was reported, but the duration of follow-up was not indicated[35]. Radiofrequency ablation can therefore advantageously compete with surgery when HAML is relatively small (< 5 cm), and when the location in the liver or the patient's comorbidities are not amenable to safe hepatic surgery.

Arterial embolization[33] is sometimes necessary in the presence of hemorrhagic HAML. There are only eight reported cases of HAML presenting as spontaneous rupture and hemorrhage; the median size of these tumors was 8.5 cm (range: 2.5 cm to 12.5 cm) and three of them were treated with arterial embolization followed by liver resection enabling formal diagnosis of HAML. The main differential diagnosis of hemorrhagic liver tumor in a non-cirrhotic liver is adenoma, which is outside the scope of this review. Therapeutic arterial embolization was used in three other patients with histologically proven HAML (size: 11, 12 and 17 cm) in a retrospective American series[19], and no progression was observed after an average follow-up of 12.7 mo (range, 1-36 mo). The risk of spontaneous hemorrhage seems to be lower for HAML than for kidney AML, which are usually supplied by a single vessel and associated with aneurysms[19]. We propose a decisional algorithm for the management of HAML (Figure 5).

## CONCLUSION

HAML is a rare but not exceptional tumor, and usually has a benign course. However, this tumor may display more aggressive behavior with recurrence or metastasis, although there are no robust histological or radiological characteristics to predict the natural course of this type of tumor. Radiological diagnosis is often hazardous due to the variable proportions of the tissues that comprise HAML. Therefore, histological analysis of the tumor and multidisciplinary consultation, whenever possible in an

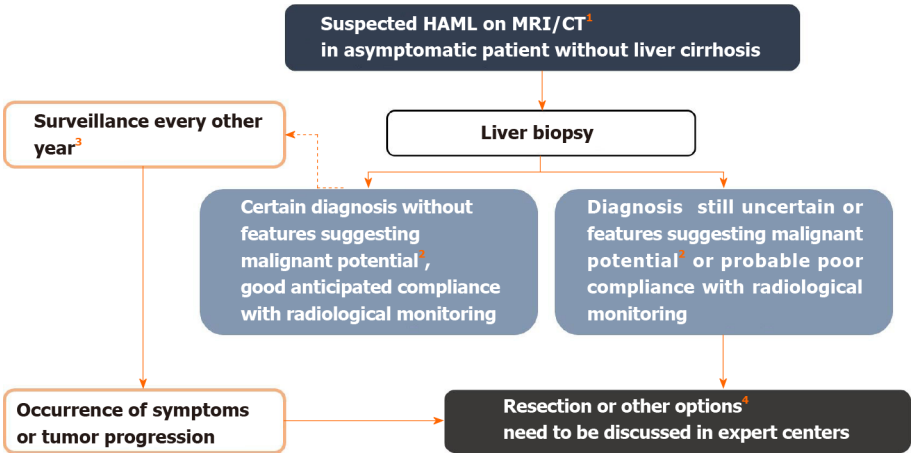



**Figure 5 Management algorithm for suspected hepatic angiomyolipoma on imaging.** [1]Hepatic angiomyolipoma diagnosis is suggested in the presence of fatty tissue within the solid lesion or presence of wash-out. In the presence of tumor-related symptoms, surgical resection is considered first. [2]Features suggesting malignant potential are reported in Table 2. Some authors also recommend surgery in the case of epithelioid-type hepatic angiomyolipoma, which would be at greater risk of progression. Likewise, an association with tuberous sclerosis complex is a condition that increases the risk of malignant transformation, by analogy with renal angiomyolipoma[3]. [3]Monitoring maintained despite the benign nature of the initial diagnosis because the aggressive behavior of the tumor is difficult to predict. [4]Other possible therapeutic options include mTOR inhibitors, radiofrequency ablation, arterial embolization in cases of hemorrhagic rupture, and liver transplantation. Citation: Klompenhouwer AJ, Verver D, Janki S, Bramer WM, Doukas M, Dwarkasing RS, de Man RA, IJzermans JNM. Management of hepatic angiomyolipoma: A systematic review. Liver Int 2017; 37(9): 1272-1280. Copyright ©The Author(s) 2017. Published by John Wiley and Sons[3]. MRI: Magnetic resonance imaging; CT: Computed tomography.

expert center, are essential for optimal care of these patients.

## REFERENCES


1 **World Health Organization Classification of Tumours Editorial Board**. Digestive System Tumours. 5th ed. World Health Organization: International Agency for Research on Cancer Publications, 2019

2 **Ishak K**. Mesenchymal tumors of the liver. In: Okuda K, Peter R. Hepatocellular carcinoma. New York: John Wiley & Sons, 1976: 247-275

3 **Klompenhouwer AJ**, Verver D, Janki S, Bramer WM, Doukas M, Dwarkasing RS, de Man RA, IJzermans JNM. Management of hepatic angiomyolipoma: A systematic review. *Liver Int* 2017; **37**: 1272-1280 [PMID: 28177188 DOI: 10.1111/liv.13381]

4 **Huang Z**, Zhou P, Li S, Li K. Hepatic Angiomyolipoma: Clinical Features and Imaging Findings of Quantitative Contrast-Enhanced Ultrasound Perfusion Analysis and Magnetic Resonance Imaging. *J Ultrasound Med* 2020; **39**: 2111-2122 [PMID: 32383807 DOI: 10.1002/jum.15316]

5 **Jung DH**, Hwang S, Hong SM, Kim KH, Ahn CS, Moon DB, Alshahrani AA, Lee SG. Clinico-pathological correlation of hepatic angiomyolipoma: a series of 23 resection cases. *ANZ J Surg* 2018; **88**: E60-E65 [PMID: 28122404 DOI: 10.1111/ans.13880]

6 **Seow J**, McGill M, Wang W, Smith P, Goodwin M. Imaging hepatic angiomyolipomas: key features and avoiding errors. *Clin Radiol* 2020; **75**: 88-99 [PMID: 31677881 DOI: 10.1016/j.crad.2019.09.135]

7 **Liu W**, Wang J, Huang Q, Lu Q, Liang W. Comparison of MRI Features of Epithelioid Hepatic Angiomyolipoma and Hepatocellular Carcinoma: Imaging Data From Two Centers. *Front Oncol* 2018; **8**: 600 [PMID: 30619742 DOI: 10.3389/fonc.2018.00600]

8 **Ding GH**, Liu Y, Wu MC, Yang GS, Yang JM, Cong WM. Diagnosis and treatment of hepatic angiomyolipoma. *J Surg Oncol* 2011; **103**: 807-812 [PMID: 21283992 DOI: 10.1002/jso.21814]

9 **Croquet V**, Pilette C, Aubé C, Bouju B, Oberti F, Cervi C, Arnaud JP, Rousselet MC, Boyer J, Calès P. Late recurrence of a hepatic angiomyolipoma. *Eur J Gastroenterol Hepatol* 2000; **12**: 579-582 [PMID: 10833105 DOI: 10.1097/00042737-200012050-00018]

10 **Dalle I**, Sciot R, de Vos R, Aerts R, van Damme B, Desmet V, Roskams T. Malignant angiomyolipoma of the liver: a hitherto unreported variant. *Histopathology* 2000; **36**: 443-450 [PMID: 10792486 DOI: 10.1046/j.1365-2559.2000.00891.x]

11 **Flemming P**, Lehmann U, Becker T, Klempnauer J, Kreipe H. Common and epithelioid variants of hepatic angiomyolipoma exhibit clonal growth and share a distinctive immunophenotype. *Hepatology* 2000; **32**: 213-217 [PMID: 10915726 DOI: 10.1053/jhep.2000.9142]

12 **McKinney CA**, Geiger JD, Castle VP, Ruiz RE, Strouse PJ. Aggressive hepatic angiomyolipoma in a child. *Pediatr Hematol Oncol* 2005; **22**: 17-24 [PMID: 15770828 DOI: 10.1080/08880010590896206]

13 **Parfitt JR**, Bella AJ, Izawa JI, Wehrli BM. Malignant neoplasm of perivascular epithelioid cells of the liver. *Arch Pathol Lab Med* 2006; **130**: 1219-1222 [PMID: 16879028]

14 **Yang CY**, Ho MC, Jeng YM, Hu RH, Wu YM, Lee PH. Management of hepatic angiomyolipoma. *J Gastrointest Surg* 2007; **11**: 452-457 [PMID: 17436129 DOI: 10.1007/s11605-006-0037-3]

15 **Deng YF**, Lin Q, Zhang SH, Ling YM, He JK, Chen XF. Malignant angiomyolipoma in the liver: a case report with pathological and molecular analysis. *Pathol Res Pract* 2008; **204**: 911-918 [PMID: 18723294 DOI: 10.1016/j.prp.2008.06.007]

16 **Nguyen TT**, Gorman B, Shields D, Goodman Z. Malignant hepatic angiomyolipoma: report of a case and review of literature. *Am J Surg Pathol* 2008; **32**: 793-798 [PMID: 18391749 DOI: 10.1097/PAS.0b013e3181607349]

17 **Xu PJ**, Shan Y, Yan FH, Ji Y, Ding Y, Zhou ML. Epithelioid angiomyolipoma of the liver: cross-sectional imaging findings of 10 immunohistochemically-verified cases. *World J Gastroenterol* 2009; **15**: 4576-4581 [PMID: 19777618 DOI: 10.3748/wjg.15.4576]

18 **Zeng JP**, Dong JH, Zhang WZ, Wang J, Pang XP. Hepatic angiomyolipoma: a clinical experience in diagnosis and treatment. *Dig Dis Sci* 2010; **55**: 3235-3240 [PMID: 20165978 DOI: 10.1007/s10620-010-1144-2]

19 **Butte JM**, Do RK, Shia J, Gönen M, D'Angelica MI, Getrajdman GI, Allen PJ, Fong Y, Dematteo RP, Klimstra DS, Jarnagin WR. Liver angiomyolipomas: a clinical, radiologic, and pathologic analysis of 22 patients from a single center. *Surgery* 2011; **150**: 557-567 [PMID: 21621235 DOI: 10.1016/j.surg.2011.03.006]

20 **Hu WG**, Lai EC, Liu H, Li AJ, Zhou WP, Fu SY, Pan ZY, Huang G, Lei Y, Lau WY, Wu MC. Diagnostic difficulties and treatment strategy of hepatic angiomyolipoma. *Asian J Surg* 2011; **34**: 158-162 [PMID: 22464831 DOI: 10.1016/j.asjsur.2011.11.005]

21 **Wang WT**, Li ZQ, Zhang GH, Guo Y, Teng MJ. Liver transplantation for recurrent posthepatectomy malignant hepatic angiomyolipoma: a case report. *World J Gastroenterol* 2015; **21**: 3755-3758 [PMID: 25834347 DOI: 10.3748/wjg.v21.i12.3755]

22 **Fukuda Y**, Omiya H, Takami K, Mori K, Kodama Y, Mano M, Nomura Y, Akiba J, Yano H, Nakashima O, Ogawara M, Mita E, Nakamori S, Sekimoto M. Malignant hepatic epithelioid angiomyolipoma with recurrence in the lung 7 years after hepatectomy: a case report and literature review. *Surg Case Rep* 2016; **2**: 31 [PMID: 27037804 DOI: 10.1186/s40792-016-0158-1]

23 **Marcuzzi A**, Haider EA, Salmi ISA. Hepatic epithelioid angiomyolipoma with renal metastasis: radiologic-pathologic correlation. *Radiol Case Rep* 2018; **13**: 829-833 [PMID: 29955240 DOI: 10.1016/j.radcr.2018.05.007]

24 **Yan Z**, Grenert JP, Joseph NM, Ren C, Chen X, Shafizadeh N, Kakar S. Hepatic angiomyolipoma: mutation analysis and immunohistochemical pitfalls in diagnosis. *Histopathology* 2018; **73**: 101-108 [PMID: 29512829 DOI: 10.1111/his.13509]

25 **Huber C**, Treutner KH, Steinau G, Schumpelick V. Ruptured hepatic angiolipoma in tuberous sclerosis complex. *Langenbecks Arch Chir* 1996; **381**: 7-9 [PMID: 8717168 DOI: 10.1007/BF00184248]

26 **Guidi G**, Catalano O, Rotondo A. Spontaneous rupture of a hepatic angiomyolipoma: CT findings and literature review. *Eur Radiol* 1997; **7**: 335-337 [PMID: 9087353 DOI: 10.1007/s003300050162]

27 **Tsui WM**, Colombari R, Portmann BC, Bonetti F, Thung SN, Ferrell LD, Nakanuma Y, Snover DC, Bioulac-Sage P, Dhillon AP. Hepatic angiomyolipoma: a clinicopathologic study of 30 cases and delineation of unusual morphologic variants. *Am J Surg Pathol* 1999; **23**: 34-48 [PMID: 9888702 DOI: 10.1097/00000478-199901000-00004]

28 **Zhou YM**, Li B, Xu F, Wang B, Li DQ, Zhang XF, Liu P, Yang JM. Clinical features of hepatic angiomyolipoma. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 284-287 [PMID: 18522883]

29 **Occhionorelli S**, Dellachiesa L, Stano R, Cappellari L, Tartarini D, Severi S, Palini GM, Pansini GC, Vasquez G. Spontaneous rupture of a hepatic epithelioid angiomyolipoma: damage control surgery. A case report. *G Chir* 2013; **34**: 320-322 [PMID: 24342160]

30 **Aoki H**, Arata T, Morihiro T, Kanaya N, Takeda S, Sui K, Shigeyasu K, Katsuda K, Tanakaya K, Takeuchi H. Spontaneous rupture of a hepatic angiomyolipoma: Report of a case. *Clin J Gastroenterol* 2014; **7**: 429-433 [PMID: 26184024 DOI: 10.1007/s12328-014-0517-z]

31 **Tajima S**, Suzuki A, Suzumura K. Ruptured hepatic epithelioid angiomyolipoma: a case report and literature review. *Case Rep Oncol* 2014; **7**: 369-375 [PMID: 24987358 DOI: 10.1159/000363690]

32 **Kai K**, Miyosh A, Aishima S, Wakiyama K, Nakashita S, Iwane S, Azama S, Irie H, Noshiro H. Granulomatous reaction in hepatic inflammatory angiomyolipoma after chemoembolization and spontaneous rupture. *World J Gastroenterol* 2015; **21**: 9675-9682 [PMID: 26327777 DOI: 10.3748/wjg.v21.i32.9675]

33 **Kim SH**, Kang TW, Lim K, Joh HS, Kang J, Sinn DH. A case of ruptured hepatic angiomyolipoma in a young male. *Clin Mol Hepatol* 2017; **23**: 179-183 [PMID: 28449573 DOI: 10.3350/cmh.2016.0027]

34 **Klompenhouwer AJ**, Dwarkasing RS, Doukas M, Pellegrino S, Vilgrain V, Paradis V, Soubrane O, Beane JD, Geller DA, Nalesnik MA, Tripke V, Lang H, Schmelzle M, Pratschke J, Schöning W, Beal E, Sun S, Pawlik TM, de Man RA, Ijzermans JNM. Hepatic angiomyolipoma: an international multicenter analysis on diagnosis, management and outcome. *HPB (Oxford)* 2020; **22**: 622-629 [PMID: 31619346 DOI: 10.1016/j.hpb.2019.09.004]

35 **Yang X**, Lei C, Qiu Y, Shen S, Lu C, Yan L, Wang W. Selecting a suitable surgical treatment for hepatic angiomyolipoma: a retrospective analysis of 92 cases. *ANZ J Surg* 2018; **88**: E664-E669 [PMID: 29241297 DOI: 10.1111/ans.14323]

36 **Pontis A**, Piras B, Meloni A, De Lisa A, Melis GB, Angioni S. Rupture of renal angiomyolipoma in pregnancy. *J Obstet Gynaecol* 2013; **33**: 628-629 [PMID: 23919867 DOI: 10.3109/01443615.2013.810201]

37 **Yamakado K**, Tanaka N, Nakagawa T, Kobayashi S, Yanagawa M, Takeda K. Renal angiomyolipoma: relationships between tumor size, aneurysm formation, and rupture. *Radiology* 2002; **225**: 78-82 [PMID: 12354988 DOI: 10.1148/radiol.2251011477]

38 **Chang Z**, Zhang JM, Ying JQ, Ge YP. Characteristics and treatment strategy of hepatic angiomyolipoma: a series of 94 patients collected from four institutions. *J Gastrointestin Liver Dis* 2011; **20**: 65-69 [PMID: 21451800 DOI: 10.1007/s11749-010-0230-2]

39 **Eble JN**. Angiomyolipoma of kidney. *Semin Diagn Pathol* 1998; **15**: 21-40 [PMID: 9503504]

40 **Kamimura K**, Nomoto M, Aoyagi Y. Hepatic angiomyolipoma: diagnostic findings and management. *Int J Hepatol* 2012; **2012**: 410781 [PMID: 23320180 DOI: 10.1155/2012/410781]

41 **Osborne JP**, Fryer A, Webb D. Epidemiology of tuberous sclerosis. *Ann N Y Acad Sci* 1991; **615**: 125-127 [PMID: 2039137 DOI: 10.1111/j.1749-6632.1991.tb37754.x]

42 **Black ME**, Hedgire SS, Camposano S, Paul E, Harisinghani M, Thiele EA. Hepatic manifestations of tuberous sclerosis complex: a genotypic and phenotypic analysis. *Clin Genet* 2012; **82**: 552-557 [PMID: 22251200 DOI: 10.1111/j.1399-0004.2012.01845.x]

43 **Orlova KA**, Crino PB. The tuberous sclerosis complex. *Ann N Y Acad Sci* 2010; **1184**: 87-105 [PMID: 20146692 DOI: 10.1111/j.1749-6632.2009.05117.x]

44 **Fricke BL**, Donnelly LF, Casper KA, Bissler JJ. Frequency and imaging appearance of hepatic angiomyolipomas in pediatric and adult patients with tuberous sclerosis. *AJR Am J Roentgenol* 2004; **182**: 1027-1030 [PMID: 15039181 DOI: 10.2214/ajr.182.4.1821027]

45 **Folpe AL**, Kwiatkowski DJ. Perivascular epithelioid cell neoplasms: pathology and pathogenesis. *Hum Pathol* 2010; **41**: 1-15 [PMID: 19604538 DOI: 10.1016/j.humpath.2009.05.011]

46 **Folpe AL**, Mentzel T, Lehr HA, Fisher C, Balzer BL, Weiss SW. Perivascular epithelioid cell neoplasms of soft tissue and gynecologic origin: a clinicopathologic study of 26 cases and review of the literature. *Am J Surg Pathol* 2005; **29**: 1558-1575 [PMID: 16327428 DOI: 10.1097/01.pas.0000173232.22117.37]

47 **Roach ES**, Gomez MR, Northrup H. Tuberous sclerosis complex consensus conference: revised clinical diagnostic criteria. *J Child Neurol* 1998; **13**: 624-628 [PMID: 9881533 DOI: 10.1177/088307389801301206]

48 **Aydin H**, Magi-Galluzzi C, Lane BR, Sercia L, Lopez JI, Rini BI, Zhou M. Renal angiomyolipoma: clinicopathologic study of 194 cases with emphasis on the epithelioid histology and tuberous sclerosis association. *Am J Surg Pathol* 2009; **33**: 289-297 [PMID: 18852677 DOI: 10.1097/PAS.0b013e31817ed7a6]

49 **Mao JX**, Yuan H, Sun KY, Liu C, Fu H, Ding GS, Guo WY, Teng F. Pooled analysis of hepatic inflammatory angiomyolipoma. *Clin Res Hepatol Gastroenterol* 2020; **44**: e145-e151 [PMID: 32482543 DOI: 10.1016/j.clinre.2020.04.005]

50 **Bonetti F**, Pea M, Martignoni G, Zamboni G. PEC and sugar. *Am J Surg Pathol* 1992; **16**: 307-308 [PMID: 1599021 DOI: 10.1097/00000478-199203000-00013]

51 **Hornick JL**, Fletcher CD. Sclerosing PEComa: clinicopathologic analysis of a distinctive variant with a predilection for the retroperitoneum. *Am J Surg Pathol* 2008; **32**: 493-501 [PMID: 18223480 DOI: 10.1097/PAS.0b013e318161dc34]

52 **Liu J**, Zhang CW, Hong DF, Tao R, Chen Y, Shang MJ, Zhang YH. Primary hepatic epithelioid angiomyolipoma: A malignant potential tumor which should be recognized. *World J Gastroenterol* 2016; **22**: 4908-4917 [PMID: 27239117 DOI: 10.3748/wjg.v22.i20.4908]

53 **Yeh CN**, Lee KF, Chen MF. Immunohistochemical study of hepatic angiomyolipoma. *Hepatogastroenterology* 2005; **52**: 1151-1153 [PMID: 16001650]

54 **L'Hostis H**, Deminiere C, Ferriere JM, Coindre JM. Renal angiomyolipoma: a clinicopathologic, immunohistochemical, and follow-up study of 46 cases. *Am J Surg Pathol* 1999; **23**: 1011-1020 [PMID: 10478660 DOI: 10.1097/00000478-199909000-00003]

55 **Ohmori T**, Arita N, Uraga N, Tabei R, Yamamoto M, Kataoka M, Hamamoto K. Giant hepatic angiomyolipoma. *Histopathology* 1989; **15**: 540-543 [PMID: 2599515 DOI: 10.1111/j.1365-2559.1989.tb01615.x]

56 **Kumasaka S**, Arisaka Y, Tokue A, Higuchi T, Nakajima T, Tsushima Y. A case of multiple hepatic angiomyolipomas with high (18) F-fluorodeoxyglucose uptake. *BMC Med Imaging* 2014; **14**: 17 [PMID: 24885757 DOI: 10.1186/1471-2342-14-17]

57 **Dumortier J**, Guillaud O, Walter T, Ber CE, Partensky C, Boillot O, Scoazec JY. Liver transplantation for multiple angiomyolipomas complicating tuberous sclerosis complex. *Gastroenterol Clin Biol* 2010; **34**: 494-498 [PMID: 20674202 DOI: 10.1016/j.gcb.2010.06.005]

58 **Vagefi PA**, Eilers H, Hiniker A, Freise CE. Liver transplantation for giant hepatic angiomyolipoma. *Liver Transpl* 2011; **17**: 985-986 [PMID: 21462294 DOI: 10.1002/lt.22310]

59 **Bissler JJ**, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, Sauter M, Nonomura N, Brakemeier S, de Vries PJ, Whittemore VH, Chen D, Sahmoud T, Shah G, Lincy J, Lebwohl D, Budde K. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet* 2013; **381**: 817-824 [PMID: 23312829 DOI: 10.1016/S0140-6736(12)61767-X]

60 **Bissler JJ**, Kingswood JC, Radzikowska E, Zonnenberg BA, Frost M, Belousova E, Sauter M,

Nonomura N, Brakemeier S, de Vries PJ, Berkowitz N, Miao S, Segal S, Peyrard S, Budde K. Everolimus for renal angiomyolipoma in patients with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis: extension of a randomized controlled trial. *Nephrol Dial Transplant* 2016; **31**: 111-119 [PMID: 26156073 DOI: 10.1093/ndt/gfv249]

61 **Franz DN**. Everolimus in the treatment of subependymal giant cell astrocytomas, angiomyolipomas, and pulmonary and skin lesions associated with tuberous sclerosis complex. *Biologics* 2013; **7**: 211-221 [PMID: 24143074 DOI: 10.2147/BTT.S25095]

62 **Li M**, Zhou Y, Chen C, Yang T, Zhou S, Chen S, Wu Y, Cui Y. Efficacy and safety of mTOR inhibitors (rapamycin and its analogues) for tuberous sclerosis complex: a meta-analysis. *Orphanet J Rare Dis* 2019; **14**: 39 [PMID: 30760308 DOI: 10.1186/s13023-019-1012-x]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2312-2324

DOI: 10.3748/wjg.v27.i19.2312

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

*MINIREVIEWS*

# Risk of hepatitis B virus reactivation in patients with autoimmune diseases undergoing non-tumor necrosis factor-targeted biologics

Shintaro Akiyama, Thomas G Cotter, Atsushi Sakuraba

**ORCID number:** Shintaro Akiyama 0000-0003-0727-7638; Thomas G Cotter 0000-0002-0376-0107; Atsushi Sakuraba 0000-0003-2519-6129.

**Author contributions:** Akiyama S performed the literature search, figures, tables, and data collection; all authors conducted study design, data interpretation, and drafting of manuscripts.

**Conflict-of-interest statement:** The authors have nothing to disclose.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** United

**Shintaro Akiyama, Thomas G Cotter, Atsushi Sakuraba,** Section of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Chicago, Chicago, IL 60637, United States

**Corresponding author:** Shintaro Akiyama, MD, MSc, PhD, Academic Fellow, Doctor, Section of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Chicago, 5841 S. Maryland Ave. MC 4076, Chicago, IL 60637, United States.
akiyama_s@hotmail.co.jp

## Abstract

Hepatitis B virus reactivation (HBVr) can occur in patients treated with immunosuppressive medications. Risk stratification for HBVr based on hepatitis B virus (HBV) serology and viral load is an important strategy to determine appropriate HBV monitoring and antiviral prophylaxis use. Recent advances in the understanding of pathophysiology of autoimmune diseases have led the development of cytokine-targeted therapies. Tumor necrosis factor (TNF)-α inhibitors have been widely used for patients with inflammatory bowel disease, psoriasis, and rheumatic diseases. Further, the clinical benefits of interleukin (IL)-12/23, IL-17, or Janus kinases inhibitors have been demonstrated in these patients. It is well known that TNF-α inhibitor use can lead to HBVr, however, the risk of HBVr in patients undergoing non-TNF-targeted biologics have not been fully understood. In this review, we discuss the risk of HBVr in patients treated with non-TNF-targeted biologics, and immunological mechanisms of these medications causing HBVr.

**Key Words:** Hepatitis B virus; Autoimmune diseases; Biological therapy; Interleukin-23; Interleukin-17; Janus kinases

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** Although the risk of hepatitis B virus reactivation (HBVr) in patients undergoing non-tumor necrosis factor (TNF)-targeted biologics have not been fully understood, some previous studies showed that the risk of HBVr in patients with non-TNF-targeted biologics might be higher than that in patients with TNF-α inhibitors. While patients with chronic hepatitis B virus (HBV) should receive antiviral

States

**Peer-review report's scientific quality classification**
Grade A (Excellent): A, A, A
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

**Received:** January 24, 2021
**Peer-review started:** January 24, 2021
**First decision:** February 23, 2021
**Revised:** February 27, 2021
**Accepted:** April 22, 2021
**Article in press:** April 22, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Georgiev T, Karagiannakis D, Mohsenzadegan M
**S-Editor:** Fan JR
**L-Editor:** A
**P-Editor:** Wang LL

prophylaxis when they start non-TNF-targeted biologics, antiviral prophylaxis may be a favorable strategy rather than the pre-emptive strategy in patients with resolved HBV. Large-scale studies are needed to ascertain the differential risk of HBVr between patients with TNF-α inhibitors and non-TNF-targeted biologics.

**Citation:** Akiyama S, Cotter TG, Sakuraba A. Risk of hepatitis B virus reactivation in patients with autoimmune diseases undergoing non-tumor necrosis factor-targeted biologics. *World J Gastroenterol* 2021; 27(19): 2312-2324
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2312.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2312

## INTRODUCTION

Hepatitis B virus reactivation (HBVr) can occur in patients treated with immunosuppressive therapy and chemotherapy. In the current era of biologics, physicians need to understand the risk of HBVr in patients with autoimmune diseases undergoing anti-cytokine therapies.

The following three components are important for the development of HBVr: (1) The host immune response; (2) The covalently closed circular DNA of the viral genome of HBV (cccDNA); and (3) The use of immunosuppressive drugs[1]. HBV infection induces a series of innate[2] and adaptive[3] immune responses[1]. The host immune responses against hepatitis B virus (HBV) infection recruit adaptive cytotoxic T (Tc) cells to induce both cytolytic-dependent and -independent antiviral effects. In the cytolytic-independent effect, interferons (IFN) play an important role to suppress the HBV replication. To produce neutralizing antibodies to clear circulating HBV, B cells are also recruited to limit the viral spread of HBV (Figure 1)[4]. However, even when clinical resolution of HBV infection is achieved, it does not mean complete elimination of HBV-DNA because cccDNA can persist in the nucleus of hepatocytes and it can be a source of HBVr when immunosuppressive medications are used.

Tumor necrosis factor (TNF)-α is a key cytokine not only in the pathogenesis of autoimmune diseases but also in the host immune reactions against HBV infection. TNF-α is synthesized by macrophages and T cells and induce the production of a variety of inflammatory cytokines, suppressing viral replication[5]. TNF-α is also necessary for the proliferation of HBV-specific Tc cells that are essential for suppression of HBV replication[6]. Hence, TNF-α inhibitors (*e.g.*, infliximab, adalimumab, and etanercept) can inhibit the anti-HBV immune response, leading to HBV replication[7]. Indeed, the pooled prevalence of HBVr in patients with autoimmune diseases undergoing TNF-α inhibitors was reported to be 4.2% (95%CI: 1.4%-8.2%)[8].

The signaling pathways involving interleukin (IL)-12/23, IL-17, and Janus kinases (JAKs) have been highlighted as novel specific therapeutic targets for autoimmune diseases. A recent multicenter observational study for patients with psoriasis showed that HBVr was significantly more common among patients receiving anti-TNF-α therapies than IL-17 inhibitors[9]. However, there is still limited data in understanding the risk of HBVr in patients who are treated with biologics which inhibit such specific inflammatory pathways. In the present article, we aimed to review previous literatures which assessed the risk of HBVr in patients treated with non-TNF-targeted biologics and discuss how each medication can influence the development of HBVr.

## DEFINITIONS OF HBV INFECTION AND REACTIVATION

The professional societies in the United States [American Association for the Study of Liver Diseases (AASLD); American Gastroenterological Association (AGA)], Europe [European Association for the Study of the Liver (EASL)] and Asia [Asian Pacific Association for the Study of the Liver (APASL)] have published guidelines to assist providers with HBVr management[10-13]. In this review article, we divide patients into 2 risk groups which is consistent with the professional society guidelines[10-13] when assessing practical management of HBVr.

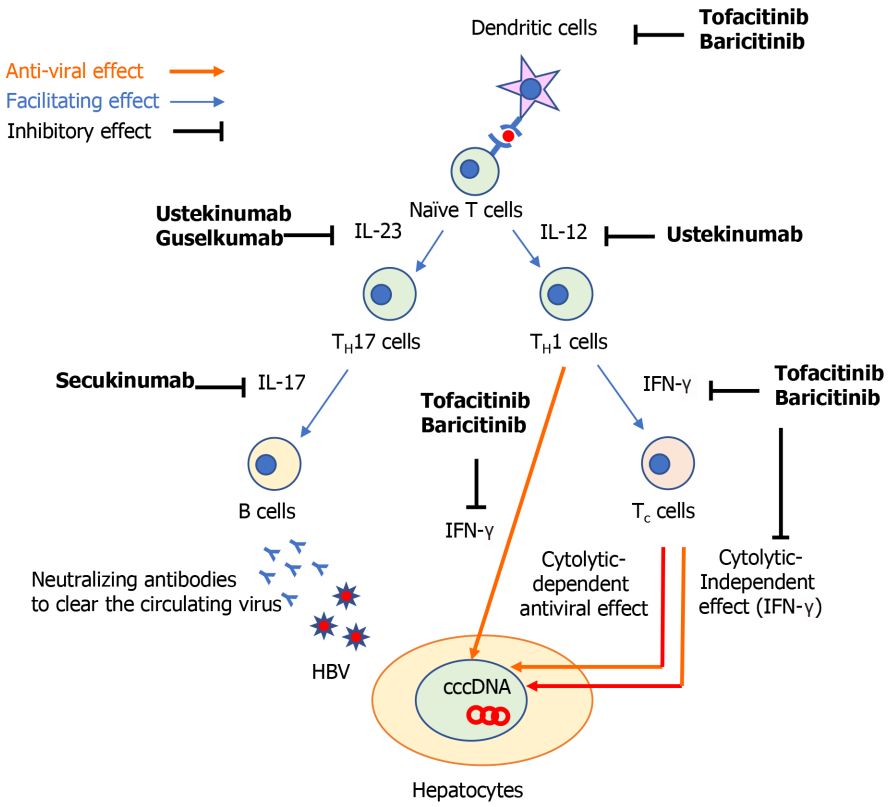



**Figure 1 The possible immunological mechanism to explain how non-tumor necrosis factor-targeted biologics can induce the development of hepatitis B reactivation.** cccDNA: Covalently closed circular DNA; HBV: Hepatitis B virus; IFN: Interferon; IL: Interleukin; $T_H17$ cells: IL-17 producing T helper cells; $T_H1$ cells: T helper 1 cells; Tc cells: Cytotoxic T cells.

### *Chronic HBV*

Chronic HBV (CHB) [*i.e.*, Hepatitis B surface antigen (HBsAg)-positive and antibody to hepatitis B core antigen (anti-HBc)-positive] which includes patients with chronic active [serum HBV DNA ≥ 2000 IU/mL and normal or elevated serum alanine transaminase (ALT)] or inactive (serum HBV DNA < 2000 IU/mL and normal ALT) HBV infection.

### *Resolved HBV*

Resolved HBV (*i.e.*, HBsAg-negative and anti-HBc-positive). Of note, there is insufficient evidence to support the use of anti-HBs titers as a decision aid when making a recommendation regarding prophylaxis[11].

There are subtle differences in the definition of HBVr among the professional society guidelines, however, the general concept is the same[10-13]. In patients with CHB, HBVr is defined by a rise in HBV DNA above baseline. In patients with resolved HBV, HBVr is defined by either the appearance of HBV DNA in the blood or conversion to the HBsAg+ state (*i.e.*, seroreversion). The heterogeneity observed in HBVr definition is also reflected in the existing studies on HBVr. The majority of studies included the following parameters: (1) An acute rise in HBV-DNA levels compared with baseline; (2) Elevated levels of serum aminotransferases; and (3) Seroreversion[1]. In this review article, we followed the criteria of HBVr described in each article.

## THE MANAGEMENT OF PATIENTS WITH HBV INFECTION UNDERGOING IMMUNOSUPPRESSION

### *Risk stratification*

Patients with CHB have an increased risk of HBVr when undergoing immunosuppressive therapy compared to patients with resolved HBV. For example, among patients treated with TNF-α inhibitors there is an estimated 5-fold increased risk of

HBVr in patients with CHB compared to patients with resolved HBV (15.4% *vs* 3.0% risk of HBVr)[8]. Further, another study showed that the pooled rate of HBVr without antiviral prophylaxis was 15.6% (95%CI: 2.3-35.7) in patients with CHB who were treated with TNF-α inhibitors. In patients with resolved HBV, the pooled rates of HBVr without antiviral prophylaxis in patients who were treated with TNF-α inhibitors and non-TNF-targeted biologics were 1.4% (95%CI: 0.5%-2.6%) and 6.1% (95%CI: 0.0%-16.6%), respectively[14]. Each of the 4 professional societal guidelines recommend testing HBV serology on all candidates for immunosuppressive therapy or chemotherapy to enable appropriate risk stratification (*i.e.* CHB *vs* resolved HBV)[10-13].

Next, the degree of expected iatrogenic immunosuppression should be assessed. Hematopoietic stem cell transplant (HSCT) recipients and B cell–depleting therapies (*e.g.*, rituximab) are high-potency regimens and confer the highest risk of HBVr[10-13]. The AGA guidelines ascertain that anthracyclines (*e.g.*, doxorubicin) and moderate- to high-dose corticosteroids (CS) (*i.e.*, ≥ 10 mg of daily prednisone or equivalent for ≥ 4 wk) confer higher risk than other immunosuppressants[11].

### *Therapeutic prophylaxis*

**CHB:** In general, the professional societal guidelines recommend HBV prophylaxis, typically entecavir or tenofovir, for all candidates for immunosuppression who have CHB, apart from patients treated with traditional immunosuppressive agents (*e.g.*, thiopurines, methotrexate), intra-articular CSs, or oral CSs ≤ 1 wk[10-13]. The AGA risk stratify this cohort of patients into moderate (1%-10%) and high risk (> 10%) groups for HBVr[11]. Antiviral prophylaxis should be started before and continued after cessation of immunosuppression, generally 12 to 18 mo if high-potency therapies are used and 6 to 12 mo for other therapies[10-13].

**Resolved HBV:** Guidelines largely agree that resolved HBV patients on high-potency immunosuppression (HSCT recipients and B cell–depleting therapies) should receive HBVr prophylaxis, with the AGA placing this group of patients in the high risk HBVr group (> 10%)[10-13].

For resolved HBV patients not on a high-potency regimen, the guidelines are more dissimilar. AGA recommend prophylaxis for resolved HBV patients at moderate risk (1%-10%) of HBVr, which include patients treated with TNF-α inhibitors, other cytokine or integrin inhibitors, tyrosine kinase inhibitors, moderate- or high-dose CSs for ≥ 4 wk and anthracycline derivatives. In contrast, AASLD, EASL and APASL recommend a pre-emptive therapy for this patient cohort, not prophylaxis, whereby serial lab monitoring (HBV DNA, HBsAg) is performed at 1- to 3-mo intervals on therapy and up to 12 mo after cessation of immunosuppression with on-demand antiviral therapy if needed[10,12,13]. Given that HBsAg seroreversion can lead to fatal acute hepatitis, antiviral therapy should be started immediately, independently of ALT level[11]. Of note, both EASL and APASL recommend treating resolved HBV patients similarly to HBsAg-positive patients if baseline serum HBV-DNA is positive[12,13].

AGA classify resolved HBV patients who are treated with traditional immunosuppressive agents (*e.g.*, thiopurines, methotrexate), low-dose CSs ≥ 4 wk, intra-articular CSs, or any dose of oral CSs for ≤ 1 wk, as low-risk (< 1%) for HBVr and do not recommend prophylaxis, similar to the other society guidelines[10-13].

### *The risk of HBVr in patients who are treated with non-TNF-targeted biologics*

Given the paucity of data on the HBVr risk among patients treated with non-TNF-targeted biologics, we reviewed the existing literature on the risk of HBVr in patients with autoimmune diseases who received non-TNF-targeted biologics and summarized the findings in Tables 1-3. A majority of articles focused on patients with CHB or resolved infection. According to the AGA guideline, CHB and resolved HBV patients treated with non-TNF-targeted therapies are categorized into the moderate-risk HBVr group and therapeutic prophylaxis is recommended. However, AASLD, EASL and APASL recommend serial monitoring among resolved HBV (if HBV DNA is negative) with pre-emptive prophylaxis if HBVr is observed. Therefore, it is important to determine the precise HBVr with non-TNF-targeted biologics to ascertain if a strategy of monitoring/pre-emptive may be too lax, and perhaps a uniform strategy of prophylaxis may be more optimal as recommended by the AGA. Given that patients with resolved infection should be treated similarly to those with CHB patients if their serum HBV-DNA tests are positive at baseline[12,13], we present their baseline HBV-DNA in Tables 1-3.

**Table 1 The risk of hepatitis B virus reactivation in patients treated with interleukin-12/23 or interleukin-23 inhibitors**

| Ref. | Number of HBV patients | HBV status | Disease | Drugs | Prophylaxis | Follow-up | HBV reactivation |
|---|---|---|---|---|---|---|---|
| Ting *et al*[27], 2018 | 54 | (1) 10 CHB; and (2) 44 resolved HBV. HBV-DNA at baseline (-) | Psoriasis | Ustekinumab | Yes: 2 patients with CHB | 24 mo | (1) 2 patients with CHB without prophylaxis. (no hepatitis); and (2) 1 patient with resolved HBV (mild hepatitis) |
| Solay *et al*[5], 2018 | 29 | 29 resolved HBV. HBV-DNA at baseline (-) | Psoriasis/HS/AS/RA/CD | Ustekinumab ( *n* = 7) | NA | 22 wk | 1 patient with psoriasis without prophylaxis (no data regarding hepatitis) |
| Sanz-Bueno *et al*[68], 2015 | 20 | 20 resolved HBV. HBV-DNA at baseline (-) but viral load was assessed in 7 of 20 patients | Psoriasis | Ustekinumab ( *n* = 6) | No | 40 mo | 0 |
| Chiu *et al*[28], 2013 | 14 | (1) 11 CHB; and (2) 3 resolved HBV. HBV-DNA at baseline was not available | Psoriasis | Ustekinumab | Yes: 4 patients with CHB | 10 mo | (1) 2 patients with CHB without prophylaxis (No hepatitis); and (2) 0 |
| Navarro *et al*[69], 2013 | 5 | 5 CHB | Psoriasis | Ustekinumab ( *n* = 1) | Yes | 25 mo | 0 |
| Hayashi *et al*[70], 2014 | 5 | 5 resolved HBV. HBV-DNA at baseline was not available | Psoriasis | Ustekinumab | No | 52 wk | 0 |
| Koskinas *et al*[41], 2013 | 1 | Resolved HBV. HBV-DNA at baseline was not available | Psoriasis | Ustekinumab | No | 16 mo | 1 with hepatitis (ALT 65 IU/mL) |
| Steglich *et al*[71], 2014 | 1 | Resolved HBV. HBV-DNA at baseline (-) | Psoriasis | Ustekinumab | Yes | 36 mo | 0 |
| Duncan *et al*[43], 2019 | 1 | Resolved HBV. HBV-DNA at baseline was not available | Palmoplantar Psoriasis | Guselkumab | No | 12 mo | 0 |

If a study included both of patients with chronic hepatitis B virus (HBV) and those with resolved HBV, we labeled the former with (1) and the latter with (2) in the column with HBV status. HBV: Hepatitis B virus; AS: Ankylosing spondylitis; CD: Crohn's disease; CHB: Chronic HBV; HS: Hidradenitis suppurativa; RA: Rheumatoid arthritis; NA: Not available; ALT: Alanine transaminase.

## IL-12/23 INHIBITORS

### *Mechanism of HBV reactivation*

The cytokine IL-12 contributes to the differentiation of naïve T cells to T helper 1 ($T_H1$) cells and IL-23 maintains and expand IL-17 producing T helper ($T_H17$) cells (Figure 1) [15]. These two cytokines play a central role to regulate T cell-mediated immune responses, which are dysregulated in various autoimmune diseases including psoriasis and Crohn's disease (CD)[15,16]. The clinical benefit of IL-12 and IL-23 inhibition has been demonstrated in psoriasis, CD, and ulcerative colitis by ustekinumab[17-19], which is an antibody against p40, the common subunit of IL-12 and IL-23. IL-12 plays an important role in achieving sustained control of HBV replication. IL-12 can promote cell-mediated immunity by facilitating the production of IFN-γ production by $T_H1$ cells, resulting in the inhibition of HBV replication[20,21] and the induction of antiviral effects of HBV-specific Tc cells[22,23]. Indeed, patients with CHB who were treated with recombinant human IL-12 exhibited a high proportion of HBV clearance in a dose-dependent manner[24] and the addition of IL-12 to lamivudine enhanced T cell reactivity to HBV and IFN-γ production[25]. Furthermore, patients with CHB responding to IFN-α treatment were shown to have higher IL-12 and IFN-γ expression levels during the treatment[26]. These findings suggest that ustekinumab might theoretically increase the risk of HBVr.

**Table 2 The risk of hepatitis B virus reactivation in patients with interleukin-17 inhibitors**

| Ref. | Number of HBV patients | HBV status | Disease | Drugs | Prophylaxis | Follow-up | HBV reactivation |
|---|---|---|---|---|---|---|---|
| Chiu *et al*[52], 2018 | 49 | (1) 25 CHB; and (2) 24 resolved HBV. HBV-DNA at baseline (-) in 11 patients with resolved HBV | Psoriasis | Secukinumab | Yes: 3 patients with CHB | 3 mo | (1) 6 patients with CHB without prophylaxis. (no hepatitis); and (2) 1 patient with resolved HBV with positive viral load at baseline (no hepatitis) |
| Moneva-Leniz *et al*[72], 2020 | 4 | (1) 2 CHB; and (2) 2 resolved HBV. HBV-DNA at baseline (-) | Psoriasis/palmoplantar psoriasis | Secukinumab | Yes: 1 patient with CHB and 1 patient with resolved HBV | 20 mo | (1) 0; and (2) 0 |
| Feaster *et al*[73], 2018 | 1 | A carrier of congenital HBV infection[1] | Psoriasis and PsA | Secukinumab | No | 24 mo | 0 |
| Bevans *et al*[74], 2018 | 1 | Seropositive hepatitis[1] | Palmoplantar psoriasis and AS | Secukinumab | No | 14 mo | 0 |
| Yanagihara *et al*[75], 2017 | 1 | CHB | Psoriasis vulgaris | Secukinumab | Yes | 9 mo | 0 |
| Peccerillo *et al*[76], 2018 | 1 | Resolved HBV. HBV-DNA at baseline (-) | Psoriasis | Secukinumab | Yes | 14 mo | 0 |
| Koike *et al*[53], 2019 | 1 | CHB | Psoriasis and PsA | Ixekizumab | Yes | 18 mo | 0 |
| Lora *et al*[54], 2019 | 1 | Resolved HBV. HBV-DNA at baseline (-) | Psoriasis | Ixekizumab | Yes | 12 mo | 0 |

[1]A diagnosis in an article was used due to lack of data regarding hepatitis B virus (HBV) serology and viral load. If a study included both of patients with chronic HBV and those with resolved HBV, we labeled the former with (1) and the latter with (2) in the column with HBV status. HBV: Hepatitis B virus; AS: Ankylosing spondylitis; CHB: Chronic HBV; PsA: Psoriatic arthritis.

**Table 3 The risk of hepatitis B virus reactivation in patients with Janus kinase inhibitors**

| Ref. | Number of HBV patients | Serology for HBV infectious | Disease | Drugs | Prophylaxis | Follow-up | HBV reactivation |
|---|---|---|---|---|---|---|---|
| Chen *et al*[66], 2018 | 81 | (1) 6 CHB; and (2) 75 resolved HBV. HBV-DNA at baseline (-) but viral load was assessed in 53 patients with resolved HBV | RA | Tofacitinib | Yes: 2 patients with CHB | 3-6 mo | (1) 2 patients with CHB without prophylaxis (1 patient developed hepatitis); and (2) 0 |
| Serling-Boyd *et al*[67], 2021 | 8 | 8 resolved HBV. HBV-DNA was assessed in 6 patients, but viral loads were not available | 7 RA, 1 PsA | Tofacitinib | Yes: 2 patients | 3.1 yr | 0 |
| Harigai *et al*[62], 2020 | 215 | 215 resolved HBV. HBV-DNA (-) at baseline in 30 patients with resolved HBV who had detectable post-baseline HBV-DNA | RA | Baricitinib | NA | 2.7 yr | 8 patients with resolved HBV had HBV-DNA ≥ 29 IU/mL (4 patients med the criteria of HBVr in this study, no hepatitis) |

If a study included both of patients with chronic hepatitis B virus (HBV) and those with resolved HBV, we labeled the former with (1) and the latter with (2) in the column with HBV status. HBV: Hepatitis B virus; CHB: Chronic HBV; PsA: Psoriatic arthritis; RA: Rheumatoid arthritis; NA: Not available; HBVr: Hepatitis B virus reactivation.

### *Clinical studies in patients with autoimmune diseases*

Several studies have assessed the risk of HBVr in patients treated with ustekinumab (Table 1). A retrospective study showed that no HBVr occurred among 2 patients with CHB taking antiviral prophylaxis after starting ustekinumab, whereas, 2 patients (25%) developed HBVr without hepatitis among 8 patients with CHB without antiviral prophylaxis[27]. Another study demonstrated a 29% rate of HBVr after ustekinumab initiation without antiviral prophylaxis in patients with CHB[28]. Given that patients

with CHB have a high risk of HBVr without antiviral prophylaxis, these patients require antiviral prophylaxis and appropriate monitoring for HBV-DNA and serology tests after initiating ustekinumab treatment.

A retrospective study on 44 patients with resolved HBV who initiated ustekinumab without antiviral prophylaxis found that 1 patient (2.3%) developed HBVr complicated with mild hepatitis[27]. This patient discontinued concurrent methotrexate when reactivation occurred and HBV-DNA became undetectable without antiviral therapy in 6 mo. In another study involving 7 patients with resolved HBV on ustekinuamb, 1 patient (14.3%) developed HBVr. This patient was not on antiviral prophylaxis and started entecavir for treatment of HBVr[5]. These data suggest that there is a certain risk of HBVr in patients with resolved HBV even without detectable HBV-DNA at baseline after starting ustekinumab, suggesting that these patients might need antiviral prophylaxis as is the AGA guidelines preferred option. While the guidelines of AASLD, EASL, APASL recommend pre-emptive therapy if HBV DNA is negative, further studies are warranted in order to understand if these patients require antiviral prophylaxis.

## IL-23 INHIBITORS

### *Mechanism of HBV reactivation*

IL-23-specific antagonists, such as tildrakizumab[29,30], risankizumab[31,32], guselkumab[33,34], and brazikumab[35], have been shown to be effective for psoriasis and CD. These medications bind to the p19 subunit on IL-23 and inhibit its interaction with the IL-23 receptors[36]. The potential mechanism of HBVr in patients treated with IL-23 inhibitors is still unclear. Previous studies found that $T_H17$ cells, which are expanded by IL-23 (Figure 1), increase with the severity of liver damage in patients with CHB[37-39]. An observational, clinical-controlled study also demonstrated that the expression levels of IL-23 and IL-17 were associated with increased possibilities of hepatitis B e-antigen (HBeAg) clearance and HBsAg decline in patients with HBeAg-positive CHB during pegylated IFN therapy[40]. This study also found that high serum IL-23 Levels can predict the response to IFN therapy in patients with HBeAg-positive CHB[40]. Given that $T_H17$ cells promote the differentiation and function of B cells[41,42], IL-23 might activate the humoral immune response against circulating HBV and play a role to facilitate HBV clearance by IFN therapy (Figure 1). Although this hypothesis suggest that IL-23 inhibitors may abrogate the HBV clearance, it still remains to be elucidated whether these medications contribute to the development of HBVr.

### *Clinical studies in patients with autoimmune diseases*

The data regarding the safety of IL-23 inhibitors in patients with HBV infection is limited. A case report showed that a patient with resolved HBV infection did not develop HBVr 1 year after starting guselkumab (Table 1)[43]. There have been no reported studies focusing on the risk of HBVr in patients treated with other IL-23 inhibitors.

## IL-17 INHIBITORS

### *Mechanism of HBV reactivation*

IL-17 is a major effector cytokine of $T_H17$ cells and mediate host defense mechanisms[44]. Inhibition of IL-17 with secukinumab, ixekizumab, and brodalumab have demonstrated clinical benefits in patients with psoriasis[45-47], psoriatic arthritis[48], and ankylosing spondylitis[49]. $T_H17$/IL-17 axis is involved in the process of fibrogenesis and increases the expression of proinflammatory cytokines, promoting the recruitment of inflammatory cells in patients with CHB[50]. A previous study showed that Th17 cells were significantly increased in patients with CHB, as well as the expression level of IL-17[51]. They also demonstrated that the suppression of viral replication induced by IFN-α resulted in a decrease in $T_H17$ cells and IL-17 expression, suggesting that $T_H17$ cells might play an important role during IFN-α treatment to eliminate HBV[51]. As we described above, $T_H17$ cells also facilitate B cells[41,42] and would enhance the humoral response to clear circulating HBV (Figure 1). These findings implicate that $T_H17$/IL-17 axis might be associated with HBV clearance and its inhibition may increase the risk of HBVr.

### *Clinical studies in patients with autoimmune diseases*

A prospective multicenter study on 22 patients with CHB with no antiviral prophylaxis after starting secukinumab showed that 6 patients (27.3%) developed HBVr (Table 2)[52]. Three patients with HBVr started antiviral treatments and their viral loads decreased rapidly within 3 mo. The remaining three patients with HBVr were followed without antiviral drugs and their viral loads remained low without acute hepatitis. Notably, none of the 3 patients with CHB who received antiviral prophylaxis developed HBVr[52]. Hence, this study reinforced the importance of antiviral prophylaxis in patients with CHB starting treatment with IL-17 inhibitors. This study also included 24 patients with resolved HBV who did not receive antiviral prophylaxis and identified one patient (4.2%) with a positive viral load at baseline who developed HBVr without acute hepatitis[52]. This study re-affirmed the EASL and APASL guidelines which recommend antiviral prophylaxis in patients with resolved HBV if their baseline viral loads are positive.

A case report on a patient with CHB treated with ixekizumab and entecavir simultaneously did not develop HBVr after 18 mo of treatment[53]. Another report showed that a patient with resolved HBV did not experience HBVr during follow-up (Table 2)[54]. Given that data regarding the risk of HBVr in patients treated with ixekizumab or brodalumab are still limited, further studies with larger sample sizes are warranted.

## JAK INHIBITORS

### *Mechanism of HBV reactivation*

JAKs bind to type I and II cytokine receptors and transmit extracellular cytokine signals to activate various signal transducers and activators of transcription, which drive the proinflammatory machinery of the cellular immune response[55]. The clinical benefit of JAK inhibitors has been demonstrated in patients with rheumatoid arthritis[56,57], psoriatic arthritis[58,59], and ulcerative colitis[60,61]. Important signaling pathways in host-defense include innate antiviral responses *via* IFN-α/β mediated by JAK1-tyrosine kinase 2 complexes, and IFN-γ mediated by JAK1-JAK2 complexes[55]. Hence, JAK inhibitors might counteract the suppressive effects of IFN on viral replication[62,63]. Further, dendritic cells and effective T cell lineages including $T_H$ cells and Tc cells play important roles to defense against HBV-infection (Figure 1)[64]. A previous study demonstrated that a JAK inhibitor can block the differentiation and function of dendritic cells, leading to impaired T cell activation (Figure 1)[65]. Thus, it was suggested that JAK inhibitors might negatively interact with the defense mechanism against HBV infection. Further studies investigating how JAK inhibitors influence the development of HBVr are warranted.

### *Clinical studies in patients with autoimmune diseases*

A retrospective cohort study including 6 patients with CHB showed that 2 out of 4 patients (50%) without antiviral prophylaxis developed HBVr after starting tofacitinib. One patient had an elevated ALT level and started entecavir, resulting in declines in HBV-DNA and ALT levels. Another patient started entecavir and did not develop acute hepatitis. Both patients continued tofacitinib after the development of HBVr. Meanwhile, 2 patients with CHB who received antiviral prophylaxis did not develop HBVr after initiating tofacitinib. Further, in this study, none of 75 patients with resolved HBV received antiviral prophylaxis and no HBVr was observed in this group[66]. Another study also demonstrated that patients with resolved HBV did not develop HBVr after starting tofacitinib (Table 3)[67].

A study assessing data which was integrated from four phase 3 trials of baricitinib in patients showed that, among 215 patients with resolved HBV, 8 patients (3.7%) had a single quantifiable result of HBV-DNA viral load (HBV-DNA level ≥ 29 IU/mL) after initiating baricitinib. Among these 8 patients, 4 patients met the definition of HBVr (HBV-DNA ≥ 100 IU/mL), but no patients developed hepatitis. HBV-DNA at baseline was assessed in 6 patients and all examined patients did not have detectable HBV-DNA level. Antiviral therapy was not used in 5 of 8 patients[62].

All these findings suggest that patients with CHB should receive antiviral prophylaxis when they start JAK inhibitors. As for patients with resolved HBV infection, given that HBVr was occasionally reported even if their HBV-DNA levels were not detected at baseline, an appropriate consultation with hepatologists is necessary. There has been limited data regarding the risk of HBVr in patients with autoimmune diseases who are treated with other JAK inhibitors (*e.g.*, upadacitinib,

filgotinib, peficitinib).

## CONCLUSION

In summary, considering antiviral prophylaxis with an appropriate risk stratification is necessary when we start non-TNF-targeted biologics for patients with autoimmune diseases. The frequencies of HBVr without antiviral prophylaxis in patients with CHB on IL-12/23, IL-17, and JAK inhibitors are up to 29%, 27%, and 50%, respectively. A meta-analysis demonstrated that the pooled rate of HBVr without antiviral prophylaxis was 15.6% (95%CI: 2.3-35.7) in patients with CHB who were treated with TNF-α inhibitors, suggesting that non-TNF-targeted biologics, particularly JAK inhibitors, may have a higher risk of HBVr compared with TNF-α inhibitors. Given that no patients who received antiviral prophylaxis developed HBVr, HBVr is preventable with antiviral therapy in patients with CHB on non-TNF-targeted biologics. As all of professional societies recommended in their guidelines, patients with CHB should receive antiviral prophylaxis when they start non-TNF-targeted biologics. In patients with resolved HBV, the rates of HBVr without antiviral prophylaxis in patients on IL-12/23, IL-17, and JAK inhibitors are up to 2.3%, 4.2%, and 0%, respectively. The meta-analysis showed that the pooled rates of HBVr without antiviral prophylaxis in patients who were treated with TNF-α inhibitors and non-TNF-targeted biologics were 1.4% (95%CI: 0.5%-2.6%) and 6.1% (95%CI: 0.0%-16.6%), respectively[14]. These data supported that the risk of HBVr in patients treated with non-TNF-targeted biologics might be higher than that in patients with TNF-α inhibitors even if their HBV status is resolved HBV. According to the AGA guideline, patients with resolved HBV who are treated with non-TNF-targeted biologics are categorized into the moderate risk group and antiviral prophylaxis are recommended for this patient cohort[11]. However, as stated previously, AASLD, EASL and APASL recommend the pre-emptive therapeutic strategy for this cohort, although APASL and EASL do include the caveat of potentially using HBV DNA assessment to aid decision-making[10,12,13]. Given the higher risk of HBVr with non-TNF-targeted biologics compared with TNF-α inhibitors, antiviral prophylaxis may be a favorable strategy rather than the pre-emptive strategy to prevent HBVr in patients with resolved HBV. Large-scale studies are needed to ascertain the differential risk of HBVr between patients with TNF-α inhibitors and non-TNF-targeted biologics and to stratify the risk of HBVr by the type of non-TNF-targeted biologics. While HBsAg seroreversion can lead to fatal acute hepatitis, a consultation with hepatologists or infectious disease specialists is recommended.

## REFERENCES


1 **Gentile G**, Antonelli G. HBV Reactivation in Patients Undergoing Hematopoietic Stem Cell Transplantation: A Narrative Review. *Viruses* 2019; **11** [PMID: 31717647 DOI: 10.3390/v11111049]

2 **Maini MK**, Gehring AJ. The role of innate immunity in the immunopathology and treatment of HBV infection. *J Hepatol* 2016; **64**: S60-S70 [PMID: 27084038 DOI: 10.1016/j.jhep.2016.01.028]

3 **Bertoletti A**, Ferrari C. Adaptive immunity in HBV infection. *J Hepatol* 2016; **64**: S71-S83 [PMID: 27084039 DOI: 10.1016/j.jhep.2016.01.026]

4 **Guidotti LG**, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol* 2006; **1**: 23-61 [PMID: 18039107 DOI: 10.1146/annurev.pathol.1.110304.100230]

5 **Solay AH**, Acar A, Eser F, Kuşcu F, Tütüncü EE, Kul G, Şentürk GÇ, Gürbüz Y. Reactivation rates in patients using biological agents, with resolved HBV infection or isolated anti-HBc IgG positivity. *Turk J Gastroenterol* 2018; **29**: 561-565 [PMID: 30260778 DOI: 10.5152/tjg.2018.18032]

6 **Kasahara S**, Ando K, Saito K, Sekikawa K, Ito H, Ishikawa T, Ohnishi H, Seishima M, Kakumu S, Moriwaki H. Lack of tumor necrosis factor alpha induces impaired proliferation of hepatitis B virus-specific cytotoxic T lymphocytes. *J Virol* 2003; **77**: 2469-2476 [PMID: 12551985 DOI: 10.1128/jvi.77.4.2469-2476.2003]

7 **Manzano-Alonso ML**, Castellano-Tortajada G. Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. *World J Gastroenterol* 2011; **17**: 1531-1537 [PMID: 21472116 DOI: 10.3748/wjg.v17.i12.1531]

8 **Cantini F**, Boccia S, Goletti D, Iannone F, Leoncini E, Panic N, Prignano F, Gaeta GB. HBV Reactivation in Patients Treated with Antitumor Necrosis Factor-Alpha (TNF-α) Agents for Rheumatic and Dermatologic Conditions: A Systematic Review and Meta-Analysis. *Int J Rheumatol* 2014; **2014**: 926836 [PMID: 25114684 DOI: 10.1155/2014/926836]

9 **Chiu HY**, Chiu YM, Chang Liao NF, Chi CC, Tsai TF, Hsieh CY, Hsieh TY, Lai KL, Chiu TM, Wu NL, Hui RC, Lee CN, Wang TS, Chen PH, Yang CC, Huang YH. Predictors of hepatitis B and C

virus reactivation in patients with psoriasis treated with biological agent: A nine-year multicenter cohort study. *J Am Acad Dermatol* 2019 [PMID: 31821860 DOI: 10.1016/j.jaad.2019.12.001]

10 **Terrault NA**, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, Brown RS Jr, Bzowej NH, Wong JB. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. *Hepatology* 2018; **67**: 1560-1599 [PMID: 29405329 DOI: 10.1002/hep.29800]

11 **Reddy KR**, Beavers KL, Hammond SP, Lim JK, Falck-Ytter YT; American Gastroenterological Association Institute. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology* 2015; **148**: 215-9; quiz e16 [PMID: 25447850 DOI: 10.1053/j.gastro.2014.10.039]

12 **European Association for the Study of the Liver**. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]

13 **Sarin SK**, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, Chen DS, Chen HL, Chen PJ, Chien RN, Dokmeci AK, Gane E, Hou JL, Jafri W, Jia J, Kim JH, Lai CL, Lee HC, Lim SG, Liu CJ, Locarnini S, Al Mahtab M, Mohamed R, Omata M, Park J, Piratvisuth T, Sharma BC, Sollano J, Wang FS, Wei L, Yuen MF, Zheng SS, Kao JH. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016; **10**: 1-98 [PMID: 26563120 DOI: 10.1007/s12072-015-9675-4]

14 **Lin TC**, Yoshida K, Tedeschi SK, de Abreu MM, Hashemi N, Solomon DH. Risk of Hepatitis B Virus Reactivation in Patients With Inflammatory Arthritis Receiving Disease-Modifying Antirheumatic Drugs: A Systematic Review and Meta-Analysis. *Arthritis Care Res (Hoboken)* 2018; **70**: 724-731 [PMID: 28834412 DOI: 10.1002/acr.23346]

15 **Yen D**, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W, Murphy E, Sathe M, Cua DJ, Kastelein RA, Rennick D. IL-23 is essential for T cell-mediated colitis and promotes inflammation *via* IL-17 and IL-6. *J Clin Invest* 2006; **116**: 1310-1316 [PMID: 16670770 DOI: 10.1172/JCI21404]

16 **Lee E**, Trepicchio WL, Oestreicher JL, Pittman D, Wang F, Chamian F, Dhodapkar M, Krueger JG. Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. *J Exp Med* 2004; **199**: 125-130 [PMID: 14707118 DOI: 10.1084/jem.20030451]

17 **Papp KA**, Langley RG, Lebwohl M, Krueger GG, Szapary P, Yeilding N, Guzzo C, Hsu MC, Wang Y, Li S, Dooley LT, Reich K; PHOENIX 2 study investigators. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet* 2008; **371**: 1675-1684 [PMID: 18486740 DOI: 10.1016/S0140-6736(08)60726-6]

18 **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]

19 **Sands BE**, Sandborn WJ, Panaccione R, O'Brien CD, Zhang H, Johanns J, Adedokun OJ, Li K, Peyrin-Biroulet L, Van Assche G, Danese S, Targan S, Abreu MT, Hisamatsu T, Szapary P, Marano C; UNIFI Study Group. Ustekinumab as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2019; **381**: 1201-1214 [PMID: 31553833 DOI: 10.1056/NEJMoa1900750]

20 **Zeuzem S**, Carreño V. Interleukin-12 in the treatment of chronic hepatitis B and C. *Antiviral Res* 2001; **52**: 181-188 [PMID: 11672828 DOI: 10.1016/s0166-3542(01)00183-8]

21 **Cavanaugh VJ**, Guidotti LG, Chisari FV. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. *J Virol* 1997; **71**: 3236-3243 [PMID: 9060687 DOI: 10.1128/JVI.71.4.3236-3243.1997]

22 **Guidotti LG**. The role of cytotoxic T cells and cytokines in the control of hepatitis B virus infection. *Vaccine* 2002; **20** Suppl 4: A80-A82 [PMID: 12477433 DOI: 10.1016/s0264-410x(02)00392-4]

23 **McClary H**, Koch R, Chisari FV, Guidotti LG. Relative sensitivity of hepatitis B virus and other hepatotropic viruses to the antiviral effects of cytokines. *J Virol* 2000; **74**: 2255-2264 [PMID: 10666256 DOI: 10.1128/jvi.74.5.2255-2264.2000]

24 **Carreño V**, Zeuzem S, Hopf U, Marcellin P, Cooksley WG, Fevery J, Diago M, Reddy R, Peters M, Rittweger K, Rakhit A, Pardo M. A phase I/II study of recombinant human interleukin-12 in patients with chronic hepatitis B. *J Hepatol* 2000; **32**: 317-324 [PMID: 10707873 DOI: 10.1016/s0168-8278(00)80078-1]

25 **Rigopoulou EI**, Suri D, Chokshi S, Mullerova I, Rice S, Tedder RS, Williams R, Naoumov NV. Lamivudine plus interleukin-12 combination therapy in chronic hepatitis B: antiviral and immunological activity. *Hepatology* 2005; **42**: 1028-1036 [PMID: 16250037 DOI: 10.1002/hep.20888]

26 **Rossol S**, Marinos G, Carucci P, Singer MV, Williams R, Naoumov NV. Interleukin-12 induction of Th1 cytokines is important for viral clearance in chronic hepatitis B. *J Clin Invest* 1997; **99**: 3025-3033 [PMID: 9185527 DOI: 10.1172/JCI119498]

27 **Ting SW**, Chen YC, Huang YH. Risk of Hepatitis B Reactivation in Patients with Psoriasis on Ustekinumab. *Clin Drug Investig* 2018; **38**: 873-880 [PMID: 29968197 DOI: 10.1007/s40261-018-0671-z]

28 **Chiu HY**, Chen CH, Wu MS, Cheng YP, Tsai TF. The safety profile of ustekinumab in the treatment of patients with psoriasis and concurrent hepatitis B or C. *Br J Dermatol* 2013; **169**: 1295-1303

[PMID: 23746170 DOI: 10.1111/bjd.12461]
29 **Reich K**, Papp KA, Blauvelt A, Tyring SK, Sinclair R, Thaçi D, Nograles K, Mehta A, Cichanowitz N, Li Q, Liu K, La Rosa C, Green S, Kimball AB. Tildrakizumab *vs* placebo or etanercept for chronic plaque psoriasis (reSURFACE 1 and reSURFACE 2): results from two randomised controlled, phase 3 trials. *Lancet* 2017; **390**: 276-288 [PMID: 28596043 DOI: 10.1016/S0140-6736(17)31279-5]
30 **Papp K**, Thaçi D, Reich K, Riedl E, Langley RG, Krueger JG, Gottlieb AB, Nakagawa H, Bowman EP, Mehta A, Li Q, Zhou Y, Shames R. Tildrakizumab (MK-3222), an anti-interleukin-23p19 monoclonal antibody, improves psoriasis in a phase IIb randomized placebo-controlled trial. *Br J Dermatol* 2015; **173**: 930-939 [PMID: 26042589 DOI: 10.1111/bjd.13932]
31 **Gordon KB**, Strober B, Lebwohl M, Augustin M, Blauvelt A, Poulin Y, Papp KA, Sofen H, Puig L, Foley P, Ohtsuki M, Flack M, Geng Z, Gu Y, Valdes JM, Thompson EHZ, Bachelez H. Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials. *Lancet* 2018; **392**: 650-661 [PMID: 30097359 DOI: 10.1016/S0140-6736(18)31713-6]
32 **Feagan BG**, Sandborn WJ, D'Haens G, Panés J, Kaser A, Ferrante M, Louis E, Franchimont D, Dewit O, Seidler U, Kim KJ, Neurath MF, Schreiber S, Scholl P, Pamulapati C, Lalovic B, Visvanathan S, Padula SJ, Herichova I, Soaita A, Hall DB, Böcher WO. Induction therapy with the selective interleukin-23 inhibitor risankizumab in patients with moderate-to-severe Crohn's disease: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet* 2017; **389**: 1699-1709 [PMID: 28411872 DOI: 10.1016/S0140-6736(17)30570-6]
33 **Gordon KB**, Duffin KC, Bissonnette R, Prinz JC, Wasfi Y, Li S, Shen YK, Szapary P, Randazzo B, Reich K. A Phase 2 Trial of Guselkumab *vs* Adalimumab for Plaque Psoriasis. *N Engl J Med* 2015; **373**: 136-144 [PMID: 26154787 DOI: 10.1056/NEJMoa1501646]
34 **Reich K**, Armstrong AW, Foley P, Song M, Wasfi Y, Randazzo B, Li S, Shen YK, Gordon KB. Efficacy and safety of guselkumab, an anti-interleukin-23 monoclonal antibody, compared with adalimumab for the treatment of patients with moderate to severe psoriasis with randomized withdrawal and retreatment: Results from the phase III, double-blind, placebo- and active comparator-controlled VOYAGE 2 trial. *J Am Acad Dermatol* 2017; **76**: 418-431 [PMID: 28057361 DOI: 10.1016/j.jaad.2016.11.042]
35 **Sands BE**, Chen J, Feagan BG, Penney M, Rees WA, Danese S, Higgins PDR, Newbold P, Faggioni R, Patra K, Li J, Klekotka P, Morehouse C, Pulkstenis E, Drappa J, van der Merwe R, Gasser RA Jr. Efficacy and Safety of MEDI2070, an Antibody Against Interleukin 23, in Patients With Moderate to Severe Crohn's Disease: A Phase 2a Study. *Gastroenterology* 2017; **153**: 77-86. e6 [PMID: 28390867 DOI: 10.1053/j.gastro.2017.03.049]
36 **Frieder J**, Kivelevitch D, Haugh I, Watson I, Menter A. Anti-IL-23 and Anti-IL-17 Biologic Agents for the Treatment of Immune-Mediated Inflammatory Conditions. *Clin Pharmacol Ther* 2018; **103**: 88-101 [PMID: 28960267 DOI: 10.1002/cpt.893]
37 **Zhang JY**, Zhang Z, Lin F, Zou ZS, Xu RN, Jin L, Fu JL, Shi F, Shi M, Wang HF, Wang FS. Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B. *Hepatology* 2010; **51**: 81-91 [PMID: 19842207 DOI: 10.1002/hep.23273]
38 **Xia L**, Tian D, Huang W, Zhu H, Wang J, Zhang Y, Hu H, Nie Y, Fan D, Wu K. Upregulation of IL-23 expression in patients with chronic hepatitis B is mediated by the HBx/ERK/NF-κB pathway. *J Immunol* 2012; **188**: 753-764 [PMID: 22174449 DOI: 10.4049/jimmunol.1101652]
39 **Ge J**, Wang K, Meng QH, Qi ZX, Meng FL, Fan YC. Implication of Th17 and Th1 cells in patients with chronic active hepatitis B. *J Clin Immunol* 2010; **30**: 60-67 [PMID: 19756987 DOI: 10.1007/s10875-009-9328-2]
40 **Yu C**, Gong X, Yang Q, Lian J, Xu K, Ruan B, Li L. The serum IL-23 Level predicts the response to pegylated interferon therapy in patients with chronic hepatitis B. *Liver Int* 2015; **35**: 1549-1556 [PMID: 25312687 DOI: 10.1111/liv.12701]
41 **Koskinas J**, Tampaki M, Doumba PP, Rallis E. Hepatitis B virus reactivation during therapy with ustekinumab for psoriasis in a hepatitis B surface-antigen-negative anti-HBs-positive patient. *Br J Dermatol* 2013; **168**: 679-680 [PMID: 23121260 DOI: 10.1111/bjd.12120]
42 **Mitsdoerffer M**, Lee Y, Jäger A, Kim HJ, Korn T, Kolls JK, Cantor H, Bettelli E, Kuchroo VK. Proinflammatory T helper type 17 cells are effective B-cell helpers. *Proc Natl Acad Sci USA* 2010; **107**: 14292-14297 [PMID: 20660725 DOI: 10.1073/pnas.1009234107]
43 **Duncan JR**, Orlowski TJ, Elewski BE. Safety of guselkumab in hepatitis B virus infection. *Dermatol Online J* 2019; **25** [PMID: 31735016]
44 **Curtis MM**, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. *Immunology* 2009; **126**: 177-185 [PMID: 19125888 DOI: 10.1111/j.1365-2567.2008.03017.x]
45 **Langley RG**, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, Puig L, Nakagawa H, Spelman L, Sigurgeirsson B, Rivas E, Tsai TF, Wasel N, Tyring S, Salko T, Hampele I, Notter M, Karpov A, Helou S, Papavassilis C; ERASURE Study Group; FIXTURE Study Group. Secukinumab in plaque psoriasis--results of two phase 3 trials. *N Engl J Med* 2014; **371**: 326-338 [PMID: 25007392 DOI: 10.1056/NEJMoa1314258]
46 **Gordon KB**, Blauvelt A, Papp KA, Langley RG, Luger T, Ohtsuki M, Reich K, Amato D, Ball SG, Braun DK, Cameron GS, Erickson J, Konrad RJ, Muram TM, Nickoloff BJ, Osuntokun OO, Secrest RJ, Zhao F, Mallbris L, Leonardi CL; UNCOVER-1 Study Group; UNCOVER-2 Study Group; UNCOVER-3 Study Group. Phase 3 Trials of Ixekizumab in Moderate-to-Severe Plaque Psoriasis. *N*

*Engl J Med* 2016; **375**: 345-356 [PMID: 27299809 DOI: 10.1056/NEJMoa1512711]

47 **Lebwohl M**, Strober B, Menter A, Gordon K, Weglowska J, Puig L, Papp K, Spelman L, Toth D, Kerdel F, Armstrong AW, Stingl G, Kimball AB, Bachelez H, Wu JJ, Crowley J, Langley RG, Blicharski T, Paul C, Lacour JP, Tyring S, Kircik L, Chimenti S, Callis Duffin K, Bagel J, Koo J, Aras G, Li J, Song W, Milmont CE, Shi Y, Erondu N, Klekotka P, Kotzin B, Nirula A. Phase 3 Studies Comparing Brodalumab with Ustekinumab in Psoriasis. *N Engl J Med* 2015; **373**: 1318-1328 [PMID: 26422722 DOI: 10.1056/NEJMoa1503824]

48 **Nash P**, Kirkham B, Okada M, Rahman P, Combe B, Burmester GR, Adams DH, Kerr L, Lee C, Shuler CL, Genovese M; SPIRIT-P2 Study Group. Ixekizumab for the treatment of patients with active psoriatic arthritis and an inadequate response to tumour necrosis factor inhibitors: results from the 24-week randomised, double-blind, placebo-controlled period of the SPIRIT-P2 phase 3 trial. *Lancet* 2017; **389**: 2317-2327 [PMID: 28551073 DOI: 10.1016/S0140-6736(17)31429-0]

49 **Baeten D**, Sieper J, Braun J, Baraliakos X, Dougados M, Emery P, Deodhar A, Porter B, Martin R, Andersson M, Mpofu S, Richards HB; MEASURE 1 Study Group; MEASURE 2 Study Group. Secukinumab, an Interleukin-17A Inhibitor, in Ankylosing Spondylitis. *N Engl J Med* 2015; **373**: 2534-2548 [PMID: 26699169 DOI: 10.1056/NEJMoa1505066]

50 **Paquissi FC**. Immunity and Fibrogenesis: The Role of Th17/IL-17 Axis in HBV and HCV-induced Chronic Hepatitis and Progression to Cirrhosis. *Front Immunol* 2017; **8**: 1195 [PMID: 29033929 DOI: 10.3389/fimmu.2017.01195]

51 **Feng H**, Yin J, Han YP, Zhou XY, Chen S, Yang L, Yan JR, Zhang GX. Sustained Changes of Treg and Th17 Cells During Interferon-α Therapy in Patients with Chronic Hepatitis B. *Viral Immunol* 2015; **28**: 412-417 [PMID: 26266573 DOI: 10.1089/vim.2015.0024]

52 **Chiu HY**, Hui RC, Huang YH, Huang RY, Chen KL, Tsai YC, Lai PJ, Wang TS, Tsai TF. Safety Profile of Secukinumab in Treatment of Patients with Psoriasis and Concurrent Hepatitis B or C: A Multicentric Prospective Cohort Study. *Acta Derm Venereol* 2018; **98**: 829-834 [PMID: 29972221 DOI: 10.2340/00015555-2989]

53 **Koike Y**, Fujiki Y, Higuchi M, Fukuchi R, Kuwatsuka S, Murota H. An interleukin-17 inhibitor successfully treated a complicated psoriasis and psoriatic arthritis patient with hepatitis B virus infection and end-stage kidney disease on hemodialysis. *JAAD Case Rep* 2019; **5**: 150-152 [PMID: 30733983 DOI: 10.1016/j.jdcr.2018.11.016]

54 **Lora V**, Graceffa D, De Felice C, Morrone A, Bonifati C. Treatment of severe psoriasis with ixekizumab in a liver transplant recipient with concomitant hepatitis B virus infection. *Dermatol Ther* 2019; **32**: e12909 [PMID: 30964590 DOI: 10.1111/dth.12909]

55 **Winthrop KL**. The emerging safety profile of JAK inhibitors in rheumatic disease. *Nat Rev Rheumatol* 2017; **13**: 234-243 [PMID: 28250461 DOI: 10.1038/nrrheum.2017.23]

56 **Burmester GR**, Blanco R, Charles-Schoeman C, Wollenhaupt J, Zerbini C, Benda B, Gruben D, Wallenstein G, Krishnaswami S, Zwillich SH, Koncz T, Soma K, Bradley J, Mebus C; ORAL Step investigators. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. *Lancet* 2013; **381**: 451-460 [PMID: 23294500 DOI: 10.1016/S0140-6736(12)61424-X]

57 **Kremer J**, Li ZG, Hall S, Fleischmann R, Genovese M, Martin-Mola E, Isaacs JD, Gruben D, Wallenstein G, Krishnaswami S, Zwillich SH, Koncz T, Riese R, Bradley J. Tofacitinib in combination with nonbiologic disease-modifying antirheumatic drugs in patients with active rheumatoid arthritis: a randomized trial. *Ann Intern Med* 2013; **159**: 253-261 [PMID: 24026258 DOI: 10.7326/0003-4819-159-4-201308200-00006]

58 **Gladman D**, Rigby W, Azevedo VF, Behrens F, Blanco R, Kaszuba A, Kudlacz E, Wang C, Menon S, Hendrikx T, Kanik KS. Tofacitinib for Psoriatic Arthritis in Patients with an Inadequate Response to TNF Inhibitors. *N Engl J Med* 2017; **377**: 1525-1536 [PMID: 29045207 DOI: 10.1056/NEJMoa1615977]

59 **Mease P**, Hall S, FitzGerald O, van der Heijde D, Merola JF, Avila-Zapata F, Cieślak D, Graham D, Wang C, Menon S, Hendrikx T, Kanik KS. Tofacitinib or Adalimumab *vs* Placebo for Psoriatic Arthritis. *N Engl J Med* 2017; **377**: 1537-1550 [PMID: 29045212 DOI: 10.1056/NEJMoa1615975]

60 **Sandborn WJ**, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, Danese S, Feagan BG, Reinisch W, Niezychowski W, Friedman G, Lawendy N, Yu D, Woodworth D, Mukherjee A, Zhang H, Healey P, Panés J; OCTAVE Induction 1, OCTAVE Induction 2, and OCTAVE Sustain Investigators. Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2017; **376**: 1723-1736 [PMID: 28467869 DOI: 10.1056/NEJMoa1606910]

61 **Sandborn WJ**, Ghosh S, Panes J, Vranic I, Su C, Rousell S, Niezychowski W; Study A3921063 Investigators. Tofacitinib, an oral Janus kinase inhibitor, in active ulcerative colitis. *N Engl J Med* 2012; **367**: 616-624 [PMID: 22894574 DOI: 10.1056/NEJMoa1112168]

62 **Harigai M**, Winthrop K, Takeuchi T, Hsieh TY, Chen YM, Smolen JS, Burmester G, Walls C, Wu WS, Dickson C, Liao R, Genovese MC. Evaluation of hepatitis B virus in clinical trials of baricitinib in rheumatoid arthritis. *RMD Open* 2020; **6** [PMID: 32098857 DOI: 10.1136/rmdopen-2019-001095]

63 **Kirito K**, Sakamoto M, Enomoto N. Elevation of the Hepatitis B Virus DNA during the Treatment of Polycythemia Vera with the JAK Kinase Inhibitor Ruxolitinib. *Intern Med* 2016; **55**: 1341-1344 [PMID: 27181544 DOI: 10.2169/internalmedicine.55.5529]

64 **Rehermann B**, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. *Nat Rev Immunol* 2005; **5**: 215-229 [PMID: 15738952 DOI: 10.1038/nri1573]

65 **Heine A**, Held SA, Daecke SN, Wallner S, Yajnanarayana SP, Kurts C, Wolf D, Brossart P. The

JAK-inhibitor ruxolitinib impairs dendritic cell function *in vitro* and in vivo. *Blood* 2013; **122**: 1192-1202 [PMID: 23770777 DOI: 10.1182/blood-2013-03-484642]

66 **Chen YM**, Huang WN, Wu YD, Lin CT, Chen YH, Chen DY, Hsieh TY. Reactivation of hepatitis B virus infection in patients with rheumatoid arthritis receiving tofacitinib: a real-world study. *Ann Rheum Dis* 2018; **77**: 780-782 [PMID: 28663308 DOI: 10.1136/annrheumdis-2017-211322]

67 **Serling-Boyd N**, Mohareb AM, Kim AY, Hyle EP, Wallace ZS. The use of tocilizumab and tofacitinib in patients with resolved hepatitis B infection: a case series. *Ann Rheum Dis* 2021; **80**: 274-276 [PMID: 32732241 DOI: 10.1136/annrheumdis-2020-218289]

68 **Sanz-Bueno J**, Vanaclocha F, García-Doval I, Torrado R, Carretero G, Daudén E, Patricia Ruiz-Genao D, Alsina-Gibert MM, Pérez-Zafrilla B, Pérez-Rial G, Rivera R; members of the BIOBADADERM group. Risk of Reactivation of Hepatitis B Virus Infection in Psoriasis Patients Treated With Biologics: A Retrospective Analysis of 20 Cases From the BIOBADADERM Database. *Actas Dermosifiliogr* 2015; **106**: 477-482 [PMID: 25776200 DOI: 10.1016/j.ad.2015.01.010]

69 **Navarro R**, Vilarrasa E, Herranz P, Puig L, Bordas X, Carrascosa JM, Taberner R, Ferrán M, García-Bustinduy M, Romero-Maté A, Pedragosa R, García-Diez A, Daudén E. Safety and effectiveness of ustekinumab and antitumour necrosis factor therapy in patients with psoriasis and chronic viral hepatitis B or C: a retrospective, multicentre study in a clinical setting. *Br J Dermatol* 2013; **168**: 609-616 [PMID: 22985451 DOI: 10.1111/bjd.12045]

70 **Hayashi M**, Umezawa Y, Fukuchi O, Ito T, Saeki H, Nakagawa H. Efficacy and safety of ustekinumab treatment in elderly patients with psoriasis. *J Dermatol* 2014; **41**: 974-980 [PMID: 25346301 DOI: 10.1111/1346-8138.12653]

71 **Steglich RB**, Meneghello LP, Carvalho AV, Cheinquer H, Muller FM, Reginatto FP. The use of ustekinumab in a patient with severe psoriasis and positive HBV serology. *An Bras Dermatol* 2014; **89**: 652-654 [PMID: 25054756 DOI: 10.1590/abd1806-4841.20143013]

72 **Moneva-Leniz LM**, Sahuquillo-Torralba A, Vila-Payeras A, Mateu-Puchades A. Risk of Hepatitis B Virus Reactivation in Patients on Secukinumab for Psoriasis: A Series of 4 Cases. *Actas Dermosifiliogr* 2020; **111**: 613-614 [PMID: 32589963 DOI: 10.1016/j.ad.2019.02.022]

73 **Feaster B**, Cline A, Feldman SR. Secukinumab for psoriasis in a patient with hepatitis B. *Dermatol Online J* 2018; **24** [PMID: 30677838]

74 **Bevans SL**, Mayo TT, Elewski BE. Safety of secukinumab in hepatitis B virus. *J Eur Acad Dermatol Venereol* 2018; **32**: e120-e121 [PMID: 28960490 DOI: 10.1111/jdv.14608]

75 **Yanagihara S**, Sugita K, Yoshida Y, Tsuruta D, Yamamoto O. Psoriasis vulgaris in a hepatitis B virus carrier successfully treated with secukinumab and entecavir combination therapy. *Eur J Dermatol* 2017; **27**: 185-186 [PMID: 27965188 DOI: 10.1684/ejd.2016.2939]

76 **Peccerillo F**, Odorici G, Pellacani G, Conti A. Secukinumab: A positive outcome in a patient with severe psoriasis and HBV-HCV co-infection. *Dermatol Ther* 2018; **31**: e12601 [PMID: 29633448 DOI: 10.1111/dth.12601]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2325-2340

DOI: 10.3748/wjg.v27.i19.2325

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

*MINIREVIEWS*

# Burden of venous thromboembolism in patients with pancreatic cancer

Corinne Frere

**ORCID number:** Corinne Frere 0000-0001-6303-4732.

**Author contributions:** Frere C performed the literature review and drafted the manuscript.

**Conflict-of-interest statement:** The author declare she has no conflict of interest relevant to the content of this paper to disclose.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** France

**Peer-review report's scientific quality classification**

**Corinne Frere,** Department of Haematology, Pitié-Salpêtrière Hospital, Assistance Publique Hôpitaux de Paris, Paris F-75013, France

**Corinne Frere,** INSERM UMRS_1166, Institute of Cardiometabolism And Nutrition, GRC 27 GRECO, Sorbonne Université, Paris F-75013, France

**Corinne Frere,** Groupe Francophone Thrombose et Cancer, Saint-Louis Hospital, Paris F-75010, France

**Corresponding author:** Corinne Frere, MD, PhD, Assistant Professor, Department of Haematology, Pitié-Salpêtrière Hospital, Assistance Publique Hôpitaux de Paris, 47-83, boulevard de l'Hôpital, Paris F-75013, France. corinne.frere@aphp.fr

## Abstract

Pancreatic cancer (PC) is a devastating malignancy with fewer than 10% of patients being alive at 5 years after diagnosis. Venous thromboembolism (VTE) occurs in approximatively 20% of patients with PC, resulting in increased morbidity, mortality and significant health care costs. The management of VTE is particularly challenging in these frail patients. Adequate selection of the most appropriate anticoagulant for each individual patient according to the current international guidelines is warranted for overcoming treatment challenges. The International Initiative on Thrombosis and Cancer multi-language web-based mobile application (downloadable for free at www.itaccme.com) has been developed to help clinicians in decision making in the most complex situations. In this narrative review, we will discuss the contemporary epidemiology and burden of VTE in PC patients, the performances and limitations of current risk assessment models to predict the risk of VTE, as well as evidence from recent clinical trials for the primary prophylaxis and treatment of cancer-associated VTE that support updated clinical practice guidelines.

**Key Words:** Pancreatic cancer; Venous thromboembolism; Low-molecular weight heparin; Direct oral anticoagulant; Multi-language mobile application; Risk-assessment models; Thromboprophylaxis

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

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

**Received:** January 24, 2021
**Peer-review started:** January 24, 2021
**First decision:** February 22, 2021
**Revised:** February 28, 2021
**Accepted:** April 22, 2021
**Article in press:** April 22, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Li CG, Nari GA, Oria I, Roselli M, Tran B, Yang F
**S-Editor:** Gao CC
**L-Editor:** A
**P-Editor:** Ma YJ

**Core Tip:** Venous thromboembolism (VTE) is a common but potentially life-threatening complication in patients with Pancreatic cancer (PC). There is an urgent need to raise awareness on this underrecognized issue. This review discusses the incidence and risk factors of VTE in PC patients, and the results from recent clinical trials for the primary prophylaxis and treatment of VTE in cancer patients supporting the most recent clinical practice guidelines.

**Citation:** Frere C. Burden of venous thromboembolism in patients with pancreatic cancer. *World J Gastroenterol* 2021; 27(19): 2325-2340
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2325.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2325

## INTRODUCTION

Pancreatic cancer (PC) is a devastating disease with fewer than 10% of patients being alive at 5 years[1]. Its prevalence continues to increase worldwide[2]. In most cases, there is no effective treatment. Given its dismal prognosis[3], there is an urgent need to improve patient quality of life by integrating best supportive care[4,5].

Venous thromboembolism (VTE) is a frequent but still underrecognized complication in PC patients[6]. According to a recent large population-based cohort study[7], the incidence of PC-associated VTE has doubled from 1997 to 2017, due to increase in PC prevalence, improved survival, advanced age of PC patients, and better detection of incidental VTE with the routine use of computed tomography scans. Primary thromboprophylaxis is a supportive care with a well-documented clinical benefit, which remains unfortunately underused nowadays. Since 2013, the International Initiative on Thrombosis and Cancer (ITAC), a multidisciplinary group of experts from across the globe committed to improve the management of patients with cancer-associated thrombosis through dissemination of educational initiatives to health professionals, strives to raise awareness on this important issue[8].

Anticoagulation therapy is the mainstay of the VTE prevention and treatment, but its management is particularly challenging for the treating physicians in these patients who already suffer from multiple co-morbidities such as renal failure, hepatic failure, thrombocytopenia, and who undergo complex cancer treatment protocols[9,10].

Herein, we discuss the most recent data on the incidence and risk factors of VTE in PC patients, as well as evidence from recent clinical trials of low-molecular-weight heparin (LMWH) and direct oral anticoagulants (DOAC) for the primary prophylaxis and treatment of cancer-associated VTE that support current clinical practices guidelines (CPGs)[8,11,12].

## EPIDEMIOLOGY OF VTE IN PC AND IMPACT ON SURVIVAL

Cancer has been demonstrated to be an independent major risk factor for VTE[13]. The extent of this risk mainly depends on cancer type and disease stage. Among all cancer types, PC carries the highest risk for VTE[7,14]. In retrospective cohorts of PC patients, the reported incidence of VTE varies broadly from 5% to 57%[15-33], depending on the study population, the duration of follow-up, the definition of VTE and the methods used for diagnosing VTE.

Due to their large sample sizes, multicenter prospective design, and systematic follow-up, phase 3 randomized control trials (RCTs) conducted in PC patients are expected to provide reliable data on the true incidence of VTE. However, a recent systematic review and meta-analysis of chemotherapeutic and thromboprophylaxis RCTs conducted in PC patients highlighted that VTE events were underreported in chemotherapeutic RCTs[6]. The pooled rate of VTE in chemotherapy studies ($n$ = 13, 5694 patients) was 5.9% [95% confidence interval (CI): 3.9-9.0; $I^2$ = 94%] and significantly lower than the corresponding 16.5% (95%CI: 11.7-23.3; $P$ < 0.001) reported in thromboprophylaxis studies ($n$ = 9, 631 patients, $I^2$ = 69%). Importantly, 30 eligible chemotherapy RCTs ($n$ = 9000 patients) were excluded from this meta-analysis because they did not report VTE as adverse events[6], which reveals quite clearly a lack of awareness on the burden of VTE among oncologists.

The incidence and risk factors for VTE was recently assessed in a large prospective multicenter cohort of patients with newly diagnosed PC[34], providing real-life contemporary estimates. In this study, 152 out of 731 (20.79%) patients developed a VTE event, with a median time from PC diagnosis to VTE of 4 mo. In competing-risk analysis, the cumulative rates of VTE were approximatively 13% and 20% at 6 mo and 1 year, respectively[34].

The most common VTE events occurring in PC patients are deep vein thrombosis (DVT) and pulmonary embolism (PE)[35], but incidental PE and incidental visceral vein thrombosis (VVT) are increasingly diagnosed, accounting now for approximatively 50% of all reported VTE events[23,30,34,36]. In the BACAP-VTE study[34], DVT, PE, VVT, and combined events were observed in 26%, 17%, 30% and 21% of patients, respectively. Overall, 46% of VTE events were symptomatic and 54% of them were asymptomatic[34].

Early retrospective studies reported no association between VTE and overall survival (OS) in PC patients[21,27]. However, all patients included in these studies had metastatic disease with a short life expectancy. By contrast, later studies reported that the onset of VTE was associated with a poorer prognosis. In a retrospective cohort of 227 patients with unresectable PC, VTE during the course of chemotherapy was associated with a 2.5-fold decrease in progression-free survival (PFS) and a 1.6-fold risk decrease in OS[17]. Similarly, in a small cohort of 135 PC patients, the onset of VTE was significantly associated with increased mortality[23]. Importantly, survival was significantly improved in patients with VTE receiving anticoagulant therapy compared to those who did not receive anticoagulant therapy [hazard ratio (HR) 0.30, 95%CI: 0.12-0.74, $P = 0.009$][23]. Retrospective studies focusing on incidental VTE in PC patients also reported an association between VVT and mortality[36,37]. Similarly, in a prospective cohort of 731 newly diagnosed PC, patients who developed asymptomatic or symptomatic VTE during follow-up had significantly shorter PFS (HR 1.74; 95%CI: 1.19-2.54; $P = 0.004$) and OS (HR 2.02; 95%CI: 1.57-2.60; $P < 0.001$) compared to those who did not developed VTE[34].

## RISK FACTORS FOR VTE AND RISK STRATIFICATION IN PC PATIENTS

Several studies have demonstrated that the most important risk factor for VTE in PC patients is the presence of a metastatic disease[16,18,27,31,34,38,39]. In a recent retrospective cohort of 165 PC patients, metastatic disease was associated with a 4.8-fold increase in the risk for VTE; 41 out of 51 patients who developed VTE had metastasis at diagnosis[39]. Similarly, in the BACAP-VTE study[34], metastatic tumors were associated with a 2.5-fold increased risk for VTE compared to non-metastatic tumors.

Major abdominal surgery is also an important risk factor for VTE in PC patients. In an early observational study of 1915 patients with exocrine PC, 127 out of 383 (33.1%) patients requiring pancreatic surgery developed postsurgical VTE[22]. Similarly, 31 out of 209 (14.8%) patients requiring pancreatic surgery developed postsurgical VTE in a large retrospective study of 1,115 conducted in East Asian population[27].

Chemotherapy increases the risk of VTE in cancer patients[40]. Nevertheless, as recently highlighted by Chiasakul *et al*[6], the rates of VTE were underreported in PC chemotherapy RCTs and data on the respective risk of various chemotherapy regimens remain scarce. In recent retrospective or prospective cohorts of PC patients, the rate of VTE did not differ between those receiving gemcitabine-based chemotherapy and those receiving FOLFIRINOX[30,34]. In the subgroup of 273 PC patients included in the CASSINI trial[41], the rates of VTE did not differ between patients treated with 5-fluorouracil-based regimen *vs* gemcitabine-based regimen.

Systematic screening of VTE is not recommended in daily clinical practice. However, all PC patients should receive verbal and written information on the risk factors for VTE, as well as on the signs and symptoms of VTE to promote self-diagnosis and reporting of VTE symptoms.

Over the past ten years, many efforts have been made to develop risk assessment models (RAM) aiming to select cancer patients at highest risk for VTE, and therefore expected to have the best benefit from thromboprophylaxis. However, none of these RAM was designed to specifically assess this risk in PC patients.

The Caprini score is the most widely RAM to assess the risk of VTE in patients undergoing surgery. It has been validated in several types of cancers[42]. However, this model was unable to identify patients at highest risk for VTE in a retrospective cohort of 426 PC patients undergoing preoperative treatment followed by surgical

resection[43].

Furthermore, the Khorana score[44] is the most widely used RAM to assess the risk of VTE in ambulatory cancer patients. It was developed ten years ago[44]. It assigns 1 to 2 points to 5 simple clinical and laboratory variables (primary tumor site, platelet count ≥ 350 × $10^9$/L, hemoglobin concentration ≤ 10 g/dL or use of erythropoiesis-stimulating agents, leukocyte count ≥ 11 × $10^9$/L, body mass index (BMI) ≥ 35 kg/$m^2$. Patients are classified as being at "low-risk" (Khorana score = 0), "intermediate-risk" (Khorana score = 1-2), or "high-risk" (Khorana score ≥ 3). All PC patients are classified as being at intermediate- or high-risk. Unfortunately, this model did not discriminate between these two risk categories, neither in retrospective studies of PC patients undergoing chemotherapy[25,28-30,32,39,45], nor in the large prospective BACAP-VTE study[34], nor in the subgroup of 273 PC patients included in the recent CASSINI trial[41] (Table 1), questioning its relevance in this specific population.

Several modifications to this RAM by the addition of other variables to the model have been proposed. The PROTECHT score[46], which includes treatment with cisplatin or carboplatin-based chemotherapy or gemcitabine was found to perform better than the Khorana score in a retrospective analysis of the PROTECHT study, decreasing the number needed to treat (NTT) from 50 to 17. However, this score has not been externally validated in PC patients. More recently, the ONKOTEV score[47] was developed in a prospective cohort of 843 various cancers patients in Italy and Germany, including 253 patient with gastroenteric cancer. The ONKOTEV score assigns one point to four variables, namely: a Khorana score > 2, a history of previous VTE, a metastatic disease, and a compression of vascular structures by the tumor. The ONKOTEV score demonstrated a significantly higher predictive power compared to the Khorana score in the original development cohort and was recently externally validated in a retrospective single-center cohort of 165 PC patients treated in Portugal with promising results[39]. Ninety-two (55.8%) patients had a metastatic disease at diagnosis and 109 (66.1%) received systemic chemotherapy. At inclusion, 18.2% of patients had an ONKOTEV score of 0, 38.2% of patients had an ONKOTEV score of 1, 33.3% of patients had an ONKOTEV score of 2, and 10.3% of patients had an ONKOTEV score > 2. During a median observation period of 6.3 mo, 51 out 165 (30.9%) PC patients developed VTE. The cumulative incidence of VTE was 82.4% in patients with an ONKOTEV > 2 compared to 3.3% in those with an ONKOTEV score of 0[39]. These results suggest that the ONKOTEV score could be of help to better stratify PC patients having the highest risk for VTE but deserve further confirmation in prospective cohorts of ambulatory PC patients.

Integration of relevant biomarkers into current RAMs might improve their ability to predict VTE. Faille *et al*[38] recently assessed the diagnosis performances of several biomarkers to predict VTE in a prospective cohort of 50 PC patients, including Factor VIII, D-dimers, von Willebrand factor, free tissue factor pathway inhibitor, microvesicle-tissue factor (MV-TF) activity and CA 19.9. In multivariate analysis, baseline D-dimers ≥ 2.16 μg/mL (HR 4.9; 95%CI: 1.0-23.1), baseline MV-TF activity 2.37 pg/mL (HR 10.5; 95%CI: 1.5-72.4), and baseline CA 19.9 ≥ 2153 U/mL (HR 9.5; 95%CI: 1.5-60.2) were significantly associated with VTE after adjustment for age and sex, with the best sensitivity and specificity in predicting VTE obtained for CA 19-9[38]. However, these associations were no more significant after adjustment for the presence of metastasis, suggesting once again that the presence of a metastatic disease is the most important risk factor for VTE in PC patients.

The clinical-genetic Thrombo inCode-Oncology (TiC-Onco) score was developed in a prospective cohort of 391 ambulatory patients with various cancers initiating systemic chemotherapy, including 72 (18.5%) patients with PC[48]. Seventy-one out of 391 (18%) patients developed VTE within 6 mo. The prespecified variable selection process selected both clinical variables (tumor site, family history of VTE, BMI ≥ 25 kg/$m^2$) and genetic variables (germline polymorphisms in the *F5*, *F13* and *SERPINA10* genes) for inclusion in the score. In the derivation cohort, the TiC-Onco score performed better than the Khorana score in predicting VTE at 6 mo (sensitivity 49% *vs* 22%, specificity 81% *vs* 82%, positive predictive value 37% *vs* 22%, and negative predictive value 88% *vs* 82%)[48]. Importantly, patients suffering from PC had higher rates of VTE (40%) than patients with other type of cancers (18%), suggesting that PC has a major impact on the accuracy of the TiC-Onco score. However, this model has not yet been externally validated in a cohort of PC patients.

The CATS/MICA score[49] includes two variables, namely: tumour-site risk category (very high *vs* high and high *vs* low or intermediate) and continuous D-dimer levels. It was developed in the prospective Vienna Cancer and Thrombosis Study (CATS) cohort of 1423 ambulatory cancer patients undergoing chemotherapy, including 118 (8%) patients with PC[49]. During a median follow-up of 6 mo, 80 out of

**Table 1 Studies assessing the predictive values of risk assessment models in pancreatic cancer patients**

| Ref. | Study design | Country | Patients analyzed/included | VTE screening at study entry | RAMs | Number of patients in each group | Study or median observation period | Patients with VTE during the overall follow-up, *n* (%) | Rates of VTE |
|---|---|---|---|---|---|---|---|---|---|
| Pelzer *et al*[45], 2013 | Retrospective analysis of theCONKO-004 RCT | Germany | 144/312, APC included in the CONKO-004 trial (control arm) | No | Khorana score | Intermediate risk: 55/144 (38.2%); High risk: 89/144 (61.8%) | 12 mo | 21/144 (14.6%) | At 6 mo: Intermediate risk: 4/55 (7.2%); High risk: 17/89 (19.1%) |
| Muñoz Martín *et al*[25], 2014 | Retrospective | Spain | 73/84, ambulatory PC patientsreceiving chemotherapy | No | Khorana score | Intermediate risk: 36/84 (43%); High risk: 48/84 (57%) | 2008-2011 | 30/84 (35.7%) | At 6 mo: Intermediate risk: 4/37 (10.8%); High risk: 10/36 (27.8%) |
| van Es *et al*[29], 2017 | Retrospective | Netherlands | 147/178, ambulatory PC patientsstarting chemotherapy | No | Khorana score | Intermediate risk: 101/147 (69%); High risk: 46/147 (31%) | 2003-2014 | 20/147(13.6%) | At 6 mo: Intermediate risk: 9/101 (8.9%); High risk: 4/46 (8.7%) |
| Kruger *et al*[28], 2017 | Retrospective | Germany | 111/172, APC patients undergoing palliative chemotherapy | No | Khorana score | Intermediate risk: 69/111 (38%); High risk: 42/111 (62%) | 2002-2012 | 16/111 (14.4%) | At 6 mo: Intermediate risk: 6/69 (8.6%)High risk: 5/42 (11.9%); During the overall observation period; Intermediate risk: 8/69 (11.6%); High risk: 8/42 (19.0%); $P = 0.4$ |
| Berger *et al*[30], 2017 | Retrospective | Germany | 150, PC patients receiving chemotherapy | No | Khorana score | Intermediate risk: 87/150 (58%); High risk: 63/150 (42%) | 2010-2014 | 37/150 (24.7%) | Unspecified; During the overall observation period: no difference between groups ($P = 0.44$) |
| Godinho *et al*[39], 2020 | Retrospective | Portugal | 165 newly diagnosed PC patients | No | Khorana score; Onkotev score | Khorana score: Intermediate risk: 106/165 (64%); High risk: 59/165 (36%). Onkotev score: Score 0: 30/165 (18.2%); Score 1: 63/165 (38.2%); Score 2: 55/165 (33.3%); Score ≥ 3: 17/165 (10.3%) | 6.3 mo | 51/165 (31%) | During the overall observation period: Khorana score: Intermediate risk: 28/106 (26.4%); High risk: 23/59 (38.9%). Onkotev score: Score 0: 1/30 (< 10%); Score 1: 8/63 (< 10%); Score 2: 28/55 (41.8%); Score ≥ 3: 14/17 (70.6%) |
| Kim *et al*[32], 2018 | Retrospective | Korea | 216 metastatic PC patients receiving palliative chemotherapy | No | Khorana score | Intermediate risk: 135/216 (62.5%); High risk: 81/21 (37.5%) | 2005-2015 | 50/216 (23.1%) | During the overall observation period: Intermediate risk: 30/135 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | (22.2%); High risk: 20/81 (24.7%); *P* = 0.677 |
| Frere *et al*[34], 2020 | Prospective | France | 675 newly diagnosed PC patients | Yes, patients excluded if VTE at diagnosis | Khorana score | Intermediate risk: 492/675 (73%); High risk: 183/675 (27%) | 2014-2019; 19.3 mo | 141/675 (20.8%) | During the total follow-up: Intermediate risk: 108/492 (22%); High risk: 33/183 (18%); *P* = 0.26 |
| Vadhan-Raj *et al*[41], 2020 | Retrospective subgroup analysis of the CASSINI RCT | International | 138 PC patients undergoing chemotherapy included in the CASSINI trial (control arm) | Yes, patients excluded if VTE at diagnosis | Khorana score | Intermediate risk: 100/138 (72.5%); High risk: 38/138 (27.5%) | 6 mo | 18/138 (13.0%) | At 6 mo: Intermediate risk: 14/100 (14.0%); High risk: 4/38 (10.5%) |

APC: Advanced Pancreatic cancer; PC: Pancreatic cancer; RAM: Risk assessment model; VTE: Venous thromboembolism.

1423 patients (6%) developed VTE. In the CATS cohort, the C-index of the model was 0.66 (95%CI: 0.63-0.67) compared to 0.61 (95%CI: 0.51-0.70) for the Khorana score[49]. The score was then validated in the prospective Multinational Cohort Study to Identify Cancer Patients at High Risk of Venous Thromboembolism (MICA) cohort ($n$ = 832), including 116 (14%) patients with PC[49]. Using this RAM, all PC patients are classified at intermediate or high risk of VTE. Of note, the CATS/MICA score has not yet been externally validated in a cohort of PC patients.

Finally, machine learning methods are increasingly used for the development of prediction models. Two recent studies conducted in various cancer patients[50] or in ovarian cancer patients[51] have demonstrated that such models could improve the prediction of VTE compared to conventional methods.

## WHEN SHOULD WE CONSIDER PRIMARY PROPHYLAXIS IN PC PATIENTS?

### *Surgical PC patients*

Prolonged thromboprophylaxis following major abdominal surgery has been shown to decrease the rate of VTE by approximately 50%[52]. Accordingly, all current CPGs recommend using thromboprophylaxis in surgical PC patients[8,11]. In those undergoing laparotomy or laparoscopic surgery without contraindications to LMWH, the highest LMWH prophylactic dose should be used for an extended duration of 4 wk (Grade 1A)[8]. External compression devices alone should be used only in patients with contraindications to anticoagulants (Grade 2B)[8]. Inferior vena cava filters should not be used systematically in this setting (Grade 1A)[8]. The risks of VTE should be balanced by the competing risk of bleeding. Numerous factors such as advanced or metastatic disease, older age, anemia, thrombocytopenia, renal impairment, liver dysfunction, and concomitant anticancer therapies may potentiate the overall bleeding risk and should be taken into account. The careful evaluation of each individual profile is warranted for overcoming management challenges.

### *Hospitalized PC patients*

Acute medical illness and bed rest constitute transient factors increasing the risk of VTE in hospitalized cancer patients. Although there is no large RCT specifically demonstrating the benefit of thromboprophylaxis in cancer inpatients, RCTs conducted in non-cancer inpatients have demonstrated that LMWH improves survival and reduces VTE in general medical patients hospitalized with acute medical conditions, and recommendations for cancer patients have been extrapolated from these RCTs. The ITAC CPGs[8] recommend using LMWH at prophylactic doses or unfractionated heparin (UFH) or Fondaparinux in PC inpatients without contraindications to anticoagulants (Grade 1B)[8]. Due to the lack of data on the efficacy and safety of DOAC in this setting, they should not be used (Best clinical practice)[8].

### *Ambulatory PC patients*

Most cancer patients develop VTE in the outpatient setting[53]. The net clinical benefit of primary thromboprophylaxis in advanced PC patients has been firmly established in two pivotal RCTs[54,55] which specifically addressed the efficacy and safety of LMWH in this setting (Table 2). Based on the results of these two trials, the ITAC CPGs recommend using primary thromboprophylaxis with LMWH in ambulatory advanced PC patients receiving chemotherapy with a Grade 1B evidence level since 2013[8,56,57].

The FRAGEM trial randomized 123 advanced PC patients to receive gemcitabine plus weight-adjusted therapeutic doses of dalteparin for 12 wk or gemcitabine alone[54]. The coprimary endpoints were the rate of symptomatic or incidentally diagnosed VTE events during the 12-wk anticoagulation period and the rate of symptomatic or incidentally diagnosed VTE events during the overall follow-up period. The rate of VTE was significantly lower in the dalteparin arm (3.4% *vs* 23% in the control arm, risk ratio 0.145, 95%CI: 0.035-0.612, $P = 0.002$), resulting in a NNT of 6 patients to prevent 1 VTE event. No VTE-related deaths occurred in the dalteparin arm compared to 5 (8.3%) VTE-related deaths in the control arm. The rates of major bleeding did not differ between the 2 arms and were lower than 3%, with only 2 patients experiencing a major bleeding requiring anticoagulation discontinuation. Of note, patients in the dalteparin arm experienced more minor bleeding such as skin bruising or epistaxis (9% *vs* 3% in the gemcitabine alone arm)[54]. There was no difference in PFS or OS between the two arms.

The PROSPECT-CONKO 004 trial randomized 312 advanced PC patients to receive supra-prophylactic doses of enoxaparin during the first 3 mo of chemotherapy or chemotherapy alone[55]. Unlike in FRAGEM, incidental VTE events were excluded from the analysis. The cumulative incidence rate of symptomatic VTE within the first 3 mo was 1.3% in the enoxaparin arm compared to 10.2% in the control arm (HR 0.12, 95%CI: 0.03-0.52), resulting in a NNT of 11 patients to prevent 1 VTE event. The rates of major bleeding events were similar in both arms. PFS and OS did not differ between the 2 arms[55].

Two additional phase III double-blinded placebo-controlled trials (the PROTECHT [58] and the SAVE-ONCO studies[59]) evaluated the efficacy and safety of primary thromboprophylaxis with prophylactic doses of other LMWH in ambulatory cancer patients receiving chemotherapy. In the PROTECHT study ($n = 1150$)[58], while nadroparin reduced the rate of VTE from 3.9% to 2.0% ($P = 0.02$) without difference in major bleeding in the overall population, the rates of VTE did not differ between the two arms in the subgroup of 53 PC patients ($P = 0.755$). In the SAVE-ONCO study ($n = 3221$)[59] the rate of VTE was 1.2% in the semuloparin arm compared to 3.4% in the placebo arm (HR 0.36, 95%CI: 0.21-0.60; $P < 0.001$) in the overall population, without difference in major bleeding (HR 1.05, 95%CI: 0.55-1.99). The absolute VTE risk reduction with semuloparin appeared to be much higher in the subgroup of 254 PC patients. The magnitude of the VTE risk reduction was similar to that obtained with therapeutic doses of dalteparin in the FRAGEM study[54] or with supra-prophylactic doses of enoxaparin in the PROSPECT-CONKO 004 study[55].

More recently, two randomized placebo-controlled trials assessed the efficacy and safety of primary thromboprophylaxis with prophylactic doses of DOACs (apixaban 2.5 mg twice daily for up to 6 mo in the AVERT trial[60]; rivaroxaban 10 mg once daily for up to 6 mo in the CASSINI trial[61]) in cancer patients with a Khorana score ≥ 2 undergoing chemotherapy. Results from a subgroup of PC patients were reported only for the CASSINI trial[41]. Among the 273 PC patients included in this prespecified subgroup analysis, 214 (78%) had a locally advanced or metastatic PC and 271 (99.3%) were receiving cytotoxic chemotherapy (fluorouracil-based in 47.6% of cases and gemcitabine-based in 44.7% of cases). Rivaroxaban did not significantly reduce the rates of the primary efficacy endpoint of symptomatic DVT, asymptomatic proximal DVT, any PE and VTE-related death within the 6 mo observation period (absolute difference of 3.4%, $P$ = not significant). However, most of VTE events occurred after discontinuation of rivaroxaban (61.5%) compared to placebo (22.2%). During the intervention period, rivaroxaban significantly reduced the rates of the primary efficacy endpoint from 10.1% to 3.7% (absolute difference of 6.4%, HR 0.35, 95%CI: 0.13-0.97, $P = 0.034$), resulting in a NTT of 16 patients to prevent 1 event. Importantly, 2 out of 5 events in the rivaroxaban arm and 5 out of 14 events in the placebo arm were asymptomatic lower-extremity proximal DVT diagnosed by ultrasound screening during the follow-up, leading to overestimate the rates of VTE in both arms. The rates of major bleeding and all-cause mortality did not differ between the two arms[41].

**Table 2 Studies assessing the clinical benefit of anticoagulants for the prevention of venous thromboembolism in ambulatory pancreatic cancer patients**

| | PROTECHT | SAVE ONCO | FRAGEM | CONKO-0004 | CASSINI |
|---|---|---|---|---|---|
| | Agnelli *et al*[58], 2009 | Agnelli *et al*[59], 2012 | Maraveyas *et al*[54], 2012 | Pelzer *et al*[55], 2015 | Khorana *et al*[61], 2019 and Vadhan-Raj *et al*[41], 2020 |
| Population | Ambulatory patients > 18 yr on chemotherapy with metastatic or locally advanced lung, gastrointestinal, breast, ovarian, or head and neck cancer | Patients with metastatic or locally advanced lung, pancreatic, gastric, colorectal, bladder, and ovarian cancer beginning to receive a course of chemotherapy | Patients aged 18 yr or older; Histologically/cytologically confirmed advanced or metastatic pancreatic cancer; KPS: 60-100 | Patients with histologically proven advanced pancreatic cancer were randomly assigned to ambulant first-line chemotherapy | Adult ambulatory patients with various cancers initiating a new systemic regimen and at increased risk for VTE (defined as Khorana score ≥ 2) |
| Study design | Randomized, placebo-controlled, double-blind, multicenter study | Randomized, placebo-controlled, double-blind, multicenter study | Randomized, controlled Phase 2b study | Prospective, open label, randomized, multicenter and group-sequential 2b trial | Double-blind, randomized, placebo-controlled, parallel-group, multicenter study |
| Intervention | Arm A: nadroparin 3800 IU/d; Arm B: placebo; For duration of chemotherapy (up to 4 mo maximum) | Arm A: Semuloparin, 20 mg/d; Arm B: placebo; For duration of chemotherapy (median: 3.5 mo) | Arm A: Gemcitabine + Dalteparin 200 IU/kg s.c., o.d., for 4 wk, followed by a step-down regimen to 150 IU/kg for a further 8 wk); Arm B: Gemcitabine alone; For up to 12 wk | Arm A: Enoxaparin 1 mg/kg per day; Arm B: No enoxaparin | Arm A: rivaroxaban 10 mg o.d. up to day 180; Arm B: placebo up to day 180 |
| Number of patients analyzed | Overall population: Arm A: 769 patients; Arm B: 381 patients. PC subgroup: Arm A: 36 patients; Arm B: 17 patients | Overall population: Arm A: 1608 patients; Arm B: 1604 patients. PC subgroup: Arm A: 126 patients; Arm B: 128 patients | Arm A: 59 patients; Arm B: 62 patients | Arm A: 160 patients; Arm B: 152 patients | Overall population: Arm A: 420 patients; Arm B: 404 patients. PC patients: Arm A: 135 patients; Arm B: 138 patients |
| Follow-up | 120 d | 3 mo | 3 mo | 3 mo | 6 mo |
| Thromboembolic endpoint events | Overall population: Arm A: 11/769 (1.4%); Arm B: 11/381 (2.9%); $P = 0.02$. PC subgroup: Arm A: 3/36 (8.3%); Arm B: 1/17 (5.9%); $P = 0.755$ | Overall population: Arm A:20/1608 (1.2%); Arm B: 55/1064 (1.2%); HR 0.36 (95%CI: 0.21-0.60); $P < 0.001$. PC subgroup: Arm A: 3/126 (2.4%); Arm B: 14/128 (10.9%); HR 0.22 (95%CI: 0.06-0.76); $P = 0.015$. At 3 mo: Arm A: 2/59 (3%); Arm B: 14/62 (23%); RR 0.145 (95%CI: 0.035-0.612); $P = 0.002$ | At 3 mo: Arm A: 2/160 (1.25%); Arm B: 15/152 (9.8%); HR 0.12 (95%CI: 0.03-0.52); $P = 0.001$. Entire study: Arm A: 7/59 (12%); Arm B: 17/62 (28%); RR 0.419 (95%CI: 0.187-0.935); $P = 0.039$ | Cumulative incidence rates: Arm A: 6.4%; Arm B: 15.1%; HR 0.40 (95%CI: 0.19-0.83); $P = 0.01$ | Overall population: Up-to-day-180 observation period: Arm A: 25/420 (5.95%); Arm B: 37/421 (8.79%); HR 0.66 (95%CI: 0.40-1.09); $P = 0.101$; NNT =3 5. Intervention period: Arm A: 11/420 (2.62%); Arm B: 27/421 (6.41%); HR 0.40 (95%CI: 0.20-0.80); $P = 0.007$; NNT = 26. PC subgroup: Up-to-day-180 observation period: Arm A: 13/135 (9.6%); Arm B: 18/138 (13.0%); HR 0.70 (95%CI: 0.34-1.43); $P = 0.329$. Intervention period: Arm A: 5/135 (3.7%); Arm B: 14/138 (10.1%); HR 0.35 (95%CI: 0.130-0.96); $P = 0.043$; NNT = 16 |
| Bleeding | Overall population: Major bleeding: Arm A: 5/769 (0.7%); Arm B: 0/381; $P = 0.18$. Minor bleeding: Arm A: 57/769 (7.4%); Arm B: 30/381 (7.9%); $P$ = not significant. PC subgroup: $P$ = not significant | Overall population: Major bleeding: Arm A: 19/1589 (1.2%); Arm B: 18/1583 (1.1%); OR 1.05 (95%CI: 0.55-2.04). CRNMB: Arm A: 26/1589 (2.8%); Arm B: 14/1583 (0.9%); OR 1.86 (95%CI: 0.98-3.68). PC subgroup: $P$ = not significant | ISTH severe: Arm A: 2/59 (3%); Arm B: 2/62 (3%). ISTH non severe: Arm A: 5/59 (9%); Arm B: 2/62 (3%) | Major bleeding: Arm A: 8.3%; Arm B: 6.9%; HR 1.23 (95%CI: 0.54-2.79); $P = 0.63$ | Overall population: Major bleeding: Arm A: 8/405 (1.98%); Arm B: 4/404 (0.99%); HR 1.96 (95%CI: 0.59-6.49) $P = 0.265$; NNH = 101. CRNMB: Arm A: 2.72%; Arm B: 1.98%; HR 1.96 (95%CI: 0.59-6.49); $P = 0.265$; NNH = 101. PC subgroup: Major bleeding: Arm A: 2/130 (1.5%); Arm B: 3/131(2.3%); HR 0.67 (95%CI: 0.11-3.99); $P = 0.654$. CRNMB: Arm A: 3/131(2.3%); Arm B: 2/130 (1.5%); HR 2.47 (95%CI: 0.48-12.72); $P = 0.264$ |
| Survival | Overall population: Arm A: 33/769 | Not significant | Arm A: 8.7 mo; Arm B: 9.7 mo | Arm A: 8.2 mo; Arm B: 8.51 mo; | Overall population: All-cause mortality: Arm A: 20.0%; |

| | | | |
|---|---|---|---|
| | (4·3%); Arm B: 16/381 (4.2%); *P* = not significant. PC subgroup: not significant | HR 1.01 (95%CI: 0.87-1.38); *P* = 0.44 | Arm B: 23.8%; HR 0.83 (95%CI 0.62-1.11); *P* = 0.213. PC subgroup: Arm A: 34/135 (25.2%); Arm B: 33/138 (23.9%) |

CI: Confidence interval; CRNMB: Clinically relevant non-major bleeding; HR: Hazard ratio; ISTH: International society of thrombosis and haemostasis; KPS: Karnofsky performance status; o.d.: Once daily; NTT: Number needed to treat; OR: Odds ratio; PC: Pancreatic cancer; RR: Risk ratio.

Recently, a systematic review and meta-analysis aggregated the data from the 1003 PC patients enrolled in the 5 above-mentioned RCTs[62]. Primary thromboprophylaxis was estimated to significantly reduce the risk of symptomatic VTE by approximately 69%, resulting in a NTT of 11.9 to prevent one VTE event, without increase in the risk of major bleeding. Sensitivity analyzes showed that primary prophylaxis with LMWH or DOAC, and prophylactic doses or supra-prophylactic doses of anticoagulants reduced the risk of VTE with the same magnitude.

In light of the results from the AVERT and CASSINI trials, the ITAC[8] and ASCO[11] CPGs now recommend thromboprophylaxis with apixaban or rivaroxaban in cancer outpatients undergoing chemotherapy having a Khorana score ≥ 2, no bleeding risk and no drug-drug interactions (Grade 1B)[8]. Since the Khorana score assigns + 2 points for PC, thromboprophylaxis with DOAC or LMWH may be now offered in all ambulatory PC patients. Decisions to initiate thromboprophylaxis should be made based on a multidisciplinary patient-centered approach, after close discussion with the patient.

Nevertheless, primary thromboprophylaxis has not been yet widely adopted in PC outpatients, mainly due to fear of bleeding in otherwise frail subjects and inherent costs for such therapy.

## HOW TO TREAT VTE IN PC PATIENTS?

### *A step-based adapted approach*

For many years, monotherapy with LMWH has been the standard of care to treat cancer-associated VTE, based on the results of 5 landmark RCTs comparing LMWH to vitamin K antagonists[63-67]. However, positive results from 4 recent RCTs comparing DOAC to LMWH monotherapy for the treatment of cancer-associated thrombosis [68-71] (Table 3) prompted current updated CPGs to include DOACs as a new first-line option in selected patients, but not all[8,11].

The ability to now use oral-only anticoagulation strategies, precluding the need for long-term daily injection and dose adjustment, may seem appealing but adds to the complexity of decision making. Appropriate selection of anticoagulants appears more than ever as a critical element of high-quality care for cancer patients with VTE, and numerous factors must be taken into consideration when choosing one anticoagulant rather than the other[72]. A personalized approach is warranted.

The ITAC CPGs recommend using LMWH for the initial and long-term treatment of established VTE when creatinine clearance is ≥ 30 mL per min (Grade 1B)[8]. For patients without risk of gastrointestinal or genitourinary bleeding, rivaroxaban (in the first 10 d) or edoxaban (started after at least 5 d of parenteral anticoagulation) can also be used (Grade 1B)[8]. UFH provides an alternative option when LMWH or DOACs are contraindicated, or not available (Grade 2C)[8]. Anticoagulation should be continued for at least 6 mo (Grade 1A) or indefinitely while cancer is active or treated [8].

LMWH are the preferred option in patients with VVT due their short half-life and possible dose reduction in case of esophageal varices.

Briefly, DOAC are a reasonable option in ambulatory PC patients with DVT or PE with an intact upper gastrointestinal tract, without nausea or vomiting, with a low risk of bleeding, with a platelet count > 50000/mm$^3$, with a creatinine clearance > 30 mL/min, without severe hepatic impairment and for whom no surgical intervention is planned. They should not be used in patients with creatinine clearance < 30 mL/min, luminal gastrointestinal lesion, platelet count < 50000/mm$^3$, high bleeding risk, recent or planned surgery, or potential drug-drug interactions[73,74].

A step-based adapted approach (Figure 1), incorporating tumor type, careful examination of bleeding risk, potential drug–drug interactions, and patient preferences, has been proposed by several authors[73,74]. The multi-language web-

**Table 3 Randomized trials assessing the efficacy and safety of direct oral anticoagulants in cancer patients with venous thromboembolism**

| | HOKUSAI-CANCER VTE[68] (*n* = 1050) | | SELECT-D[69] (*n* = 406) | | ADAM-VTE[70] (*n* = 300) | | CARAVAGGIO[71] (*n* = 1155) | |
|---|---|---|---|---|---|---|---|---|
| | Edoxaban | Dalteparin | Rivaroxaban | Dalteparin | Apixaban | Dalteparin | Apixaban | Dalteparin |
| Dose | LMWH × 5 d, then 60 mg OD | 200 IU/kg × 1 mo, then 150 U/kg daily | 15 mg BID × 3 wk, then 20 mg OD × 6mo | 200 IU/kg × 1 mo, then 150 U/kg daily | 10 mg BID × 7 d, then 5 mg BID × 6 mo | 200 IU/kg × 1 mo, then 150 U/kg daily | 10 mg BID × 7 d, then 5 mg BID × 6 mo | 200 IU/kg × 1 mo, then 150 U/kg daily |
| Patients | Patients with active cancer and symptomatic or incidental popliteal, femoral or iliac or IVC DVT, symptomatic or incidental PE | | Patients with active cancer and symptomatic DVT, symptomatic PE, or incidental PE | | Active cancer patients with acute DVT (including upper extremity), PE, splanchnic or cerebral vein thrombosis | | Patients with active or recent cancer and acute DVT or PE | |
| PrimaryEndpoint | Composite of recurrent VTE/major bleeding at 12 mo | | VTE recurrence over 6 mo | | Primary safety: Major bleeding at 6mo; secondary efficacy: VTE at 6 mo | | Efficacy: Recurrent VTE at 6 mo; Safety: Major bleeding at 6 mo | |
| Follow-up | 12 mo | | 6 mo | | 6 mo | | 6 mo | |
| Recurrent VTE (%) | 41/525 (7.9) | 59/525 (11.3) | 8/203 (4) | 18/203 (11) | 1/145 (0.7) | 9/142 (6.3) | 32/576 (5.6) | 46/579 (7.9) |
| HR (95%CI) for recurrent VTE | 0.71 (0.48-1.06), *P* = 0.006 | | 0.43 (0.19-0.99) | | 0.099 (0.013-0.780), *P* = 0.03 | | 0.63 (0.37-1.07, *P* < 0.001) | |
| Major bleeding (%) | 36/525 (6.9) | 21/525 (4.0) | 11/203 (4) | 6/203 (6) | 0/145 (0) | 2/142 (1.4) | 22/576 (3.8) | 23/579 (4) |
| HR (CI) for major bleeding | 1.77 (1.03-3.04) | | 1.83 (0.68-4.96) | | Not estimable | | 0.82 (0.40-1.69, *P* = 0.6) | |
| CRNMB (%) | 76/525 (14.6) | 58/525 (11.1) | 25/203 (12.3) | 7/203 (3.4) | 9/145 (6.2) | 7/142 (4.9) | 52/576 (9) | 35/579 (6.0) |
| HR (95%CI) for CRNMB | 1.38 (0.98-1.94) | | 3.76 (1.63-8.69) | | 0.931 (0.43-2.02), *P* = 0.88 | | 1.42 (0.88-2.30) | |

DVT: Deep vein thrombosis; PE: Pulmonary embolism; VTE: Venous thromboembolism; CI: Confidence interval; CRNMB: Clinically relevant non-major bleeding; HR: Hazard ratio; LMWH: Low-molecular-weight heparin.

based mobile application developed by the ITAC (downloadable for free at www.itaccme.com) based on such decision-tree algorithms is paramount to help clinicians in decision making[8].

Patients should be actively involved in treatment decisions and those treated with anticoagulants should be educated on the rationale for their treatment, the potential treatment safety concerns, and the risk of drug-drug interactions to ensure optimal adherence and treatment outcomes.

Incidental VTE is associated with high risks of recurrent VTE and VTE-related mortality[75-77] and should be treated as symptomatic VTE[8].

## CONCLUSION

VTE is a common and potentially life-threatening complication in PC patients. Strict adherence to current evidence-based guidelines and dedicated patient education programs are warranted to optimize both the primary thromboprophylaxis and the treatment of VTE in PC patients. Clinical innovative tools, such as the multi-language web-based mobile application developed by the ITAC (downloadable for free at www.itaccme.com) will be paramount to assist clinicians in rigorously implementing updated CPGs and further decrease the burden of VTE in PC patients.

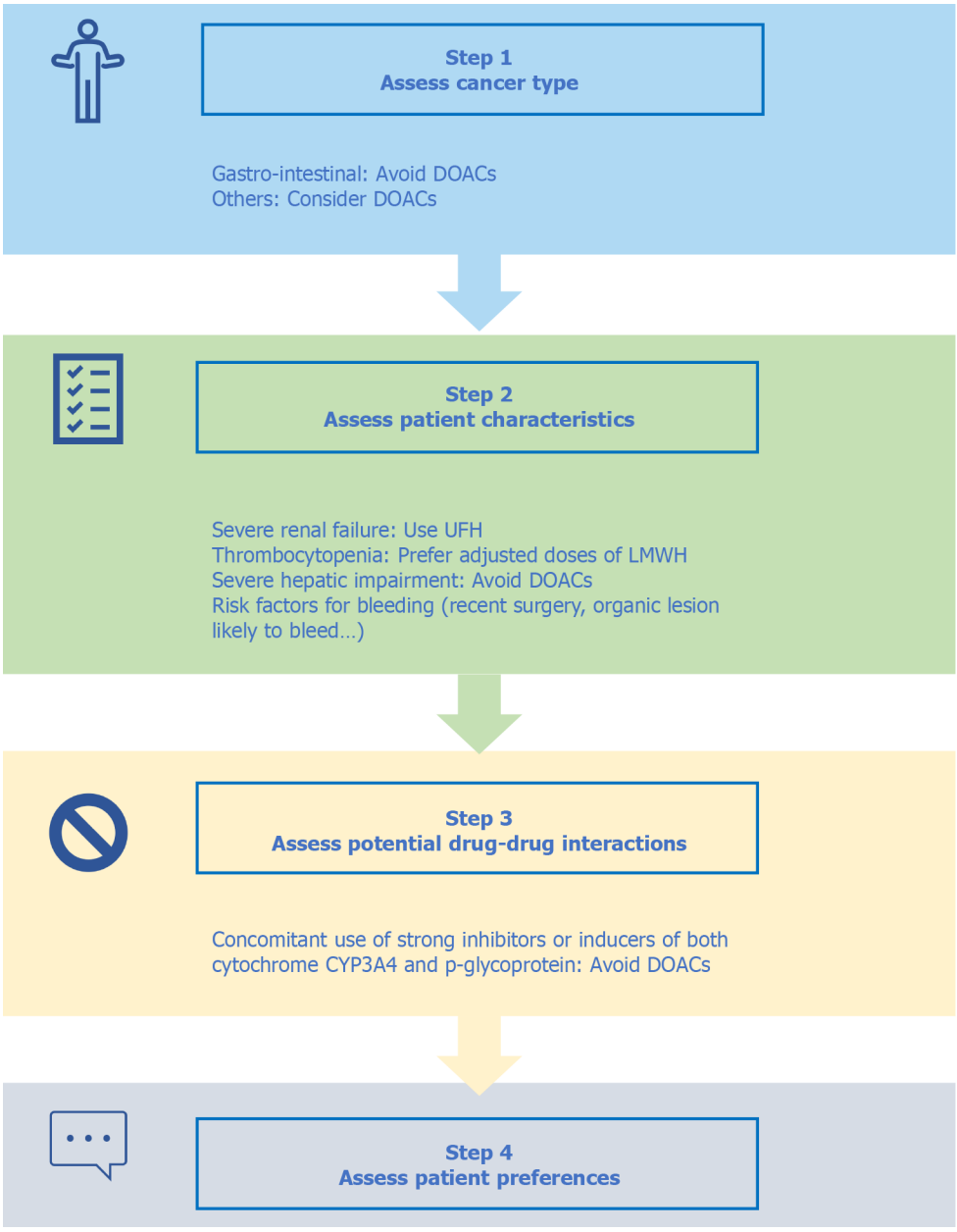



**Figure 1 Four step adapted approach for the treatment of cancer-associated venous thromboembolism.** DOACs: Direct oral anticoagulants; UFH: Unfractionated heparin; LMWH: Low-molecular-weight heparin; CYP3A4: Cytochrome P450 3A4.

## REFERENCES


1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7-30 [PMID: 29313949 DOI: 10.3322/caac.21442]

2 **Rahib L**, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* 2014; **74**: 2913-2921 [PMID: 24840647 DOI: 10.1158/0008-5472.CAN-14-0155]

3 **Lambert A**, Schwarz L, Borbath I, Henry A, Van Laethem JL, Malka D, Ducreux M, Conroy T. An update on treatment options for pancreatic adenocarcinoma. *Ther Adv Med Oncol* 2019; **11**: 1758835919875568 [PMID: 31598142 DOI: 10.1177/1758835919875568]

4 **Moffat GT**, Epstein AS, O'Reilly EM. Pancreatic cancer-A disease in need: Optimizing and integrating supportive care. *Cancer* 2019; **125**: 3927-3935 [PMID: 31381149 DOI: 10.1002/cncr.32423]

5 **Farge D**, Bournet B, Conroy T, Vicaut E, Rak J, Zogoulous G, Barkun J, Ouaissi M, Buscail L, Frere C. Primary Thromboprophylaxis in Pancreatic Cancer Patients: Why Clinical Practice Guidelines Should Be Implemented. *Cancers (Basel)* 2020; **12** [PMID: 32155940 DOI: 10.3390/cancers12030618]

6 **Chiasakul T**, Patell R, Maraveyas A, Carrier M, Zwicker JI. Discordant reporting of VTE in

pancreatic cancer: A systematic review and meta-analysis of thromboprophylaxis *vs* chemotherapeutic trials. *J Thromb Haemost* 2021; **19**: 489-501 [PMID: 33174368 DOI: 10.1111/jth.15175]

7 **Mulder FI**, Horváth-Puhó E, van Es N, van Laarhoven HWM, Pedersen L, Moik F, Ay C, Büller HR, Sørensen HT. Venous thromboembolism in cancer patients: a population-based cohort study. *Blood* 2021; **137**: 1959-1969 [PMID: 33171494 DOI: 10.1182/blood.2020007338]

8 **Farge D**, Frere C, Connors JM, Ay C, Khorana AA, Munoz A, Brenner B, Kakkar A, Rafii H, Solymoss S, Brilhante D, Monreal M, Bounameaux H, Pabinger I, Douketis J; International Initiative on Thrombosis and Cancer (ITAC) advisory panel. 2019 international clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer. *Lancet Oncol* 2019; **20**: e566-e581 [PMID: 31492632 DOI: 10.1016/S1470-2045(19)30336-5]

9 **Prandoni P**, Lensing AW, Piccioli A, Bernardi E, Simioni P, Girolami B, Marchiori A, Sabbion P, Prins MH, Noventa F, Girolami A. Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. *Blood* 2002; **100**: 3484-3488 [PMID: 12393647 DOI: 10.1182/blood-2002-01-0108]

10 **Abdulla A**, Davis WM, Ratnaweera N, Szefer E, Ballantyne Scott B, Lee AYY. A Meta-Analysis of Case Fatality Rates of Recurrent Venous Thromboembolism and Major Bleeding in Patients with Cancer. *Thromb Haemost* 2020; **120**: 702-713 [PMID: 32289865 DOI: 10.1055/s-0040-1708481]

11 **Key NS**, Khorana AA, Kuderer NM, Bohlke K, Lee AYY, Arcelus JI, Wong SL, Balaban EP, Flowers CR, Francis CW, Gates LE, Kakkar AK, Levine MN, Liebman HA, Tempero MA, Lyman GH, Falanga A. Venous Thromboembolism Prophylaxis and Treatment in Patients With Cancer: ASCO Clinical Practice Guideline Update. *J Clin Oncol* 2020; **38**: 496-520 [PMID: 31381464 DOI: 10.1200/JCO.19.01461]

12 **National Comprehensive Cancer Network**. NCCN guideline on cancer-associated venous thromboembolic disease. Version 1. 2020. [cited 3 January 2021]. In: National Comprehensive Cancer Network [Internet]. Available from: https://www.nccn.org/professionals/physician_gls/pdf/vte.pdf

13 **Levitan N**, Dowlati A, Remick SC, Tahsildar HI, Sivinski LD, Beyth R, Rimm AA. Rates of initial and recurrent thromboembolic disease among patients with malignancy *vs* those without malignancy. Risk analysis using Medicare claims data. *Medicine (Baltimore)* 1999; **78**: 285-291

14 **Horsted F**, West J, Grainge MJ. Risk of venous thromboembolism in patients with cancer: a systematic review and meta-analysis. *PLoS Med* 2012; **9**: e1001275 [PMID: 22859911 DOI: 10.1371/journal.pmed.1001275]

15 **Sproul EE**. Carcinoma and Venous Thrombosis: The Frequency of Association of Carcinoma in the Body or Tail of the Pancreas with Multiple Venous Thrombosis. *Am J Cancer* 1938; **34**: 566-585

16 **Blom JW**, Osanto S, Rosendaal FR. High risk of venous thrombosis in patients with pancreatic cancer: a cohort study of 202 patients. *Eur J Cancer* 2006; **42**: 410-414 [PMID: 16321518 DOI: 10.1016/j.ejca.2005.09.013]

17 **Mandalà M**, Reni M, Cascinu S, Barni S, Floriani I, Cereda S, Berardi R, Mosconi S, Torri V, Labianca R. Venous thromboembolism predicts poor prognosis in irresectable pancreatic cancer patients. *Ann Oncol* 2007; **18**: 1660-1665 [PMID: 17660490 DOI: 10.1093/annonc/mdm284]

18 **Mitry E**, Taleb-Fayad R, Deschamps A, Mansencal N, Lepère C, Declety G, Lièvre A, Vaillant JN, Lesur G, Cramer E, Dubourg O, Rougier P. Risk of venous thrombosis in patients with pancreatic adenocarcinoma. *Gastroenterol Clin Biol* 2007; **31**: 1139-1142 [PMID: 18176374 DOI: 10.1016/s0399-8320(07)78352-5]

19 **Oh SY**, Kim JH, Lee KW, Bang SM, Hwang JH, Oh D, Lee JS. Venous thromboembolism in patients with pancreatic adenocarcinoma: lower incidence in Asian ethnicity. *Thromb Res* 2008; **122**: 485-490 [PMID: 18234292 DOI: 10.1016/j.thromres.2007.12.015]

20 **Poruk KE**, Firpo MA, Huerter LM, Scaife CL, Emerson LL, Boucher KM, Jones KA, Mulvihill SJ. Serum platelet factor 4 is an independent predictor of survival and venous thromboembolism in patients with pancreatic adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2605-2610 [PMID: 20729288 DOI: 10.1158/1055-9965.EPI-10-0178]

21 **Shaib W**, Deng Y, Zilterman D, Lundberg B, Saif MW. Assessing risk and mortality of venous thromboembolism in pancreatic cancer patients. *Anticancer Res* 2010; **30**: 4261-4264 [PMID: 21036750]

22 **Epstein AS**, Soff GA, Capanu M, Crosbie C, Shah MA, Kelsen DP, Denton B, Gardos S, O'Reilly EM. Analysis of incidence and clinical outcomes in patients with thromboembolic events and invasive exocrine pancreatic cancer. *Cancer* 2012; **118**: 3053-3061 [PMID: 21989534 DOI: 10.1002/cncr.26600]

23 **Menapace LA**, Peterson DR, Berry A, Sousou T, Khorana AA. Symptomatic and incidental thromboembolism are both associated with mortality in pancreatic cancer. *Thromb Haemost* 2011; **106**: 371-378 [PMID: 21713322 DOI: 10.1160/TH10-12-0789]

24 **Afsar CU**, Gunaldi M, Kum P, Sahin B, Erkisi M, Kara IO, Paydas S, Duman BB, Ercolak V, Karaca F, Uyeturk U, Guner SI. Pancreatic carcinoma, thrombosis and mean platelet volume: single center experience from the southeast region of Turkey. *Asian Pac J Cancer Prev* 2014; **15**: 9143-9146 [PMID: 25422192 DOI: 10.7314/apjcp.2014.15.21.9143]

25 **Muñoz Martín AJ**, García Alfonso P, Rupérez Blanco AB, Pérez Ramírez S, Blanco Codesido M, Martín Jiménez M. Incidence of venous thromboembolism (VTE) in ambulatory pancreatic cancer patients receiving chemotherapy and analysis of Khorana's predictive model. *Clin Transl Oncol* 2014; **16**: 927-930 [PMID: 24643701 DOI: 10.1007/s12094-014-1165-y]

26 **Krepline AN**, Christians KK, George B, Ritch PS, Erickson BA, Tolat P, Evans DB, Tsai S. Venous thromboembolism prophylaxis during neoadjuvant therapy for resectable and borderline resectable pancreatic cancer-Is it indicated? *J Surg Oncol* 2016; **114**: 581-586 [PMID: 27760280 DOI: 10.1002/jso.24361]

27 **Lee JC**, Ro YS, Cho J, Park Y, Lee JH, Hwang JH, Choi HJ, Lee S. Characteristics of Venous Thromboembolism in Pancreatic Adenocarcinoma in East Asian Ethnics: A Large Population-Based Observational Study. *Medicine (Baltimore)* 2016; **95**: e3472 [PMID: 27124043 DOI: 10.1097/MD.0000000000003472]

28 **Kruger S**, Haas M, Burkl C, Goehring P, Kleespies A, Roeder F, Gallmeier E, Ormanns S, Westphalen CB, Heinemann V, Rank A, Boeck S. Incidence, outcome and risk stratification tools for venous thromboembolism in advanced pancreatic cancer - A retrospective cohort study. *Thromb Res* 2017; **157**: 9-15 [PMID: 28675831 DOI: 10.1016/j.thromres.2017.06.021]

29 **van Es N**, Franke VF, Middeldorp S, Wilmink JW, Büller HR. The Khorana score for the prediction of venous thromboembolism in patients with pancreatic cancer. *Thromb Res* 2017; **150**: 30-32 [PMID: 28002757 DOI: 10.1016/j.thromres.2016.12.013]

30 **Berger AK**, Singh HM, Werft W, Muckenhuber A, Sprick MR, Trumpp A, Weichert W, Jäger D, Springfeld C. High prevalence of incidental and symptomatic venous thromboembolic events in patients with advanced pancreatic cancer under palliative chemotherapy: A retrospective cohort study. *Pancreatology* 2017; **17**: 629-634 [PMID: 28462862 DOI: 10.1016/j.pan.2017.04.012]

31 **Chen JS**, Hung CY, Chang H, Liu CT, Chen YY, Lu CH, Chang PH, Hung YS, Chou WC. Venous Thromboembolism in Asian Patients with Pancreatic Cancer Following Palliative Chemotherapy: Low Incidence but a Negative Prognosticator for Those with Early Onset. *Cancers (Basel)* 2018; **10** [PMID: 30544670 DOI: 10.3390/cancers10120501]

32 **Kim JS**, Kang EJ, Kim DS, Choi YJ, Lee SY, Kim HJ, Seo HY, Kim JS. Early venous thromboembolism at the beginning of palliative chemotherapy is a poor prognostic factor in patients with metastatic pancreatic cancer: a retrospective study. *BMC Cancer* 2018; **18**: 1260 [PMID: 30558603 DOI: 10.1186/s12885-018-5154-3]

33 **Ouaissi M**, Frasconi C, Mege D, Panicot-Dubois L, Boiron L, Dahan L, Debourdeau P, Dubois C, Farge D, Sielezneff I. Impact of venous thromboembolism on the natural history of pancreatic adenocarcinoma. *Hepatobiliary Pancreat Dis Int* 2015; **14**: 436-442 [PMID: 26256090 DOI: 10.1016/s1499-3872(15)60397-6]

34 **Frere C**, Bournet B, Gourgou S, Fraisse J, Canivet C, Connors JM, Buscail L, Farge D; BACAP Consortium. Incidence of Venous Thromboembolism in Patients With Newly Diagnosed Pancreatic Cancer and Factors Associated With Outcomes. *Gastroenterology* 2020; **158**: 1346-1358. e4 [PMID: 31843588 DOI: 10.1053/j.gastro.2019.12.009]

35 **Khorana AA**, Fine RL. Pancreatic cancer and thromboembolic disease. *Lancet Oncol* 2004; **5**: 655-663 [PMID: 15522652 DOI: 10.1016/S1470-2045(04)01606-7]

36 **Mier-Hicks A**, Raj M, Do RK, Yu KH, Lowery MA, Varghese A, O'Reilly EM. Incidence, Management, and Implications of Visceral Thrombosis in Pancreatic Ductal Adenocarcinoma. *Clin Colorectal Cancer* 2018; **17**: 121-128 [PMID: 29477452 DOI: 10.1016/j.clcc.2018.01.008]

37 **Afzal A**, Suhong L, Gage BF, Schoen MW, Carson K, Thomas T, Sanfilippo K. Splanchnic vein thrombosis predicts worse survival in patients with advanced pancreatic cancer. *Thromb Res* 2020; **185**: 125-131 [PMID: 31812026 DOI: 10.1016/j.thromres.2019.11.023]

38 **Faille D**, Bourrienne MC, de Raucourt E, de Chaisemartin L, Granger V, Lacroix R, Panicot-Dubois L, Hammel P, Lévy P, Ruszniewski P, Ajzenberg N, Rebours V. Biomarkers for the risk of thrombosis in pancreatic adenocarcinoma are related to cancer process. *Oncotarget* 2018; **9**: 26453-26465 [PMID: 29899870 DOI: 10.18632/oncotarget.25458]

39 **Godinho J**, Casa-Nova M, Moreira-Pinto J, Simões P, Paralta Branco F, Leal-Costa L, Faria A, Lopes F, Teixeira JA, Passos-Coelho JL. ONKOTEV Score as a Predictive Tool for Thromboembolic Events in Pancreatic Cancer-A Retrospective Analysis. *Oncologist* 2020; **25**: e284-e290 [PMID: 32043787 DOI: 10.1634/theoncologist.2019-0510]

40 **Heit JA**. Epidemiology of venous thromboembolism. *Nat Rev Cardiol* 2015; **12**: 464-474 [PMID: 26076949 DOI: 10.1038/nrcardio.2015.83]

41 **Vadhan-Raj S**, McNamara MG, Venerito M, Riess H, O'Reilly EM, Overman MJ, Zhou X, Vijapurkar U, Kaul S, Wildgoose P, Khorana AA. Rivaroxaban thromboprophylaxis in ambulatory patients with pancreatic cancer: Results from a pre-specified subgroup analysis of the randomized CASSINI study. *Cancer Med* 2020; **9**: 6196-6204 [PMID: 32663379 DOI: 10.1002/cam4.3269]

42 **Cronin M**, Dengler N, Krauss ES, Segal A, Wei N, Daly M, Mota F, Caprini JA. Completion of the Updated Caprini Risk Assessment Model (2013 Version). *Clin Appl Thromb Hemost* 2019; **25**: 1076029619838052 [PMID: 30939900 DOI: 10.1177/1076029619838052]

43 **Boone BA**, Zenati MS, Rieser C, Hamad A, Al-Abbas A, Zureikat AH, Hogg ME, Neal MD, Zeh HJ 3rd. Risk of Venous Thromboembolism for Patients with Pancreatic Ductal Adenocarcinoma Undergoing Preoperative Chemotherapy Followed by Surgical Resection. *Ann Surg Oncol* 2019; **26**: 1503-1511 [PMID: 30652227 DOI: 10.1245/s10434-018-07148-z]

44 **Khorana AA**, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood* 2008; **111**: 4902-4907 [PMID: 18216292 DOI: 10.1182/blood-2007-10-116327]

45 **Pelzer U**, Sinn M, Stieler J, Riess H. [Primary pharmacological prevention of thromboembolic events in ambulatory patients with advanced pancreatic cancer treated with chemotherapy? *Dtsch Med*

*Wochenschr* 2013; **138**: 2084-2088 [PMID: 24085361 DOI: 10.1055/s-0033-1349608]
46 **Verso M**, Agnelli G, Barni S, Gasparini G, LaBianca R. A modified Khorana risk assessment score for venous thromboembolism in cancer patients receiving chemotherapy: the Protecht score. *Intern Emerg Med* 2012; **7**: 291-292 [PMID: 22547369 DOI: 10.1007/s11739-012-0784-y]
47 **Cella CA**, Di Minno G, Carlomagno C, Arcopinto M, Cerbone AM, Matano E, Tufano A, Lordick F, De Simone B, Muehlberg KS, Bruzzese D, Attademo L, Arturo C, Sodano M, Moretto R, La Fata E, De Placido S. Preventing Venous Thromboembolism in Ambulatory Cancer Patients: The ONKOTEV Study. *Oncologist* 2017; **22**: 601-608 [PMID: 28424324 DOI: 10.1634/theoncologist.2016-0246]
48 **Muñoz Martín AJ**, Ortega I, Font C, Pachón V, Castellón V, Martínez-Marín V, Salgado M, Martínez E, Calzas J, Rupérez A, Souto JC, Martín M, Salas E, Soria JM. Multivariable clinical-genetic risk model for predicting venous thromboembolic events in patients with cancer. *Br J Cancer* 2018; **118**: 1056-1061 [PMID: 29588512 DOI: 10.1038/s41416-018-0027-8]
49 **Pabinger I**, van Es N, Heinze G, Posch F, Riedl J, Reitter EM, Di Nisio M, Cesarman-Maus G, Kraaijpoel N, Zielinski CC, Büller HR, Ay C. A clinical prediction model for cancer-associated venous thromboembolism: a development and validation study in two independent prospective cohorts. *Lancet Haematol* 2018; **5**: e289-e298 [PMID: 29885940 DOI: 10.1016/S2352-3026(18)30063-2]
50 **Ferroni P**, Zanzotto FM, Scarpato N, Riondino S, Guadagni F, Roselli M. Validation of a Machine Learning Approach for Venous Thromboembolism Risk Prediction in Oncology. *Dis Markers* 2017; **2017**: 8781379 [PMID: 29104344 DOI: 10.1155/2017/8781379]
51 **Fresard ME**, Erices R, Bravo ML, Cuello M, Owen GI, Ibanez C, Rodriguez-Fernandez M. Multi-Objective Optimization for Personalized Prediction of Venous Thromboembolism in Ovarian Cancer Patients. *IEEE J Biomed Health Inform* 2020; **24**: 1500-1508 [PMID: 31562113 DOI: 10.1109/JBHI.2019.2943499]
52 **Felder S**, Rasmussen MS, King R, Sklow B, Kwaan M, Madoff R, Jensen C. Prolonged thromboprophylaxis with low molecular weight heparin for abdominal or pelvic surgery. *Cochrane Database Syst Rev* 2019; **8**: CD004318 [PMID: 31449321 DOI: 10.1002/14651858.CD004318.pub5]
53 **Spencer FA**, Lessard D, Emery C, Reed G, Goldberg RJ. Venous thromboembolism in the outpatient setting. *Arch Intern Med* 2007; **167**: 1471-1475 [PMID: 17646600 DOI: 10.1001/archinte.167.14.1471]
54 **Maraveyas A**, Waters J, Roy R, Fyfe D, Propper D, Lofts F, Sgouros J, Gardiner E, Wedgwood K, Ettelaie C, Bozas G. Gemcitabine *vs* gemcitabine plus dalteparin thromboprophylaxis in pancreatic cancer. *Eur J Cancer* 2012; **48**: 1283-1292 [PMID: 22100906 DOI: 10.1016/j.ejca.2011.10.017]
55 **Pelzer U**, Opitz B, Deutschinoff G, Stauch M, Reitzig PC, Hahnfeld S, Müller L, Grunewald M, Stieler JM, Sinn M, Denecke T, Bischoff S, Oettle H, Dörken B, Riess H. Efficacy of Prophylactic Low-Molecular Weight Heparin for Ambulatory Patients With Advanced Pancreatic Cancer: Outcomes From the CONKO-004 Trial. *J Clin Oncol* 2015; **33**: 2028-2034 [PMID: 25987694 DOI: 10.1200/JCO.2014.55.1481]
56 **Farge D**, Debourdeau P, Beckers M, Baglin C, Bauersachs RM, Brenner B, Brilhante D, Falanga A, Gerotzafias GT, Haim N, Kakkar AK, Khorana AA, Lecumberri R, Mandala M, Marty M, Monreal M, Mousa SA, Noble S, Pabinger I, Prandoni P, Prins MH, Qari MH, Streiff MB, Syrigos K, Bounameaux H, Büller HR. International clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer. *J Thromb Haemost* 2013; **11**: 56-70 [PMID: 23217107 DOI: 10.1111/jth.12070]
57 **Farge D**, Bounameaux H, Brenner B, Cajfinger F, Debourdeau P, Khorana AA, Pabinger I, Solymoss S, Douketis J, Kakkar A. International clinical practice guidelines including guidance for direct oral anticoagulants in the treatment and prophylaxis of venous thromboembolism in patients with cancer. *Lancet Oncol* 2016; **17**: e452-e466 [PMID: 27733271 DOI: 10.1016/S1470-2045(16)30369-2]
58 **Agnelli G**, Gussoni G, Bianchini C, Verso M, Mandalà M, Cavanna L, Barni S, Labianca R, Buzzi F, Scambia G, Passalacqua R, Ricci S, Gasparini G, Lorusso V, Bonizzoni E, Tonato M; PROTECHT Investigators. Nadroparin for the prevention of thromboembolic events in ambulatory patients with metastatic or locally advanced solid cancer receiving chemotherapy: a randomised, placebo-controlled, double-blind study. *Lancet Oncol* 2009; **10**: 943-949 [PMID: 19726226 DOI: 10.1016/S1470-2045(09)70232-3]
59 **Agnelli G**, George DJ, Kakkar AK, Fisher W, Lassen MR, Mismetti P, Mouret P, Chaudhari U, Lawson F, Turpie AG; SAVE-ONCO Investigators. Semuloparin for thromboprophylaxis in patients receiving chemotherapy for cancer. *N Engl J Med* 2012; **366**: 601-609 [PMID: 22335737 DOI: 10.1056/NEJMoa1108898]
60 **Carrier M**, Abou-Nassar K, Mallick R, Tagalakis V, Shivakumar S, Schattner A, Kuruvilla P, Hill D, Spadafora S, Marquis K, Trinkaus M, Tomiak A, Lee AYY, Gross PL, Lazo-Langner A, El-Maraghi R, Goss G, Le Gal G, Stewart D, Ramsay T, Rodger M, Witham D, Wells PS; AVERT Investigators. Apixaban to Prevent Venous Thromboembolism in Patients with Cancer. *N Engl J Med* 2019; **380**: 711-719 [PMID: 30511879 DOI: 10.1056/NEJMoa1814468]
61 **Khorana AA**, Soff GA, Kakkar AK, Vadhan-Raj S, Riess H, Wun T, Streiff MB, Garcia DA, Liebman HA, Belani CP, O'Reilly EM, Patel JN, Yimer HA, Wildgoose P, Burton P, Vijapurkar U, Kaul S, Eikelboom J, McBane R, Bauer KA, Kuderer NM, Lyman GH; CASSINI Investigators. Rivaroxaban for Thromboprophylaxis in High-Risk Ambulatory Patients with Cancer. *N Engl J Med* 2019; **380**: 720-728 [PMID: 30786186 DOI: 10.1056/NEJMoa1814630]

62 **Frere C**, Crichi B, Bournet B, Canivet C, Abdallah NA, Buscail L, Farge D. Primary Thromboprophylaxis in Ambulatory Pancreatic Cancer Patients Receiving Chemotherapy: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Cancers (Basel)* 2020; **12** [PMID: 32722064 DOI: 10.3390/cancers12082028]

63 **Meyer G**, Marjanovic Z, Valcke J, Lorcerie B, Gruel Y, Solal-Celigny P, Le Maignan C, Extra JM, Cottu P, Farge D. Comparison of low-molecular-weight heparin and warfarin for the secondary prevention of venous thromboembolism in patients with cancer: a randomized controlled study. *Arch Intern Med* 2002; **162**: 1729-1735 [PMID: 12153376 DOI: 10.1001/archinte.162.15.1729]

64 **Lee AY**, Levine MN, Baker RI, Bowden C, Kakkar AK, Prins M, Rickles FR, Julian JA, Haley S, Kovacs MJ, Gent M; Randomized Comparison of Low-Molecular-Weight Heparin vs Oral Anticoagulant Therapy for the Prevention of Recurrent Venous Thromboembolism in Patients with Cancer (CLOT) Investigators. Low-molecular-weight heparin *vs* a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *N Engl J Med* 2003; **349**: 146-153 [PMID: 12853587 DOI: 10.1056/NEJMoa025313]

65 **Deitcher SR**, Kessler CM, Merli G, Rigas JR, Lyons RM, Fareed J; ONCENOX Investigators. Secondary prevention of venous thromboembolic events in patients with active cancer: enoxaparin alone *vs* initial enoxaparin followed by warfarin for a 180-day period. *Clin Appl Thromb Hemost* 2006; **12**: 389-396 [PMID: 17000884 DOI: 10.1177/1076029606293692]

66 **Hull RD**, Pineo GF, Brant RF, Mah AF, Burke N, Dear R, Wong T, Cook R, Solymoss S, Poon MC, Raskob G; LITE Trial Investigators. Long-term low-molecular-weight heparin *vs* usual care in proximal-vein thrombosis patients with cancer. *Am J Med* 2006; **119**: 1062-1072 [PMID: 17145251 DOI: 10.1016/j.amjmed.2006.02.022]

67 **Lee AYY**, Kamphuisen PW, Meyer G, Bauersachs R, Janas MS, Jarner MF, Khorana AA; CATCH Investigators. Tinzaparin *vs* Warfarin for Treatment of Acute Venous Thromboembolism in Patients With Active Cancer: A Randomized Clinical Trial. *JAMA* 2015; **314**: 677-686 [PMID: 26284719 DOI: 10.1001/jama.2015.9243]

68 **Raskob GE**, van Es N, Verhamme P, Carrier M, Di Nisio M, Garcia D, Grosso MA, Kakkar AK, Kovacs MJ, Mercuri MF, Meyer G, Segers A, Shi M, Wang TF, Yeo E, Zhang G, Zwicker JI, Weitz JI, Büller HR; Hokusai VTE Cancer Investigators. Edoxaban for the Treatment of Cancer-Associated Venous Thromboembolism. *N Engl J Med* 2018; **378**: 615-624 [PMID: 29231094 DOI: 10.1056/NEJMoa1711948]

69 **Young AM**, Marshall A, Thirlwall J, Chapman O, Lokare A, Hill C, Hale D, Dunn JA, Lyman GH, Hutchinson C, MacCallum P, Kakkar A, Hobbs FDR, Petrou S, Dale J, Poole CJ, Maraveyas A, Levine M. Comparison of an Oral Factor Xa Inhibitor With Low Molecular Weight Heparin in Patients With Cancer With Venous Thromboembolism: Results of a Randomized Trial (SELECT-D). *J Clin Oncol* 2018; **36**: 2017-2023 [PMID: 29746227 DOI: 10.1200/JCO.2018.78.8034]

70 **McBane RD 2nd**, Wysokinski WE, Le-Rademacher JG, Zemla T, Ashrani A, Tafur A, Perepu U, Anderson D, Gundabolu K, Kuzma C, Perez Botero J, Leon Ferre RA, Henkin S, Lenz CJ, Houghton DE, Vishnu P, Loprinzi CL. Apixaban and dalteparin in active malignancy-associated venous thromboembolism: The ADAM VTE trial. *J Thromb Haemost* 2020; **18**: 411-421 [PMID: 31630479 DOI: 10.1111/jth.14662]

71 **Agnelli G**, Becattini C, Meyer G, Muñoz A, Huisman MV, Connors JM, Cohen A, Bauersachs R, Brenner B, Torbicki A, Sueiro MR, Lambert C, Gussoni G, Campanini M, Fontanella A, Vescovo G, Verso M; Caravaggio Investigators. Apixaban for the Treatment of Venous Thromboembolism Associated with Cancer. *N Engl J Med* 2020; **382**: 1599-1607 [PMID: 32223112 DOI: 10.1056/NEJMoa1915103]

72 **Frere C**, Benzidia I, Marjanovic Z, Farge D. Recent Advances in the Management of Cancer-Associated Thrombosis: New Hopes but New Challenges. *Cancers (Basel)* 2019; **11** [PMID: 30634638 DOI: 10.3390/cancers11010071]

73 **Carrier M**, Wang TF. Direct oral anticoagulants and cancer-associated VTE: good for all, or just some? *Blood* 2020; **136**: 669-673 [PMID: 32575112 DOI: 10.1182/blood.2019004177]

74 **O'Connell C**, Escalante CP, Goldhaber SZ, McBane R, Connors JM, Raskob GE. Treatment of Cancer-Associated Venous Thromboembolism with Low-Molecular-Weight Heparin or Direct Oral Anticoagulants: Patient Selection, Controversies, and Caveats. *Oncologist* 2021; **26**: e8-e16 [PMID: 33275319 DOI: 10.1002/onco.13584]

75 **den Exter PL**, Hooijer J, Dekkers OM, Huisman MV. Risk of recurrent venous thromboembolism and mortality in patients with cancer incidentally diagnosed with pulmonary embolism: a comparison with symptomatic patients. *J Clin Oncol* 2011; **29**: 2405-2409 [PMID: 21555690 DOI: 10.1200/JCO.2010.34.0984]

76 **van der Hulle T**, den Exter PL, Planquette B, Meyer G, Soler S, Monreal M, Jiménez D, Portillo AK, O'Connell C, Liebman HA, Shteinberg M, Adir Y, Tiseo M, Bersanelli M, Abdel-Razeq HN, Mansour AH, Donnelly OG, Radhakrishna G, Ramasamy S, Bozas G, Maraveyas A, Shinagare AB, Hatabu H, Nishino M, Huisman MV, Klok FA. Risk of recurrent venous thromboembolism and major hemorrhage in cancer-associated incidental pulmonary embolism among treated and untreated patients: a pooled analysis of 926 patients. *J Thromb Haemost* 2016; **14**: 105-113 [PMID: 26469193 DOI: 10.1111/jth.13172]

77 **Kraaijpoel N**, Bleker SM, Meyer G, Mahé I, Muñoz A, Bertoletti L, Bartels-Rutten A, Beyer-Westendorf J, Porreca E, Boulon C, van Es N, Iosub DI, Couturaud F, Biosca M, Lerede T, Lacroix P, Maraveyas A, Aggarwal A, Girard P, Büller HR, Di Nisio M; UPE investigators. Treatment and

Long-Term Clinical Outcomes of Incidental Pulmonary Embolism in Patients With Cancer: An International Prospective Cohort Study. *J Clin Oncol* 2019; **37**: 1713-1720 [PMID: 31116676 DOI: 10.1200/JCO.18.01977]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2341-2352

DOI: 10.3748/wjg.v27.i19.2341

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

*MINIREVIEWS*

# Pathophysiological mechanisms underlying gastrointestinal symptoms in patients with COVID-19

Byungchang Jin, Rajan Singh, Se Eun Ha, Hannah Zogg, Paul J Park, Seungil Ro

**ORCID number:** Byungchang Jin 0000-0003-3184-5332; Rajan Singh 0000-0001-5500-1949; Se Eun Ha 0000-0001-6063-5559; Hannah Zogg 0000-0002-2413-157X; Paul J Park 0000-0003-4786-2030; Seungil Ro 0000-0003-0861-8334.

**Author contributions:** Jin B, Singh R and Ro S conceived the study; Jin B and Singh R drafted the original manuscript and are joint first authors; Jin B, Singh R, Ha SE, Zogg H, Park PJ, and Ro S revised the manuscript and approved the submitted version.

**Conflict-of-interest statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: htt

**Byungchang Jin, Rajan Singh, Se Eun Ha, Hannah Zogg, Seungil Ro,** Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV 89557, United States

**Paul J Park,** Department of Medicine, Renown Health, Reno, NV 89557, United States

**Corresponding author:** Seungil Ro, PhD, Associate Professor, Department of Physiology and Cell Biology, University of Nevada School of Medicine, Building L-207E, 1664 North Virginia Street, Reno, NV 89557, United States. sro@med.unr.edu

## Abstract

Gastrointestinal (GI) symptoms, such as diarrhea, abdominal pain, vomiting, and anorexia, are frequently observed in patients with coronavirus disease 2019 (COVID-19). However, the pathophysiological mechanisms connecting these GI symptoms to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections remain elusive. Previous studies indicate that the entry of SARS-CoV-2 into intestinal cells leads to downregulation of angiotensin converting enzyme 2 (ACE2) receptors resulting in impaired barrier function. While intestinal ACE2 functions as a chaperone for the amino acid transporter B0AT1, the B0AT1/ACE2 complex within the intestinal epithelium acts as a regulator of gut microbiota composition and function. Alternations to the B0AT1/ACE2 complex lead to microbial dysbiosis through increased local and systemic immune responses. Previous studies have also suggested that altered serotonin metabolism may be the underlying cause of GI disorders involving diarrhea. The findings of elevated plasma serotonin levels and high fecal calprotectin in COVID-19 patients with diarrhea indicate that the viral infection evokes a systemic inflammatory response that specifically involves the GI. Interestingly, the elevated proinflammatory cytokines correlate with elevated serotonin and fecal calprotectin levels further supporting the evidence of GI inflammation, a hallmark of functional GI disorders. Moreover, the finding that rectal swabs of COVID-19 patients remain positive for SARS-CoV-2 even after the nasopharynx clears the virus, suggests that viral replication and shedding from the GI tract may be more robust than that of the respiratory tract, further indicating fecal-oral transmission as another important route of viral spread. This review summarized the evidence for pathophysiological mechanisms (impaired barrier function, gut inflammation, altered serotonin metabolism and gut microbiota dysbiosis) underlying the GI symptoms in patients with COVID-19.

**Key Words:** COVID-19; Gastrointestinal symptoms; Gut microbiota dysbiosis; Impaired

p://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** United States

**Peer-review report's scientific quality classification**

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

**Received:** January 29, 2021
**Peer-review started:** January 29, 2021
**First decision:** March 6, 2021
**Revised:** March 17, 2021
**Accepted:** April 22, 2021
**Article in press:** April 22, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Luo A
**S-Editor:** Liu M
**L-Editor:** A
**P-Editor:** Ma YJ

barrier function; Serotonin; Angiotensin converting enzyme 2 receptor

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** Since the declaration of the pandemic on March 11, 2020 by the World Health Organization, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has quickly become a global health threat. In addition to respiratory symptoms, gastrointestinal (GI) symptoms have been widely observed in coronavirus disease 2019 (COVID-19) patients. Here, we have summarized the GI symptoms seen in COVID-19 patients that have been reported in nineteen studies and recapitulated potential mechanisms that are responsible for the GI symptoms in COVID-19 patients. This biochemical understanding may assist in new therapeutic approaches.

**Citation:** Jin B, Singh R, Ha SE, Zogg H, Park PJ, Ro S. Pathophysiological mechanisms underlying gastrointestinal symptoms in patients with COVID-19. *World J Gastroenterol* 2021; 27(19): 2341-2352
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2341.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2341

## INTRODUCTION

Since December 2019, an acute respiratory infection, referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused by the novel coronavirus has rapidly spread worldwide[1-3]. In the United States alone, 198589 deaths (60.3/100000) have been reported due to the coronavirus pandemic from February 13, 2020 to September 19, 2020[4].

Based on next-generation sequencing data from patient samples, SARS-CoV-2 is closely associated with two bat-derived SARS-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21 (88% identity)[5]. The binding of the SARS-CoV-2 spike protein to the host receptor, angiotensin-converting enzyme 2 (ACE2), is critical for viral invasion[6]. This viral infection may be asymptomatic or cause symptoms, such as fever, cough, headache, and myalgia[7-9]. Interestingly, up to 40% of coronavirus disease 2019 (COVID-19) patients experience gastrointestinal (GI) symptoms, including diarrhea, anorexia, nausea, vomiting, and abdominal pain (Table 1). In order to provide appropriate medical care to COVID-19 patients, it is necessary to explore pathophysiological mechanisms underlying their GI symptoms.

In this review, we summarized the studies that describe the various GI symptoms in COVID-19 patients and highlighted the likely underlying pathophysiological mechanisms. These insights offer potential new therapeutic approaches for containment of the global inflammatory response. Furthermore, we also shed light on the importance of the altered gut microbiota profile in the possible pathogenesis of COVID-19.

## CLINICAL PRESENTATION OF COVID-19 PATIENTS WITH GI SYMPTOMS

The clinical severity of COVID-19 patients may be stratified into three grades: Placid, ordinary, and grave cases. The incubation period of SARS-CoV-2 ranges from 1-14 d, but is more commonly 3-7 d. The typical clinical presentation of SARS-CoV-2 consists of fever, fatigue, dry cough, and shortness of breath. Other common symptoms involve congestion and rhinorrhea, pharyngalgia, myalgias, and diarrhea. In grave cases, the infection culminates in acute respiratory distress syndrome, which is associated with a high degree of mortality. Although the majority of symptomatic SARS-CoV-2 cases present with pulmonary symptoms, extra-pulmonary symptoms are also common, and several case studies have described the presence of digestive symptoms in the SARS-CoV-2 infection.

We identified and analyzed the GI symptoms of COVID-19 patients reported in nineteen published papers, which included diarrhea, nausea, abdominal pain, vomiting, anorexia, and bleeding (Table 1). Out of the nineteen papers, thirteen were

**Table 1 Clinical presentation of gastrointestinal symptoms among coronavirus disease 2019 patients, *n* (%)**

| Ref. | Number of patients | Diarrhea | Anorexia | Nausea | Vomiting | Abdominal pain | GI Bleeding |
|---|---|---|---|---|---|---|---|
| Ha *et al*[12] | 80 | 10 (12.5) | - | 6 (7.5) | 2 (2.5) | 7 (8.8) | 1 (1.3) |
| Jin *et al*[10] | 651 | 53 (8.1) | - | 10 (1.5) | 11 (1.7) | - | - |
| Lin *et al*[11] | 95 | 23 (24.2) | 17 (17.9) | 17 (17.9) | 4 (4.2) | 2 (2.1)[1] | 2 (2.1)[2] |
| Zhang *et al*[65] | 139 | 18 (12.9) | 17 (12.2) | 24 (17.3) | 7 (5) | 8 (5.8) | - |
| Wang *et al*[66] | 138 | 14 (10.1) | 55 (39.9) | 14 (10.1) | 5 (3.6) | 3 (2.2) | - |
| Chen *et al*[67] | 99 | 2(2) | - | 1 (1)[3] | | | |
| Chen *et al*[68] | 9 | 2 (22.2) | - | - | - | - | - |
| Young *et al*[13] | 18 | 3 (16.7) | - | - | - | - | - |
| Chang *et al*[69] | 13 | 1 (7.7) | - | - | - | - | - |
| Liu *et al*[70] | 137 | 11 (8) | - | - | - | - | - |
| Pan *et al*[71] | 204 | 35 (17.2) | 81 (39.7) | - | 4 (2) | 2 (1) | - |
| Wang *et al*[72] | 69 | 10 (14.5) | 7 (10.1) | - | 3 (4.3) | - | - |
| Yang *et al*[73] | 52 | - | - | - | 2 (3.8) | - | - |
| Spiteri *et al*[74] | 38 | 1 (2.6) | - | 1 (2.6) | - | - | - |
| Han *et al*[75] | 206 | 67 (32.5) | 32 (15.5) | - | 24 (11.7) | 9 (4.4) | - |
| Nobel *et al*[76] | 278 | 56 (20.1) | - | 63 (22.7)[3] | | | |
| Zhou *et al*[77] | 254 | 46 (18.1) | - | 21 (8.3) | 15 (5.9) | 3 (1.2) | - |
| Cholankeril *et al*[78] | 116 | 12 (10.3) | 22 (25.3) | 12 (10.3) | 5 (4.3) | 10 (8.8) | - |
| Redd *et al*[79] | 318 | 107 (33.7) | 110 (34.8) | 84 (26.4) | 49 (15.4) | 46 (14.5) | 2 (0.63) |
| Total | 2914 | 471 (16.2) | 341 (11.7) | 253 (8.7) | 131 (4.5) | 90 (3.1) | 5 (0.2) |

[1]Epigastric discomfort.
[2]Upper gastrointestinal hemorrhage.
[3]Nausea and vomiting. GI: Gastrointestinal.

from China, four were from the United States, one was from Singapore, and one was from Europe. Among GI symptoms, diarrhea was the most prevalent, accounting for 2% to 33.7% of all patients. The median duration period of diarrhea in COVID-19 patients was 4 d with a range of 1 d to 9 d[10]. Other frequently reported GI symptoms were anorexia (341/2914, 11.7%), nausea (253/2914, 8.7%), vomiting (131/2914, 4.5%), abdominal pain (90/2914, 3.1%) and bleeding (5/2914, 0.2%). GI symptoms were more frequently reported during hospitalization than at the time of admission[11]. We have also recently reported a strong correlation between diarrhea and the severity of the disease[12]. These data suggest that GI symptoms should be included in the assessment of the disease severity in COVID-19.

Previously, it was shown that RNA from SARS-CoV-2 was found in fecal samples (four out of eight patients) regardless of the presence of diarrhea[13]. Furthermore, another study demonstrated that SARS-CoV-2 RNA was found in the feces of 22/42 (52.4%) COVID-19 patients with GI symptoms. Among 23 COVID-19 patients without GI symptoms, SARS-CoV-2 RNA was found in the feces of 9 (39.1%) patients[11]. Although the clinical relevance of SARS-CoV-2 RNA in fecal material remains unclear, understanding the biochemical mechanisms behind the SARS-CoV-2 mediated induction of GI symptoms is important to gain further understanding of the pathophysiology of COVID-19. Therefore, we described potential mechanisms, by which GI symptoms might occur in COVID-19 patients and proposed new therapeutic strategies to modulate the global inflammatory response.

## PATHOPHYSIOLOGICAL MECHANISMS FOR GI SYMTOMS IN COVID-19

### *Intestinal ACE2 receptor mediated impaired barrier function*

ACE2 has emerged as a critical regulator of the renin angiotensin system (RAS) by metabolizing angiotensin (Ang) II into the beneficial peptide Ang 1-7[14]. ACE2 has also been identified as the key receptor for SARS-CoV and SARS-CoV-2[15]. Spike protein exposure led to increased Ang II and pulmonary edema, which was mediated by angiotensin II type I receptor (AT1R)[16]. Given the similarities between the SARS-CoV and SARS-CoV-2 spike proteins, a similar mechanism of spike-mediated ACE2 down-regulation most likely underlies tissue damage in COVID-19 by skewing the RAS[16].

The pathophysiology of GI symptoms in COVID-19 remains poorly understood. However, evidence points to a role of ACE2 cell-surface receptors and SARS-CoV-2 induced inflammatory processes in the GI tract[17]. A vital structural protein of SARS-CoV-2 is the spike glycoprotein (S). It consists of two functional units, S1 and S2, that bind to the host cell ACE2 receptor by membrane fusion, replicates through replication-transcription complexes, and promotes proliferation by interfering with and suppressing the host's immune response[18]. SARS-CoV-2 is highly concentrated in air droplets exhaled by infected subjects and inhalation of these particles by a noninfected individual may lead to infection of the recipient's respiratory tract *via* ACE2 receptors. The respiratory tract is one of the primary sites of viral entry. Interestingly, ACE2 receptors are also highly expressed in the digestive tract making it another potential route of SARS-CoV-2 infection[19]. In the gut, ACE2 has a completely different function independent of RAS. ACE2 stabilizes neutral amino acid transporters, such as B0AT1 and loss of ACE2 compromises intestinal uptake of certain dietary amino acids, such as tryptophan[20]. Because tryptophan plays an important role in immunity, ACE2 knockout mice exhibited altered gut microbiota and developed more severe dextran sulfate sodium–induced colitis compared with wild-type control mice[21]. These studies implicated ACE2 in SARS-CoV-2 infections in the gut.

ACE2 plays a major role in amino acid transport in the intestinal epithelium, a mechanism linked to the production of antimicrobial peptides, which suggests its role in intestinal barrier maintenance and gut microbiota equilibrium[22]. ACE2 controls expression of B0AT1 in the intestine, which is the primary apical membrane transporter in the intestine that permits $Na^+$ coupled uptake of neutral amino acids, such as tryptophan[23]. Notably, B0AT1 substrates, such as tryptophan and glutamine, signal to downregulate lymphoid pro-inflammatory cytokines, maintain the integrity of intestinal tight junctions, activate the release of antimicrobial peptides, and modulate mucosal cell autophagy as defense mechanisms[23]. Altered B0AT1 expression mediated through ACE2 in COVID-19 may be a major contributor to the leaky gut. Thus, it is possible that SARS-CoV-2 mediated disruption of the gut barrier could lead to a systemic elevation of bacterial lipopolysaccharide and peptidoglycan, further worsening GI inflammation. For instance, one study showed that the spike protein of SARS-CoV-2 (S1 subunit) interacted with the ACE2 complex and the tryptophan amino acid transporter B0AT1[24]. Furthermore, downregulated intestinal ACE2-B0AT1 cell surface expression led to a series of downstream sequelae to promote a leaky gut as well as gut microbiota dysbiosis[23,24]. Therefore, ACE2 mediated impaired barrier function in combination with microbial dysbiosis may contribute to the cytokine storm seen in patients severely ill with COVID-19 and may also be responsible for their GI symptoms.

### *Gut inflammation in COVID-19 patients with diarrhea*

Fecal calprotectin (FC) has evolved as a reliable fecal biomarker allowing detection of intestinal inflammation in inflammatory bowel disease (IBD) and infectious colitis[25]. Previous studies have shown that COVID-19 patients with diarrhea without IBD had high FC compared to patients without diarrhea, indicating that the infection evokes a significant intestinal inflammatory process[25]. Furthermore, FC levels correlated significantly with the pro-inflammatory interleukin - 6 (IL-6) serum concentrations, and a murine study showed that deficiency of ACE2 results in highly increased susceptibility to intestinal inflammation induced by epithelial damage[21]. Collectively, the aforementioned studies highlighted that GI inflammation was overrepresented in patients with COVID-19 that also had functional GI disorders (FGIDs) or post-infection (PI) GI disorders.

### *Alterations in serotonin metabolism in COVID-19 patients*

We have reported that plasma serotonin (5-hydroxytrytamine, 5-HT) levels were elevated in COVID-19 patients with diarrhea[12]. 5-HT is a hormone and neurotransmitter that has a monoamine structure. 5-HT synthesis begins with the amino acid L-tryptophan, which is converted to 5-hydroxytryptophan (5-HTP) *via* the rate-limiting enzyme tryptophan hydroxylase (TPH). 5-HTP is then rapidly decarboxylated by aromatic L-amino acid decarboxylase to produce 5-HT[26,27]. 5-HT either circulates in our body or is absorbed by the cells that express serotonin reuptake transporter to act or decompose, resulting in 5-hydroxyindoleacetic acid (5-HIAA)[28]. TPH is an enzyme specifically found in 5-HT producing cells, and there are two different isoforms, TPH1 and TPH2[29,30]. TPH1 dependent 5-HT synthesis occurs in enterochromaffin (EC) cells in GI tract, while TPH2 is involved in 5-HT synthesis in the central nervous system and enteric nervous system[31,32].

Since 95% of total 5-HT is produced by EC cells in GI tract, 5-HT has been widely studied for GI functions, especially in GI motility. Many studies have demonstrated that 5-HT is important for colonic peristaltic reflexes and GI transit[33-35]. Moreover, altered 5-HT levels are closely associated with irritable bowel syndrome (IBS), and it has been shown that platelet-depleted plasma 5-HT levels are increased in IBS patients with diarrhea[36]. Therefore, approaches to target 5-HT signaling have been proposed as a way to alleviate GI dysmotility. A total of seven classes of 5-HT receptors have been identified, and it is well-known that 5-$HT_1$, 5-$HT_2$, 5-$HT_3$, 5-$HT_4$, and 5-$HT_7$ are expressed in the GI tract to influence gut motor function[37]. 5-$HT_3$ antagonists are especially effective in treating IBS with diarrhea[38,39] and 5-$HT_4$ agonists are effective in treating IBS with constipation[40,41].

Previously, we have reported that plasma 5-HT levels are increased in COVID-19 patients and are directly correlated to the severity of COVID-19 symptoms. Moreover, COVID-19 patients with diarrhea had increased plasma 5-HT and a lower ratio of plasma 5-HIAA/5-HT levels compared to healthy subjects or COVID-19 patients without diarrhea[12]. These data suggest that 5-HT is not being broken down into 5-HIAA, and 5-HT remains in some COVID-19 patients' for a longer duration, resulting in GI symptoms such as diarrhea. Thus, regulating the amount of 5-HT might be a therapeutic modality for COVID-19 patients with diarrhea.

### *Gut microbiota dysbiosis in COVID-19 patients*

From ancient times, viral infectious diseases have been plaguing mankind through a wide-range of clinical manifestations. Moreover, scientific annals depict the occurrence of life-threatening viral diseases that are enumerated as epidemics and pandemics[42]. Examples include: The flu, polio, Ebola, acquired immune deficiency syndrome, and the very recent COVID-19. In 2020 the past several months, COVID-19 has reached pandemic status, exposing the world to iminent danger. Previously, two other similar viral infections including the Middle East respiratory syndrome virus and SARS-CoV have been reported[43]. SARS-CoV-2 is an enveloped virus in the *Coronaviridae* family. They harbor single stranded RNA as their genetic material that has positive polarity. Some studies published during the recent pandemic of COVID-19 have provided insight into parameters pertaining to the transmission, susceptibility, clinical presentation, and laboratory findings of this potential pathogen[44,45]. Although respiratory droplets and contact are the prime route of transmission for SARS-CoV2, there have been some instances where prolonged exposure to aerosols with high concentrations of the virus may facilitate transmission. Symptoms and severity of COVID-19 differ from patient to patient[46]. In general, humans of all ages are susceptible. However, individuals with an attenuated immune response including elderly, infants, children below 6 years old, patients with underlying diseases (transplants, cancers, diabetes, asthma, heart ailment, and other peril maladies) are at higher risk.

To inject their genetic material into the host, SARS-CoV-2 pierces the pulmonary epithelial cells of the lower respiratory tract thereby commandeering the host's cellular machinery[47]. Moreover, this process is enhanced by the spike (S) protein that interacts with ACE2[47,48]. Thus, the importance of the gut and its microbiome cannot be underestimated. The knowledge in gut research has augmented with a plethora of scientific annals that point towards the role of gut microbes in many degenerative and infectious diseases[49]. Gut dysbiosis has been reported in patients with COVID-19 with enrichment of pathogens and depletion of beneficial commensals[17]. An inverse correlation between the abundance of *Faecalibacterium prausnitzii* (*F. prausnitzii*) and disease severity has been observed. *F. prausnitzii* has anti-inflammatory properties, and its depletion has been related to IBS[17]. Another study showed the gut

microbiome composition was significantly altered in patients with COVID-19 compared with non-COVID-19 individuals irrespective of whether patients had received medication[50]. Several gut commensals with known immunomodulatory potential such as *F. prausnitzii*, *Eubacterium rectale* and Bifidobacteria were underrepresented in patients and remained hampered in samples collected up to 30 d after disease resolution[17,51]. Moreover, this perturbed composition exhibited stratification with disease severity concordant with elevated concentrations of inflammatory cytokines and blood markers such as C-reactive protein, lactate dehydrogenase, aspartate aminotransferase and gamma-glutamyl transferase[17]. The depletion of several bacterial species in the COVID-19 cohort was linked to increased concentrations of tumor necrosis factor-alpha, C-X-C motif chemokine ligand 10, C-C motif chemokine ligand 2 and IL-10. These studies highlighted the need to understand how gut microorganisms are involved in inflammation and COVID-19 pathogenesis[50].

Another study found a signature of active gut viral infection in a subset of patients with COVID-19 even in the absence of GI symptoms, suggesting a 'quiescent' GI infection of SARS-CoV-2[52]. The transcriptional activity of viral infection and replication persisted in the gut even after respiratory clearance of SARS-CoV-2. Fecal samples with a signature of high SARS-CoV-2 infectivity harbored a higher abundance of opportunistic pathogens, for instance, *Morganella morganii, Collinsella aerofaciens*, *Streptococcus infantis*, and *Collinsella tanakaei* and an enhanced capacity for the biosynthesis of nucleotides and amino acids, along with carbohydrate metabolism, whereas fecal samples with a signature of no SARS-CoV-2 infection had a higher abundance of short-chain fatty acid producing bacteria, for instance, *Bacteroides stercoris*, *Parabacteroides merdae*, *Lachnospiraceae bacterium*, and *Alistipes onderdonkii*[52]. This study provided evidence for active and prolonged 'quiescent' GI infection even in the absence of GI manifestations and after recovery from respiratory infection of SARS-CoV-2. The gut microbiota of patients with active SARS-CoV-2 GI infection was characterized by enrichment of opportunistic pathogens; loss of salutary bacteria and increased functional capacity for nucleotides, along with increased amino acid biosynthesis and carbohydrate metabolism[52].

In addition, bacterial groups belonging to the genus Bacteroides, known to downregulate the ACE2 expression in the murine colon, inversely correlated with fecal SARS-CoV-2 nucleic acid loads. Similarly, SARS-CoV-2 infection of GI epithelial cells has been associated with: (1) Lamina propria infiltration of plasma cells and lymphocytes, and edema in the stomach, duodenum, and rectum; (2) Increased levels of FC; (3) Higher fecal levels of IL-8 and lower levels of the anti-inflammatory IL-10 when compared with uninfected controls[53]; (4) SARS-CoV-2-specific IgA and limited inflammatory cytokines were also present in the stool of patients with acute COVID-19; and (5) Gut microbiota dysbiosis. Interestingly, gut microbiota dysbiosis persisted after the resolution of SARS-CoV-2 infection, suggesting that microbiota perturbation may contribute to the persistence of gut dysfunction and symptoms even after the infection has subsided. Indeed, the persistent microbial dysbiosis may contribute to maintaining a chronic state of low-grade GI inflammation, increased intestinal permeability, increased sensory perception, and bile acid malabsorption, which have all been previously associated with symptoms of GI motility disorders.

### Post-COVID-19 functional GI disorders

Evidence supports the development of FGIDs after a bout of viral, bacterial, or protozoal gastroenteritis or after resolution of an acute flareup of GI inflammatory diseases such as IBD[54]. Individual susceptibility to these so-called postinfectious functional gastrointestinal disorders (PI-FGIDs) involves genetic predisposition and the presence of pre-existing psychological disturbances such as anxiety and/or depression[55,56]. PI-FGIDs have also been associated with dysregulation of gut motility, visceral hypersensitivity, microbial dysbiosis, intestinal barrier dysfunction, bile acid malabsorption, and alterations in serotonin metabolism[54,57]. Current data suggest that the resolution of the SARS-CoV-2 infection may lead to persistent GI dysfunction resembling certain aspects of PI-FGIDs[17]. Transient non-specific gut inflammation is the common trigger for long-lasting symptoms of FGIDs, regardless of the initiating event (*i.e.*, viral, parasitic, bacterial, after resolution of IBD flares)[58].

### SARS-CoV-2 in stool: Suggesting fecal-oral transmission

Evidence of fecal shedding of viral RNA further supports viral replication in the digestive tract and potentially a fecal-oral route of transmission. Studies showed that more than one-half of COVID-19 patients tested positive for fecal SARS-CoV-2 RNA[59]. One study in a group of pediatric patients infected with SARS-CoV-2 had positive rectal swabs for SARS-CoV-2, even after the nasopharynx was cleared of the

virus, suggesting that viral shedding from the digestive tract might be more prolonged than that from the respiratory tract[60]. Another study showed that SARS-CoV-2 can infect the enterocytes of bats in an organoid culture system of bat intestinal epithelium [61]. One study indicated that infection by SARS-CoV-2 led to an altered fecal microbiome during hospitalization[62]. The authors showed enrichment of opportunistic pathogens and depletion of commensals during SARS-CoV-2 infection. *Coprobacillus*, *Clostridium ramosum*, and *Clostridum mathewayi* were found more commonly in patients with severe COVID-19. In contrast, the presence of *F. prausnitzii* was correlated with milder disease. Gut microbial dysbiosis persisted in the majority of COVID-19 patients in spite of clearance of the virus, suggesting that exposure to SARS-CoV-2 might be associated with more long-lasting deleterious effects to the healthy gut microbiome [23,62]. These studies support the possibility for SARS-CoV-2 fecal-oral route of transmission. Therefore, from both clinical and public health standpoints, it is critical to fully understand all routes of transmission of SARS-CoV-2. If high levels of infectious viruses are present in the intestinal lumen of infected patients, especially in asymptomatic patients, this poses risks during endoscopy and colonoscopy to gastroenterologists, endoscopy personnel and other patients. For the general public, infectious viral particles in the feces shed by infected individuals, if aerosolized, have great implications in confined environments such as cruise ships, hospitals, individual households, and densely populated housing, such as those in regions with poor sanitation[19].

## CONCLUSION

GI symptoms are overrepresented in patients with COVID-19. A proportion of patients affected by COVID-19 may develop PI-FGIDs based on the following pathophysiological mechanisms: Intestinal barrier dysfunction, chronic low-grade intestinal inflammation, altered serotonin metabolism, and gut microbiota dysbiosis. The question of whether gut inflammation is associated with gut microbiota dysbiosis in patients, which may have a central role in the COVID-19 disease progression warrants further investigation. However, there is mounting evidence that gut microorganisms are linked to GI inflammatory diseases, which highlights the urgent need to understand the specific roles of gut microorganisms that are responsible for the immune dysfunction and systemic inflammation in COVID-19.

The abundance of SARS-CoV-2 viral RNA in stool and the stability of the virus in the environment suggest that fecal contamination may be an important modality for the spread among human hosts. Fecal sources may lead to viral transmission, especially when aerosols are generated. The significance of GI involvement in COVID-19 patients requires attention in clinical practices, such as incorporation of rectal swab testing before discharging patients, as well as the importance of personal protective equipment in the endoscopy setting. These precautions will be imperative in our battle against COVID-19[63].

Considering the critical role of the ACE2 receptor in the pathogenesis of COVID-19 and the potential impact on severity of symptoms in some patients, several therapeutic approaches have been evaluated such as a soluble form of ACE2 (rhACE2), ACE2 blockers, TMPRSS2 inhibitors, and Ang 1-7 receptor agonists. Some of these therapeutic approaches appeared to show promising results and are currently in clinical trials. Another strategy to manage COVID-19 might be to restore the microbiota during the dysbiosis through prebiotic and/or probiotic interventions and dietary nutritional supplementation[64].

This review sheds light on the studies that formulate the pathophysiological mechanisms (impaired barrier function, gut inflammation, altered serotonin metabolism and gut microbiota dysbiosis) underlying GI symptoms in patients with COVID-19 (Figure 1). To the best of our knowledge we are the first to propose altered serotonin metabolism in the pathogenesis of COVID-19 associated with diarrhea. This novel insight of serotonin metabolism might be a key player underpinning GI symptoms and severity in patients with COVID-19 as altered serotonin signaling modulates the majority of pathological mechanisms in patients with FGIDs. Therapeutic modalities regulating serotonin signaling might offer potential treatment options in a subset of COVID-19 patients. Furthermore, we highlighted the important concept of post-SARS-CoV-2-FGIDs, which warrants future studies to dissect persistent GI symptoms after the clearance of SARS-CoV-2 infection. Scientists and clinicians should be aware of this new clinical scenario, and additional studies will be needed to further characterize and uncover the pathophysiological mechanisms of this

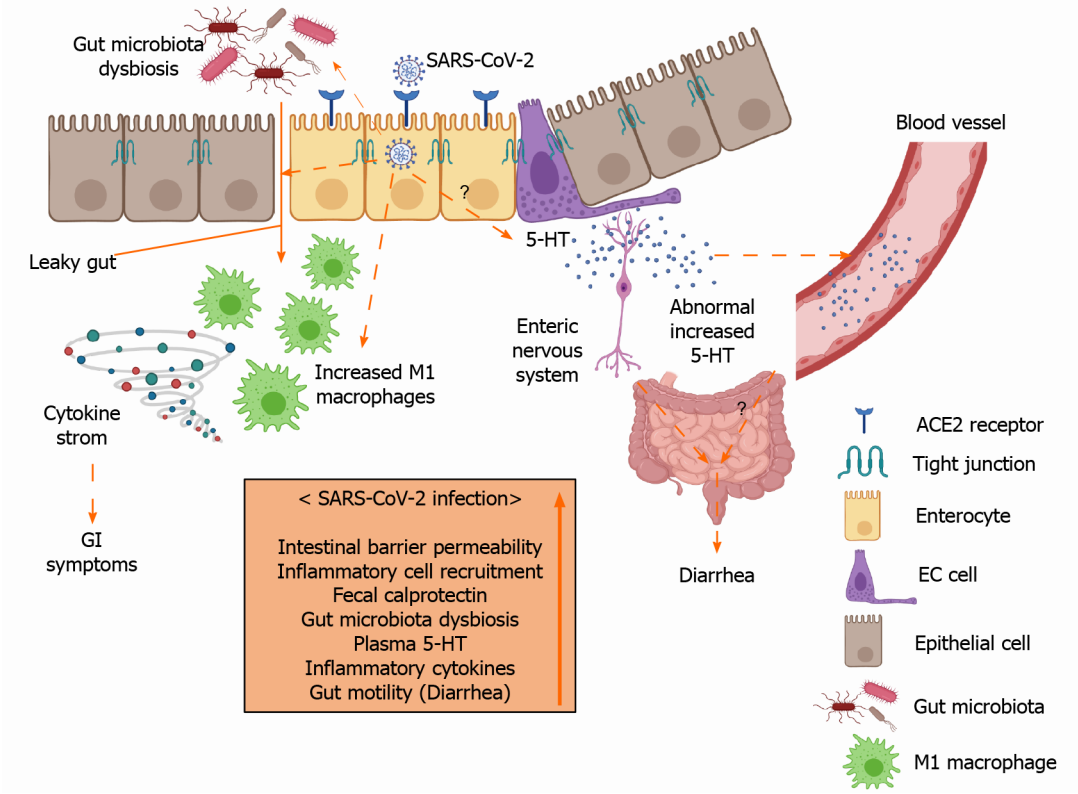



**Figure 1 A simplified diagram of the potential pathological mechanisms for gastrointestinal symptoms associated with severe acute respiratory syndrome coronavirus 2 infection.** The figure was created with BioRender.com. SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; 5-HT: 5-hydroxytrytamine; EC cell: Enterochromaffin; ACE2 cell: Angiotensin converting enzyme 2.

phenomenon. Furthermore, studies are warranted to elucidate the following: (1) the cause-and-effect relationship between changes in relative abundance of gut bacteria and COVID-19; (2) the possibility that the microbiota plays a role in illness severity; and (3) the relationship between the host's immune response (regulatory T cell response) to SARS-CoV-2 resulting in a high or low cytokine storm.

## REFERENCES


1 **Hui DS**, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A, Petersen E. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis* 2020; **91**: 264-266 [PMID: 31953166 DOI: 10.1016/j.ijid.2020.01.009]

2 **Lu H**, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. *J Med Virol* 2020; **92**: 401-402 [PMID: 31950516 DOI: 10.1002/jmv.25678]

3 **Bogoch II**, Watts A, Thomas-Bachli A, Huber C, Kraemer MUG, Khan K. Potential for global spread of a novel coronavirus from China. *J Travel Med* 2020; **27**: taaa011 [PMID: 31985790 DOI: 10.1093/jtm/taaa011]

4 **Bilinski A**, Emanuel EJ. COVID-19 and Excess All-Cause Mortality in the US and 18 Comparison Countries. *JAMA* 2020; **324**: 2100-2102 [PMID: 33044514 DOI: 10.1001/jama.2020.20717]

5 **Lu R**, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; **395**: 565-574 [PMID: 32007145 DOI: 10.1016/S0140-6736(20)30251-8]

6 **Wan Y**, Shang J, Graham R, Baric RS, Li F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J Virol* 2020; 94: e00127-20 [PMID: 31996437 DOI: 10.1128/JVI.00127-20]

7 **Dhama K**, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. Coronavirus Disease 2019-COVID-19. *Clin Microbiol Rev* 2020; **33**: e00028-20 [PMID: 32580969 DOI: 10.1128/CMR.00028-20]

8 **Vabret N**, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, Levantovsky R, Malle L, Moreira A,

Park MD, Pia L, Risson E, Saffern M, Salomé B, Esai Selvan M, Spindler MP, Tan J, van der Heide V, Gregory JK, Alexandropoulos K, Bhardwaj N, Brown BD, Greenbaum B, Gümüş ZH, Homann D, Horowitz A, Kamphorst AO, Curotto de Lafaille MA, Mehandru S, Merad M, Samstein RM; Sinai Immunology Review Project. Immunology of COVID-19: Current State of the Science. *Immunity* 2020; **52**: 910-941 [PMID: 32505227 DOI: 10.1016/j.immuni.2020.05.002]

9 **Machhi J**, Herskovitz J, Senan AM, Dutta D, Nath B, Oleynikov MD, Blomberg WR, Meigs DD, Hasan M, Patel M, Kline P, Chang RC, Chang L, Gendelman HE, Kevadiya BD. The Natural History, Pathobiology, and Clinical Manifestations of SARS-CoV-2 Infections. *J Neuroimmune Pharmacol* 2020; **15**: 359-386 [PMID: 32696264 DOI: 10.1007/s11481-020-09944-5]

10 **Jin X**, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, Hao SR, Jia HY, Cai H, Zhang XL, Yu GD, Xu KJ, Wang XY, Gu JQ, Zhang SY, Ye CY, Jin CL, Lu YF, Yu X, Yu XP, Huang JR, Xu KL, Ni Q, Yu CB, Zhu B, Li YT, Liu J, Zhao H, Zhang X, Yu L, Guo YZ, Su JW, Tao JJ, Lang GJ, Wu XX, Wu WR, Qv TT, Xiang DR, Yi P, Shi D, Chen Y, Ren Y, Qiu YQ, Li LJ, Sheng J, Yang Y. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020; **69**: 1002-1009 [PMID: 32213556 DOI: 10.1136/gutjnl-2020-320926]

11 **Lin L**, Jiang X, Zhang Z, Huang S, Fang Z, Gu Z, Gao L, Shi H, Mai L, Liu Y, Lin X, Lai R, Yan Z, Li X, Shan H. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020; **69**: 997-1001 [PMID: 32241899 DOI: 10.1136/gutjnl-2020-321013]

12 **Ha S**, Jin B, Clemmensen B, Park P, Mahboob S, Gladwill V, Lovely FM, Gottfried-Blackmore A, Habtezion A, Verma S, Ro S. Serotonin is elevated in COVID-19-associated diarrhoea. *Gut* 2021; epub ahead of print [PMID: 33402416 DOI: 10.1136/gutjnl-2020-323542]

13 **Young BE**, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, Ng OT, Marimuthu K, Ang LW, Mak TM, Lau SK, Anderson DE, Chan KS, Tan TY, Ng TY, Cui L, Said Z, Kurupatham L, Chen MI, Chan M, Vasoo S, Wang LF, Tan BH, Lin RTP, Lee VJM, Leo YS, Lye DC; Singapore 2019 Novel Coronavirus Outbreak Research Team. Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. *JAMA* 2020; **323**: 1488-1494 [PMID: 32125362 DOI: 10.1001/jama.2020.3204]

14 **Gheblawi M**, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, Raizada MK, Grant MB, Oudit GY. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. *Circ Res* 2020; **126**: 1456-1474 [PMID: 32264791 DOI: 10.1161/CIRCRESAHA.120.317015]

15 **Zhang H**, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med* 2020; **46**: 586-590 [PMID: 32125455 DOI: 10.1007/s00134-020-05985-9]

16 **Ni W**, Yang X, Yang D, Bao J, Li R, Xiao Y, Hou C, Wang H, Liu J, Xu Y, Cao Z, Gao Z. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit Care* 2020; **24**: 422 [PMID: 32660650 DOI: 10.1186/s13054-020-03120-0]

17 **Schmulson M**, Ghoshal UC, Barbara G. Managing the Inevitable Surge of Post-COVID-19 Functional Gastrointestinal Disorders. *Am J Gastroenterol* 2021; **116**: 4-7 [PMID: 33273261 DOI: 10.14309/ajg.0000000000001062]

18 **Xu X**, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci* 2020; **63**: 457-460 [PMID: 32009228 DOI: 10.1007/s11427-020-1637-5]

19 **Ding S**, Liang TJ. Is SARS-CoV-2 Also an Enteric Pathogen With Potential Fecal-Oral Transmission? *Gastroenterology* 2020; **159**: 53-61 [PMID: 32353371 DOI: 10.1053/j.gastro.2020.04.052]

20 **Camargo SMR**, Vuille-Dit-Bille RN, Meier CF, Verrey F. ACE2 and gut amino acid transport. *Clin Sci (Lond)* 2020; **134**: 2823-2833 [PMID: 33140827 DOI: 10.1042/CS20200477]

21 **Hashimoto T**, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, Wild B, Camargo SM, Singer D, Richter A, Kuba K, Fukamizu A, Schreiber S, Clevers H, Verrey F, Rosenstiel P, Penninger JM. ACE2 Links amino acid malnutrition to microbial ecology and intestinal inflammation. *Nature* 2012; **487**: 477-481 [PMID: 22837003 DOI: 10.1038/nature11228]

22 **Perlot T**, Penninger JM. ACE2 - from the renin-angiotensin system to gut microbiota and malnutrition. *Microbes Infect* 2013; **15**: 866-873 [PMID: 23962453 DOI: 10.1016/j.micinf.2013.08.003]

23 **Penninger JM**, Grant MB, Sung JJY. The Role of Angiotensin Converting Enzyme 2 in Modulating Gut Microbiota, Intestinal Inflammation, and Coronavirus Infection. *Gastroenterology* 2021; **160**: 39-46 [PMID: 33130103 DOI: 10.1053/j.gastro.2020.07.067]

24 **Yan R**, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020; **367**: 1444-1448 [PMID: 32132184 DOI: 10.1126/science.abb2762]

25 **Effenberger M**, Grabherr F, Mayr L, Schwaerzler J, Nairz M, Seifert M, Hilbe R, Seiwald S, Scholl-Buergi S, Fritsche G, Bellmann-Weiler R, Weiss G, Müller T, Adolph TE, Tilg H. Faecal calprotectin indicates intestinal inflammation in COVID-19. *Gut* 2020; **69**: 1543-1544 [PMID: 32312790 DOI: 10.1136/gutjnl-2020-321388]

26 **Boadle-Biber MC**. Regulation of serotonin synthesis. *Prog Biophys Mol Biol* 1993; **60**: 1-15 [PMID: 8480026 DOI: 10.1016/0079-6107(93)90009-9]

27 **Höglund E**, Øverli Ø, Winberg S. Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review. *Front Endocrinol (Lausanne)* 2019; **10**: 158 [PMID: 31024440 DOI: 10.3389/fendo.2019.00158]

28 **Bian X**, Patel B, Dai X, Galligan JJ, Swain G. High mucosal serotonin availability in neonatal guinea pig ileum is associated with low serotonin transporter expression. *Gastroenterology* 2007; **132**: 2438-2447 [PMID: 17570217 DOI: 10.1053/j.gastro.2007.03.103]

29 **Côté F**, Thévenot E, Fligny C, Fromes Y, Darmon M, Ripoche MA, Bayard E, Hanoun N, Saurini F, Lechat P, Dandolo L, Hamon M, Mallet J, Vodjdani G. Disruption of the nonneuronal tph1 gene demonstrates the importance of peripheral serotonin in cardiac function. *Proc Natl Acad Sci USA* 2003; **100**: 13525-13530 [PMID: 14597720 DOI: 10.1073/pnas.2233056100]

30 **Walther DJ**, Peter JU, Bashammakh S, Hörtnagl H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 2003; **299**: 76 [PMID: 12511643 DOI: 10.1126/science.1078197]

31 **Bellono NW**, Bayrer JR, Leitch DB, Castro J, Zhang C, O'Donnell TA, Brierley SM, Ingraham HA, Julius D. Enterochromaffin Cells Are Gut Chemosensors that Couple to Sensory Neural Pathways. *Cell* 2017; **170**: 185-198. e16 [PMID: 28648659 DOI: 10.1016/j.cell.2017.05.034]

32 **Israelyan N**, Del Colle A, Li Z, Park Y, Xing A, Jacobsen JPR, Luna RA, Jensen DD, Madra M, Saurman V, Rahim R, Latorre R, Law K, Carson W, Bunnett NW, Caron MG, Margolis KG. Effects of Serotonin and Slow-Release 5-Hydroxytryptophan on Gastrointestinal Motility in a Mouse Model of Depression. *Gastroenterology* 2019; **157**: 507-521. e4 [PMID: 31071306 DOI: 10.1053/j.gastro.2019.04.022]

33 **Li Z**, Chalazonitis A, Huang YY, Mann JJ, Margolis KG, Yang QM, Kim DO, Côté F, Mallet J, Gershon MD. Essential roles of enteric neuronal serotonin in gastrointestinal motility and the development/survival of enteric dopaminergic neurons. *J Neurosci* 2011; **31**: 8998-9009 [PMID: 21677183 DOI: 10.1523/JNEUROSCI.6684-10.2011]

34 **Heredia DJ**, Gershon MD, Koh SD, Corrigan RD, Okamoto T, Smith TK. Important role of mucosal serotonin in colonic propulsion and peristaltic reflexes: *in vitro* analyses in mice lacking tryptophan hydroxylase 1. *J Physiol* 2013; **591**: 5939-5957 [PMID: 24127620 DOI: 10.1113/jphysiol.2013.256230]

35 **Heredia DJ**, Dickson EJ, Bayguinov PO, Hennig GW, Smith TK. Localized release of serotonin (5-hydroxytryptamine) by a fecal pellet regulates migrating motor complexes in murine colon. *Gastroenterology* 2009; **136**: 1328-1338 [PMID: 19138686 DOI: 10.1053/j.gastro.2008.12.010]

36 **Atkinson W**, Lockhart S, Whorwell PJ, Keevil B, Houghton LA. Altered 5-hydroxytryptamine signaling in patients with constipation- and diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2006; **130**: 34-43 [PMID: 16401466 DOI: 10.1053/j.gastro.2005.09.031]

37 **De Ponti F**. Pharmacology of serotonin: what a clinician should know. *Gut* 2004; **53**: 1520-1535 [PMID: 15361507 DOI: 10.1136/gut.2003.035568]

38 **Gershon MD**, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. *Gastroenterology* 2007; **132**: 397-414 [PMID: 17241888 DOI: 10.1053/j.gastro.2006.11.002]

39 **Fayyaz M**, Lackner JM. Serotonin receptor modulators in the treatment of irritable bowel syndrome. *Ther Clin Risk Manag* 2008; **4**: 41-48 [PMID: 18728719 DOI: 10.2147/tcrm.s140]

40 **Sullivan KL**, Staffetti JF, Hauser RA, Dunne PB, Zesiewicz TA. Tegaserod (Zelnorm) for the treatment of constipation in Parkinson's disease. *Mov Disord* 2006; **21**: 115-116 [PMID: 16142776 DOI: 10.1002/mds.20666]

41 **Di Palma JA**, Cleveland MV, McGowan J, Herrera JL. A randomized, multicenter comparison of polyethylene glycol laxative and tegaserod in treatment of patients with chronic constipation. *Am J Gastroenterol* 2007; **102**: 1964-1971 [PMID: 17573794 DOI: 10.1111/j.1572-0241.2007.01365.x]

42 **Bloom DE**, Cadarette D. Infectious Disease Threats in the Twenty-First Century: Strengthening the Global Response. *Front Immunol* 2019; **10**: 549 [PMID: 30984169 DOI: 10.3389/fimmu.2019.00549]

43 **Elfiky AA**. SARS-CoV-2 Spike-Heat Shock Protein A5 (GRP78) Recognition may be Related to the Immersed Human Coronaviruses. *Front Pharmacol* 2020; **11**: 577467 [PMID: 33362542 DOI: 10.3389/fphar.2020.577467]

44 **Kakodkar P**, Kaka N, Baig MN. A Comprehensive Literature Review on the Clinical Presentation, and Management of the Pandemic Coronavirus Disease 2019 (COVID-19). *Cureus* 2020; **12**: e7560 [PMID: 32269893 DOI: 10.7759/cureus.7560]

45 **Voto C**, Berkner P, Brenner C. Overview of the Pathogenesis and Treatment of SARS-CoV-2 for Clinicians: A Comprehensive Literature Review. *Cureus* 2020; **12**: e10357 [PMID: 33062480 DOI: 10.7759/cureus.10357]

46 **Shah SJ**, Barish PN, Prasad PA, Kistler A, Neff N, Kamm J, Li LM, Chiu CY, Babik JM, Fang MC, Abe-Jones Y, Alipanah N, Alvarez FN, Botvinnik OB, Castaneda G; CZB CLIAhub Consortium, Dadasovich RM, Davis J, Deng X, DeRisi JL, Detweiler AM, Federman S, Haliburton J, Hao S, Kerkhoff AD, Kumar GR, Malcolm KB, Mann SA, Martinez S, Mary RK, Mick E, Mwakibete L, Najafi N, Peluso MJ, Phelps M, Pisco AO, Ratnasiri K, Rubio LA, Sellas A, Sherwood KD, Sheu J, Spottiswoode N, Tan M, Yu G, Kangelaris KN, Langelier C. Clinical features, diagnostics, and outcomes of patients presenting with acute respiratory illness: A retrospective cohort study of patients with and without COVID-19. *EClinicalMedicine* 2020; **27**: 100518 [PMID: 32864588 DOI: 10.1016/j.eclinm.2020.100518]

47 **Xu J**, Xu X, Jiang L, Dua K, Hansbro PM, Liu G. SARS-CoV-2 induces transcriptional signatures in

human lung epithelial cells that promote lung fibrosis. *Respir Res* 2020; **21**: 182 [PMID: 32664949 DOI: 10.1186/s12931-020-01445-6]

48 **Carvalho T**. Extrapulmonary SARS-CoV-2 manifestations. *Nat Med* 2020; **26**: 1806 [PMID: 33288941 DOI: 10.1038/s41591-020-01162-z]

49 **Villapol S**. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. *Transl Res* 2020; **226**: 57-69 [PMID: 32827705 DOI: 10.1016/j.trsl.2020.08.004]

50 **Yeoh YK**, Zuo T, Lui GC, Zhang F, Liu Q, Li AY, Chung AC, Cheung CP, Tso EY, Fung KS, Chan V, Ling L, Joynt G, Hui DS, Chow KM, Ng SSS, Li TC, Ng RW, Yip TC, Wong GL, Chan FK, Wong CK, Chan PK, Ng SC. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut* 2021; **70**: 698-706 [PMID: 33431578 DOI: 10.1136/gutjnl-2020-323020]

51 **Lordan C**, Thapa D, Ross RP, Cotter PD. Potential for enriching next-generation health-promoting gut bacteria through prebiotics and other dietary components. *Gut Microbes* 2020; **11**: 1-20 [PMID: 31116628 DOI: 10.1080/19490976.2019.1613124]

52 **Zuo T**, Liu Q, Zhang F, Lui GC, Tso EY, Yeoh YK, Chen Z, Boon SS, Chan FK, Chan PK, Ng SC. Depicting SARS-CoV-2 faecal viral activity in association with gut microbiota composition in patients with COVID-19. *Gut* 2021; **70**: 276-284 [PMID: 32690600 DOI: 10.1136/gutjnl-2020-322294]

53 **Britton GJ**, Chen-Liaw A, Cossarini F, Livanos AE, Spindler MP, Plitt T, Eggers J, Mogno I, Gonzalez-Reiche A, Siu S, Tankelevich M, Grinspan L, Dixon RE, Jha D, Martinez-Delgado G, Amanat F, Hoagland DA, tenOever B, Dubinsky MC, Merad M, van Bakel H, Krammer F, Bongers G, Mehandru S, Faith JJ. SARS-CoV-2-specific IgA and limited inflammatory cytokines are present in the stool of select patients with acute COVID-19. 2020 Preprint. Available from: medRxiv [PMID: 32909002 DOI: 10.1101/2020.09.03.20183947]

54 **Barbara G**, Grover M, Bercik P, Corsetti M, Ghoshal UC, Ohman L, Rajilić-Stojanović M. Rome Foundation Working Team Report on Post-Infection Irritable Bowel Syndrome. *Gastroenterology* 2019; **156**: 46-58. e7 [PMID: 30009817 DOI: 10.1053/j.gastro.2018.07.011]

55 **Barbara G**, Cremon C, Stanghellini V. Inflammatory bowel disease and irritable bowel syndrome: similarities and differences. *Curr Opin Gastroenterol* 2014; **30**: 352-358 [PMID: 24811054 DOI: 10.1097/MOG.0000000000000070]

56 **Marshall JK**, Thabane M, Borgaonkar MR, James C. Postinfectious irritable bowel syndrome after a food-borne outbreak of acute gastroenteritis attributed to a viral pathogen. *Clin Gastroenterol Hepatol* 2007; **5**: 457-460 [PMID: 17289440 DOI: 10.1016/j.cgh.2006.11.025]

57 **Ghoshal UC**, Rahman MM. Post-infection irritable bowel syndrome in the tropical and subtropical regions: Vibrio cholerae is a new cause of this well-known condition. *Indian J Gastroenterol* 2019; **38**: 87-94 [PMID: 31073702 DOI: 10.1007/s12664-019-00959-2]

58 **Barbara G**, Feinle-Bisset C, Ghoshal UC, Quigley EM, Santos J, Vanner S, Vergnolle N, Zoetendal EG. The Intestinal Microenvironment and Functional Gastrointestinal Disorders. *Gastroenterology* 2016; **150**: 1305-1318 [PMID: 27144620 DOI: 10.1053/j.gastro.2016.02.028]

59 **Gupta S**, Parker J, Smits S, Underwood J, Dolwani S. Persistent viral shedding of SARS-CoV-2 in faeces - a rapid review. *Colorectal Dis* 2020; **22**: 611-620 [PMID: 32418307 DOI: 10.1111/codi.15138]

60 **Donà D**, Minotti C, Costenaro P, Da Dalt L, Giaquinto C. Fecal-Oral Transmission of SARS-CoV-2 In Children: is it Time to Change Our Approach? *Pediatr Infect Dis J* 2020; **39**: e133-e134 [PMID: 32304466 DOI: 10.1097/INF.0000000000002704]

61 **Zhou J**, Li C, Liu X, Chiu MC, Zhao X, Wang D, Wei Y, Lee A, Zhang AJ, Chu H, Cai JP, Yip CC, Chan IH, Wong KK, Tsang OT, Chan KH, Chan JF, To KK, Chen H, Yuen KY. Infection of bat and human intestinal organoids by SARS-CoV-2. *Nat Med* 2020; **26**: 1077-1083 [PMID: 32405028 DOI: 10.1038/s41591-020-0912-6]

62 **Zuo T**, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, Wan Y, Chung ACK, Cheung CP, Chen N, Lai CKC, Chen Z, Tso EYK, Fung KSC, Chan V, Ling L, Joynt G, Hui DSC, Chan FKL, Chan PKS, Ng SC. Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization. *Gastroenterology* 2020; **159**: 944-955. e8 [PMID: 32442562 DOI: 10.1053/j.gastro.2020.05.048]

63 **Goh KL**, Chuah KH. COVID-19 and the digestive system: More than just a "flu". *JGH Open* 2020; **4**: 318-319 [PMID: 32514430 DOI: 10.1002/jgh3.12364]

64 **Viana SD**, Nunes S, Reis F. ACE2 imbalance as a key player for the poor outcomes in COVID-19 patients with age-related comorbidities - Role of gut microbiota dysbiosis. *Ageing Res Rev* 2020; **62**: 101123 [PMID: 32683039 DOI: 10.1016/j.arr.2020.101123]

65 **Zhang JJ**, Dong X, Cao YY, Yuan YD, Yang YB, Yan YQ, Akdis CA, Gao YD. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy* 2020; **75**: 1730-1741 [PMID: 32077115 DOI: 10.1111/all.14238]

66 **Wang D**, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020; **323**: 1061-1069 [PMID: 32031570 DOI: 10.1001/jama.2020.1585]

67 **Chen N**, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020; **395**: 507-513 [PMID: 32007143 DOI: 10.1016/S0140-6736(20)30211-7]

68 **Chen Q**, Quan B, Li X, Gao G, Zheng W, Zhang J, Zhang Z, Liu C, Li L, Wang C, Zhang G, Li J, Dai Y, Yang J, Han W. A report of clinical diagnosis and treatment of nine cases of coronavirus disease 2019. *J Med Virol* 2020; **92**: 683-687 [PMID: 32162699 DOI: 10.1002/jmv.25755]

69 **Chang**, Lin M, Wei L, Xie L, Zhu G, Dela Cruz CS, Sharma L. Epidemiologic and Clinical Characteristics of Novel Coronavirus Infections Involving 13 Patients Outside Wuhan, China. *JAMA* 2020; **323**: 1092-1093 [PMID: 32031568 DOI: 10.1001/jama.2020.1623]

70 **Liu K**, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, Li GC, Liu HG. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl)* 2020; **133**: 1025-1031 [PMID: 32044814 DOI: 10.1097/CM9.0000000000000744]

71 **Pan L**, Mu M, Yang P, Sun Y, Wang R, Yan J, Li P, Hu B, Wang J, Hu C, Jin Y, Niu X, Ping R, Du Y, Li T, Xu G, Hu Q, Tu L. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *Am J Gastroenterol* 2020; **115**: 766-773 [PMID: 32287140 DOI: 10.14309/ajg.0000000000000620]

72 **Wang Z**, Yang B, Li Q, Wen L, Zhang R. Clinical Features of 69 Cases With Coronavirus Disease 2019 in Wuhan, China. *Clin Infect Dis* 2020; **71**: 769-777 [PMID: 32176772 DOI: 10.1093/cid/ciaa272]

73 **Yang X**, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; **8**: 475-481 [PMID: 32105632 DOI: 10.1016/S2213-2600(20)30079-5]

74 **Spiteri G**, Fielding J, Diercke M, Campese C, Enouf V, Gaymard A, Bella A, Sognamiglio P, Sierra Moros MJ, Riutort AN, Demina YV, Mahieu R, Broas M, Bengnér M, Buda S, Schilling J, Filleul L, Lepoutre A, Saura C, Mailles A, Levy-Bruhl D, Coignard B, Bernard-Stoecklin S, Behillil S, van der Werf S, Valette M, Lina B, Riccardo F, Nicastri E, Casas I, Larrauri A, Salom Castell M, Pozo F, Maksyutov RA, Martin C, Van Ranst M, Bossuyt N, Siira L, Sane J, Tegmark-Wisell K, Palmérus M, Broberg EK, Beauté J, Jorgensen P, Bundle N, Pereyaslov D, Adlhoch C, Pukkila J, Pebody R, Olsen S, Ciancio BC. First cases of coronavirus disease 2019 (COVID-19) in the WHO European Region, 24 January to 21 February 2020. *Euro Surveill* 2020; **25** [PMID: 32156327 DOI: 10.2807/1560-7917.ES.2020.25.9.2000178]

75 **Han C**, Duan C, Zhang S, Spiegel B, Shi H, Wang W, Zhang L, Lin R, Liu J, Ding Z, Hou X. Digestive Symptoms in COVID-19 Patients With Mild Disease Severity: Clinical Presentation, Stool Viral RNA Testing, and Outcomes. *Am J Gastroenterol* 2020; **115**: 916-923 [PMID: 32301761 DOI: 10.14309/ajg.0000000000000664]

76 **Nobel YR**, Phipps M, Zucker J, Lebwohl B, Wang TC, Sobieszczyk ME, Freedberg DE. Gastrointestinal Symptoms and Coronavirus Disease 2019: A Case-Control Study From the United States. *Gastroenterology* 2020; **159**: 373-375. e2 [PMID: 32294477 DOI: 10.1053/j.gastro.2020.04.017]

77 **Zhou Z**, Zhao N, Shu Y, Han S, Chen B, Shu X. Effect of Gastrointestinal Symptoms in Patients With COVID-19. *Gastroenterology* 2020; **158**: 2294-2297 [PMID: 32199880 DOI: 10.1053/j.gastro.2020.03.020]

78 **Cholankeril G**, Podboy A, Aivaliotis VI, Tarlow B, Pham EA, Spencer SP, Kim D, Hsing A, Ahmed A. High Prevalence of Concurrent Gastrointestinal Manifestations in Patients With Severe Acute Respiratory Syndrome Coronavirus 2: Early Experience From California. *Gastroenterology* 2020; **159**: 775-777 [PMID: 32283101 DOI: 10.1053/j.gastro.2020.04.008]

79 **Redd WD**, Zhou JC, Hathorn KE, McCarty TR, Bazarbashi AN, Thompson CC, Shen L, Chan WW. Prevalence and Characteristics of Gastrointestinal Symptoms in Patients With Severe Acute Respiratory Syndrome Coronavirus 2 Infection in the United States: A Multicenter Cohort Study. *Gastroenterology* 2020; **159**: 765-767. e2 [PMID: 32333911 DOI: 10.1053/j.gastro.2020.04.045]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2353-2365

DOI: 10.3748/wjg.v27.i19.2353

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

ORIGINAL ARTICLE

Retrospective Cohort Study

# Risk factors and prognostic value of acute severe lower gastrointestinal bleeding in Crohn's disease

Jiyoung Yoon, Dae Sung Kim, Ye-Jee Kim, Jin Wook Lee, Seung Wook Hong, Ha Won Hwang, Sung Wook Hwang, Sang Hyoung Park, Dong-Hoon Yang, Byong Duk Ye, Jeong-Sik Byeon, Seung-Jae Myung, Suk-Kyun Yang

**ORCID number:** Jiyoung Yoon 0000-0001-7448-4296; Dae Sung Kim 0000-0002-4881-7883; Ye-Jee Kim 0000-0002-3307-2970; Jin Wook Lee 0000-0002-5312-4053; Seung Wook Hong 0000-0003-1440-9950; Ha Won Hwang 0000-0002-4065-5058; Sung Wook Hwang 0000-0002-6981-7575; Sang Hyoung Park 0000-0002-5366-5749; Dong-Hoon Yang 0000-0001-7756-2704; Byong Duk Ye 0000-0001-6647-6325; Jeong-Sik Byeon 0000-0002-9793-6379; Seung-Jae Myung 0000-0003-0585-4016; Suk-Kyun Yang 0000-0003-2772-2575.

**Author contributions:** Yoon J and Kim DS equally contributed to this article; Yoon J and Kim DS collected and analyzed data, and drafted the manuscript; Kim YJ supervised the research and contributed to analysis; Lee JW, Hong SW, Hwang HW, Hwang SW, Park SH, Yang DH, Ye BD, Byeon JS, and Myung SJ were involved in study patient enrollment, data collection, and data analysis; Yang SK was involved in study design, supervision, and critical revision of the study; all authors read and approved the final version of the manuscript.

**Institutional review board statement:** The study was

**Jiyoung Yoon, Dae Sung Kim, Jin Wook Lee, Seung Wook Hong, Ha Won Hwang, Sung Wook Hwang, Sang Hyoung Park, Dong-Hoon Yang, Byong Duk Ye, Jeong-Sik Byeon, Seung-Jae Myung, Suk-Kyun Yang,** Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, South Korea

**Ye-Jee Kim,** Department of Clinical Epidemiology and Biostatistics, Asan Medical Center, University of Ulsan College of Medicine, Seoul 05505, South Korea

**Corresponding author:** Suk-Kyun Yang, PhD, Professor, Department of Gastroenterology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, South Korea. sky@amc.seoul.kr

## Abstract

### *BACKGROUND*

Acute severe lower gastrointestinal bleeding (LGIB) is an uncommon but challenging complication of Crohn's disease (CD).

### *AIM*

To identify the predictors of acute severe LGIB and to evaluate the impact of acute severe LGIB on the subsequent clinical course in CD patients.

### *METHODS*

A retrospective inception cohort study was conducted in 75 CD patients with acute severe LGIB and 1359 CD patients without acute severe LGIB who were diagnosed between February 1991 and November 2019 at Asan Medical Center, a tertiary university hospital in Korea. Multivariable analysis with Cox proportional hazard regression was performed to identify the risk factors for acute severe LGIB. A matched analysis using 72 patients with bleeding and 267 matched patients without within the cohort was also conducted to investigate whether acute severe LGIB is a predictor of clinical outcomes of CD.

### *RESULTS*

Multivariable Cox regression analysis revealed that early use of thiopurines [hazard ratio (HR): 0.23, 95% confidence interval (CI): 0.12-0.48; $P < 0.001$] and female sex (HR: 0.51, 95%CI: 0.27-0.94; $P = 0.031$) were significantly associated

reviewed and approved by the Institutional Review Board of Asan Medical Center, No. 2020-0814.

**Informed consent statement:** The requirement for informed consent was waived by the Institutional Review Board considering the retrospective design of the study.

**Conflict-of-interest statement:** All authors have no conflict of interest related to the study.

**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. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** South Korea

**Peer-review report's scientific quality classification**

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

**Received:** February 5, 2021
**Peer-review started:** February 5, 2021
**First decision:** February 27, 2021

with a lower risk of acute severe LGIB. The cumulative risks of behavioral progression and intestinal resection were not significantly different between the two matched groups ($P$ = 0.139 and $P$ = 0.769, respectively). The hospitalization rate was higher in the bleeding group than in the matched non-bleeding group (22.1/100 *vs* 13.2/100 patient-years; $P$ = 0.012). However, if hospitalizations due to bleeding episodes were excluded from the analysis, the hospitalization rate was not significantly different between the bleeding group and the matched non-bleeding group (14.5/100 *vs* 13.2/100 patient-years; $P$ = 0.631).

*CONCLUSION*
Early use of thiopurines may reduce the risk of acute severe LGIB. History of acute severe LGIB may not have a significant prognostic value in patients with CD.

**Key Words:** Gastrointestinal hemorrhage; Lower gastrointestinal tract; Crohn's disease; Risk factors; Cohort studies; Clinical course

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** A retrospective cohort study was conducted to identify the predictors of acute severe lower gastrointestinal bleeding (LGIB) in Crohn's disease (CD) and the impact of acute severe LGIB on the subsequent clinical course thereof. In multivariable analysis, early use of thiopurines and female sex were associated with a lower risk of acute severe LGIB. Moreover, matched analyses within the cohort demonstrated that the risks of behavioral progression, intestinal resection, and hospitalization due to non-bleeding causes did not significantly differ according to bleeding, which suggests that a history of acute severe LGIB may not have a significant prognostic value in CD.

**Citation:** Yoon J, Kim DS, Kim YJ, Lee JW, Hong SW, Hwang HW, Hwang SW, Park SH, Yang DH, Ye BD, Byeon JS, Myung SJ, Yang SK. Risk factors and prognostic value of acute severe lower gastrointestinal bleeding in Crohn's disease. *World J Gastroenterol* 2021; 27(19): 2353-2365
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2353.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2353

## INTRODUCTION

Acute severe lower gastrointestinal bleeding (LGIB) is an uncommon manifestation that occurs in 0.6%-6.0% of patients with Crohn's disease (CD); however, it is a challenging problem with high rates of recurrence (21.4%-41.4%)[1-5], surgery (7.1%-39.7%)[1-4], and mortality (0%-8.2%)[2,3]. Despite this clinical importance, acute severe LGIB in CD has not been well-studied. Two studies have evaluated the risk factors for acute severe LGIB[1,3]; however, because the patient populations in these studies were not inception cohorts, it is still unknown what characteristics at the time of CD diagnosis predispose a patient to acute severe LGIB. Moreover, no previous studies have evaluated the impact of acute severe LGIB on the subsequent clinical course of CD. Therefore, by using a well-defined hospital-based inception cohort, we aimed to identify the risk factors for acute severe LGIB in CD and to investigate whether a history of acute severe LGIB is a predictor of a worse clinical course for CD.

## MATERIALS AND METHODS

### *Patients*

Between February 1991 and November 2019, a total of 4010 patients with CD were registered at the Inflammatory Bowel Disease (IBD) Center of Asan Medical Center, a tertiary university hospital in Seoul, Korea. Of these 4010 patients, 1437 had been first diagnosed and/or first treated with CD at Asan Medical Center. Among them, 78

**Revised:** March 5, 2021
**Accepted:** April 25, 2021
**Article in press:** April 25, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Brisinda G, Hosoe N, Shoreibah M
**S-Editor:** Fan JR
**L-Editor:** A
**P-Editor:** Ma YJ

patients experienced acute severe gastrointestinal (GI) bleeding and 1359 patients did not. Of the 78 patients, 75 with acute severe LGIB were finally selected for this study after those with postoperative bleeding ($n$ = 1), upper GI bleeding ($n$ = 1), or anal bleeding ($n$ = 1) were excluded.

To identify the risk factors for acute severe LGIB, we performed a retrospective cohort study in the 75 patients with acute severe LGIB and 1359 patients without acute severe LGIB. In addition, to investigate whether acute severe LGIB is a predictor of a worse clinical course of CD, a matched cohort analysis was performed by matching patients without acute severe LGIB to those with acute severe LGIB at a ratio of 4:1 in terms of sex, age at CD diagnosis (± 5 years), calendar year of CD diagnosis (± 5 years), and disease location and behavior at CD diagnosis. According to the matching conditions, a total of 72 patients with acute severe LGIB were matched to 267 patients without. The selection process for our study population is shown as a flowchart (Figure 1). The study protocol was approved by the Institutional Review Board of Asan Medical Center.

### Data collection

The patients' demographic and clinical information were retrieved from the Asan IBD Registry, which has been prospectively maintained since 1997. Information missing in the IBD registry was obtained by reviewing the medical records. To identify the risk factors for acute severe LGIB, we retrieved data on sex, date of birth, date of CD diagnosis, date of acute severe LGIB, disease location and behavior at diagnosis, smoking status at diagnosis, early use of medications including corticosteroids, thiopurines, and anti-tumor necrosis factor (TNF) agents, diagnostic modalities for identification of bleeding site, identified bleeding site, and date of final follow-up. In addition, to investigate whether acute severe LGIB is a predictor of a worse clinical course of CD, we additionally retrieved data on medications (*i.e.*, corticosteroids, thiopurines, and anti-TNF agents), progression of disease behavior, intestinal resection, and hospitalization.

### Definitions and classifications

CD was diagnosed using standard clinical, radiological, endoscopic, and histopathological criteria[6]. Acute severe LGIB was defined as acute overt rectal bleeding that resulted in (1) an abrupt decrease in the hemoglobin level to < 9 g/dL or at least 2 g/dL below the baseline; and/or (2) transfusion of at least two units of blood within 24 h[1]. The ligament of Treitz was regarded as the anatomic landmark separating LGIB from upper GI bleeding. Postoperative bleeding was defined as bleeding that occurred within 1 mo after intestinal surgery. The lesion found was defined as the bleeding site when it showed active bleeding or adherent blood clot[2]. Early use of corticosteroids was defined as the initiation of treatment within 3 mo of diagnosis[7]; early use of thiopurines or anti-TNF agents was defined as the initiation of therapy within 6 mo of diagnosis[8,9] and at least 6 mo before the first intestinal resection and acute severe LGIB episode[7,10]. In the matched analysis, each matched patient in the non-bleeding group was given an index date that corresponded to the date of acute severe LGIB in the matched patient in the bleeding group, such that the time interval between CD diagnosis and the index date of each non-bleeding patient was equal to that between CD diagnosis and the acute severe LGIB date for the matched bleeding patient. Disease location and behavior were defined according to the Montreal classification[11]. Behavioral progression was defined as the development of stricturing or penetrating disease in patients who had non-stricturing, non-penetrating disease at the start of follow-up. Hospitalization was defined as care in a hospital setting for ≥ 3 d for flare-ups or complications of CD[12]. Hospitalizations only for disease evaluation or due to conditions unrelated to CD were excluded. Index hospitalization was defined as the first hospitalization for acute severe LGIB.

### Statistical analyses

Categorical variables are expressed as numbers with percentages, and continuous variables are expressed as medians with interquartile ranges (IQRs). The Chi-squared test or Fisher's exact test was used to compare categorical variables, as appropriate, and the $t$-test was used to compare continuous variables. Multivariable analysis with Cox proportional hazard regression was performed to identify the risk factors for acute severe LGIB and to calculate their hazard ratios (HRs) and 95% confidence intervals (CIs). All variables with $P$ values < 0.2 in the univariate analysis were included in the multivariable analysis, and backward elimination was performed. In the matched analyses, the balance in the distribution of baseline characteristics

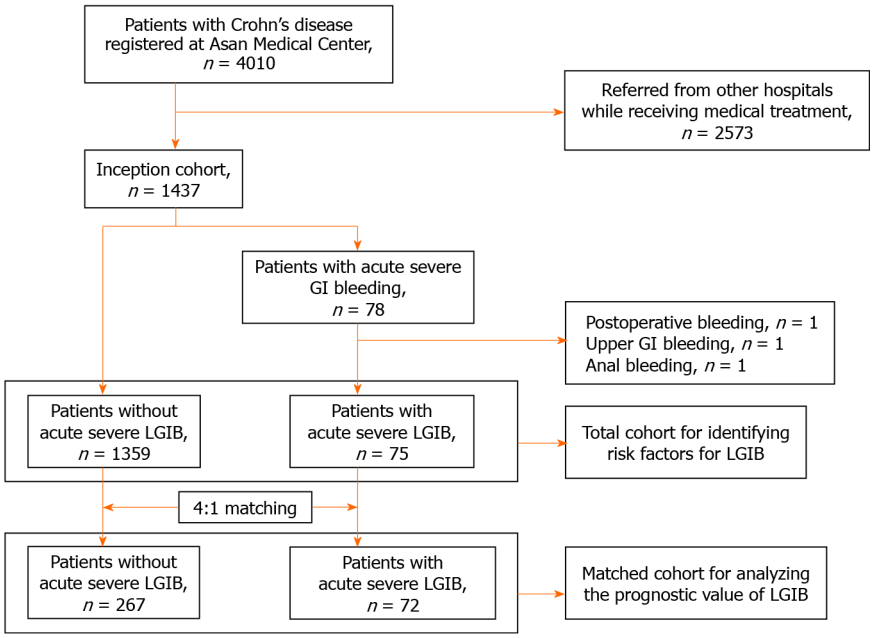



**Figure 1 Flowchart of study patients.** GI: Gastrointestinal; LGIB: Lower gastrointestinal bleeding.

between the matched groups was quantified using the standardized mean difference (SMD). An SMD < 0.1 was regarded to indicate a fair balance of confounders between the matched groups[13]. Disease courses such as medication use, behavioral progression, and intestinal resection before the bleeding/index date were compared between the matched groups using conditional logistic regression analysis. Cumulative risks of medication use, behavioral progression, and intestinal resection after the bleeding/index date were calculated using the Kaplan-Meier method, and the values were compared between the matched groups using the log-rank test. Hospitalization rates *per* 100 patient-years of follow-up were calculated in both the bleeding group and the non-bleeding group, and the relative rates and associated CIs were estimated using Poisson regression with generalized estimating equation. *P* values < 0.05 were considered statistically significant. Statistical evaluations were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, United States) and R version 3.6.2 for Windows (R Foundation for Statistical Computing, Vienna, Austria). The statistical methods of this study were reviewed by Kim YJ from the Department of Clinical Epidemiology and Biostatistics at Asan Medical Center, University of Ulsan College of Medicine.

## RESULTS

### *Characteristics of patients and bleeding episodes*

During a median follow-up of 84.8 mo (IQR 43.2-141.8), 75 (5.2%) of 1437 patients developed the first episode of acute severe LGIB. The cumulative risks of bleeding at 1, 5, 10, and 20 years after CD diagnosis were 3.0%, 4.4%, 5.9%, and 11.6%, respectively. Sixty-three of the 75 patients were men, yielding a male-to-female ratio of 5.2:1. The median age at the first bleeding episode was 27.0 years (IQR 21-34), and the median duration of CD at the first bleeding episode was 7.7 mo (IQR 0-42.1). After excluding 26 patients who were first diagnosed with CD at the time of bleeding, the median duration of disease at the first bleeding episode in the other 49 patients was 31.0 mo (IQR 8.0-66.0).

Of the 75 patients with acute severe LGIB, bleeding sites were identified in 19 (25.3%) patients through ileocolonoscopy, computed tomography, angiography, bleeding scan, and surgery. Capsule endoscopy or double-balloon enteroscopy was not used in the evaluation of acute severe LGIB. In the patients as a whole, the sites of bleeding were the jejunum in 2, the ileum in 12, and the colon in 5. Ileocolonoscopy was performed in 66 patients and revealed bleeding sites in 7 (10.6%) patients (colon, *n* = 5; terminal ileum, *n* = 2). Computed tomography was performed in 55 patients and revealed bleeding sites in 6 (10.9%) patients (jejunum, *n* = 2; ileum, *n* = 4). Mesenteric

angiography was performed in 14 patients and identified bleeding sites in the ileum in 4 (28.5%) patients. Radionuclide bleeding scan was performed in 22 patients and identified bleeding sites in the ileum in 4 (18.2%) patients. Surgery was performed to control bleeding in 4 patients and revealed ileal ulcers with adherent blood clots in 2 (50%) patients.

### *Risk factors for acute severe LGIB*

Table 1 shows the baseline demographic and clinical characteristics according to the presence of acute severe LGIB. The proportions of male patients and patients with ileal disease at diagnosis were significantly higher in the bleeding group ($P$ = 0.045 and $P$ = 0.002, respectively), whereas the proportion of patients with early use of thiopurines was significantly higher in the non-bleeding group ($P$ < 0.001). There were no significant differences between the two groups in terms of age at diagnosis, disease behavior at diagnosis, smoking status at diagnosis, early use of corticosteroids, and early use of anti-TNF agents.

Multivariable Cox regression analysis revealed that female sex (HR: 0.51, 95%CI: 0.27-0.94; $P$ = 0.031) and early use of thiopurines (HR: 0.23, 95%CI: 0.12-0.48; $P$ < 0.001) were significantly associated with a lower risk of acute severe LGIB (Table 2). In addition, patients with ileal disease at diagnosis showed a trend toward a higher risk of bleeding compared with those with colonic disease at diagnosis (HR: 6.58, 95%CI: 0.90-48.12; $P$ = 0.063).

### *Impact of acute severe LGIB on the clinical course of CD*

In the matched analysis between patients in the non-bleeding group and the bleeding group, the baseline characteristics including age at diagnosis, sex, disease location at diagnosis, and disease behavior at diagnosis were well-balanced between the matched groups (Table 3). The median duration of follow-up from CD diagnosis to the bleeding/index date was 7.1 mo (IQR 0.9-38.6) among 72 patients with acute severe LGIB and 8.7 mo (IQR 0.01-38.6) among 267 matched patients without ($P$ = 0.423). During the follow-up period before the bleeding/index date, there were no significant differences in the rates of medication use, behavioral progression, intestinal resection, and hospitalization between the two matched groups (Tables 4 and 5).

The median duration of follow-up from the bleeding/index date to the last follow-up was 105.2 mo (IQR 50.7-135.3) in the bleeding group and 84.4 mo (IQR 46.7-144.2) in the matched non-bleeding group ($P$ = 0.766). During the follow-up period after the bleeding/index date, the cumulative risks of receiving corticosteroids and thiopurines were not significantly different between the two matched groups ($P$ = 0.068 and 0.248, respectively; Figure 2A and B). In contrast, the cumulative risk of receiving anti-TNF agents was significantly higher in the bleeding group ($P$ = 0.035; Figure 2C). The cumulative risk of behavioral progression did not significantly differ between the two matched groups ($P$ = 0.139; Figure 3A). Intestinal resection was performed in 13 (18.1%) of the 72 patients in the bleeding group and 53 (19.9%) of the 267 patients in the matched non-bleeding group ($P$ = 0.86). Four (30.8%) of the 13 patients who underwent intestinal resection in the bleeding group underwent surgery due to acute severe LGIB. The cumulative risks of intestinal resection did not significantly differ between the two matched groups ($P$ = 0.769; Figure 3B). The hospitalization rate after the bleeding/index date was significantly higher in the bleeding group than in the matched non-bleeding group (22.1/100 *vs* 13.2/100 patient-years; $P$ = 0.012), even when the index hospitalization was excluded from the analysis. However, if all hospitalizations due to bleeding episodes were excluded from the analysis, the hospitalization rate did not significantly differ between the bleeding group and the matched non-bleeding group (14.5/100 *vs* 13.2/100 patient-years; $P$ = 0.631; Table 5).

## DISCUSSION

In this study, we investigated the risk factors for acute severe LGIB in CD and the impact of acute severe LGIB on the subsequent clinical course of CD by using a well-defined hospital-based inception cohort. To our knowledge, this is the first study that evaluated the prognostic value of acute severe LGIB in CD. Our results demonstrated that early use of thiopurines and female sex were negatively associated with the risk of bleeding and that there were no significant differences in the clinical course of CD including the rates of medication use (*e.g.*, corticosteroids and thiopurines), behavioral progression, intestinal resection, and hospitalization due to non-bleeding causes between patients who experienced acute severe LGIB and those who did not.

**Table 1 Demographic and clinical characteristics of patients with Crohn's disease according to the presence of acute severe lower gastrointestinal bleeding**

| | Bleeding group (*n* = 75) | Non-bleeding group (*n* = 1359) | *P* value |
|---|---|---|---|
| Age at diagnosis, years (median, IQR) | 23.6 (18.9-31.9) | 23.2 (18.6-33.5) | 0.952 |
| Sex, male, *n* (%) | 63 (84.0) | 1000 (73.6) | 0.045 |
| Disease location at diagnosis, *n* (%) | | | 0.002 |
| Ileum | 34 (45.3) | 377 (27.7) | |
| Colon | 1 (1.3) | 81 (6.0) | |
| Ileocolon | 40 (53.4) | 901 (66.3) | |
| Upper GI modifier at diagnosis, *n* (%) | 15 (20) | 355 (26.1) | 0.238 |
| Disease behavior at diagnosis, *n* (%) | | | 0.157 |
| Non-stricturing, non-penetrating | 59 (78.6) | 1012 (74.5) | |
| Stricturing | 11 (14.7) | 155 (11.4) | |
| Penetrating | 5 (6.7) | 192 (14.1) | |
| Perianal modifier at diagnosis, *n* (%) | 32 (42.7) | 686 (50.5) | 0.204 |
| Smoking status at diagnosis, *n* (%) | | | 0.540 |
| Never smokers | 51 (68.0) | 874 (64.3) | |
| Ex-smokers | 3 (4.0) | 99 (7.3) | |
| Current smokers | 21 (28.0) | 386 (28.4) | |
| Early use of medications, *n* (%) | | | |
| Corticosteroids | 21 (28.0) | 399 (29.4) | 0.840 |
| Thiopurines | 10 (13.3) | 649 (47.8) | < 0.001 |
| Anti-TNF agents | 1 (1.3) | 43 (3.2) | 0.560 |

IQR: Interquartile range; GI: Gastrointestinal; TNF: Tumor necrosis factor.

However, the use of anti-TNF agents was more common in the bleeding group.

To date, only two studies have investigated the risk factors of acute severe LGIB in CD[1,3], with Kim *et al*[1] reporting that the use of thiopurines was associated with a lower risk of bleeding[1] and Li *et al*[3] confirming this result[3]. Li *et al*[3] also reported that left colon involvement and a history of bleeding were associated with a higher risk of bleeding. Similar to the results of previous studies, our present study demonstrated that early use of thiopurines was associated with a lower risk of bleeding. Thiopurines are effective for maintaining remission in patients with CD[14,15], and some studies have also reported that early use of thiopurines is associated with a lower risk of intestinal resection in patients with CD, albeit there are some disagreements in the literature[7,10,16]. Given the results of these previous studies, it can be assumed that early use of thiopurines can lower the risk of bleeding in patients with CD.

Another risk factor for bleeding in our study was male sex. In most population-based cohort studies, sex was not associated with the risk for surgery in patients with CD[7,17,18]. As a result, sex has not been considered a prognostic factor of CD[19]. However, sex differences in the phenotypic characteristics and clinical course of CD are increasingly being recognized[20]. Mazor *et al*[21] reported that only male sex was independently associated with complicated diseases such as stricturing disease, penetrating disease, perianal disease, and abdominal surgery[21]. Some studies have also shown that male sex was significantly associated with a higher risk for surgery[22-24]. These results collectively suggest that male sex may be a real risk factor for bleeding. Further studies are required to confirm whether male sex is an independent risk factor for bleeding in patients with CD.

In the present study, patients with ileal disease at diagnosis had a higher risk of bleeding compared with those with colonic disease at diagnosis, although the result did not reach statistical significance. This is in line with the results of previous studies

**Table 2 Risk factors of acute severe lower gastrointestinal bleeding in patients with Crohn's disease**

| | Univariate analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | HR (95%CI) | *P* value | HR (95%CI) | *P* value |
| Age at diagnosis | 1.01 (0.98-1.03) | 0.663 | | |
| Sex | | | | |
| Male | Reference | | Reference | |
| Female | 0.51 (0.28-0.95) | 0.035 | 0.51 (0.27-0.94) | 0.031 |
| Disease location at diagnosis | | | | |
| Colon | Reference | | Reference | |
| Ileum | 7.56 (1.04-55.26) | 0.046 | 6.58 (0.90-48.12) | 0.063 |
| Ileocolon | 3.60 (0.50-26.22) | 0.206 | 3.86 (0.53-28.09) | 0.182 |
| Upper GI modifier at diagnosis | | | | |
| No | Reference | | | |
| Yes | 0.78 (0.45-1.38) | 0.401 | | |
| Disease behavior at diagnosis | | | | |
| Non-stricturing, non-penetrating | Reference | | | |
| Stricturing | 1.10 (0.58-2.10) | 0.763 | | |
| Penetrating | 0.45 (0.18-1.13) | 0.067 | | |
| Perianal modifier at diagnosis | | | | |
| No | Reference | | Reference | |
| Yes | 0.71 (0.45-1.12) | 0.142 | 0.74 (0.42-1.19) | 0.209 |
| Smoking status at diagnosis | | | | |
| Never smokers | Reference | | | |
| Ex-smokers | 0.59 (0.18-1.88) | 0.368 | | |
| Current smokers | 0.93 (0.56-1.55) | 0.783 | | |
| Early use of medications | | | | |
| Corticosteroids | 0.94 (0.57-1.58) | 0.840 | | |
| Thiopurines | 0.17 (0.09-0.34) | 0.001 | 0.23 (0.12-0.48) | < 0.001 |
| Anti-TNF agents | 0.41 (0.12-3.21) | 0.560 | | |

HR: Hazard ratio; CI: Confidence interval; GI: Gastrointestinal; TNF: Tumor necrosis factor.

that ileal disease is a predictor of complicated behavior[25] and surgery[17,26,27], whereas colonic disease is a predictor of a milder disease course[17,28]. However, other studies reported contrasting results in that bleeding was more common in patients with colonic involvement than in those with isolated small bowel disease[2,29]. These conflicting results warrant further targeted investigation.

The impact of acute severe LGIB on the subsequent clinical course of CD has not been investigated to date. To investigate this issue after controlling for potential confounders, we performed a matched cohort study. In our matched analyses, the cumulative risks of behavioral progression and intestinal resection after the bleeding/index date did not significantly differ between the bleeding group and the matched non-bleeding group. In particular, although 31% of the patients in the bleeding group who underwent intestinal resection received surgery due to bleeding, the overall rate of intestinal resection in the bleeding group was not significantly higher than that in the matched non-bleeding group. Moreover, when we excluded the hospitalizations due to bleeding episodes from the analysis, the hospitalization rate was not significantly different between the two matched groups. These results suggest that a history of acute severe LGIB may not have a significant prognostic value in patients with CD. However, a definite conclusion on this issue cannot be made

**Table 3 Comparison of the baseline parameters between patients with acute severe lower gastrointestinal bleeding and matched patients without**

| | Before matching | | | After matching | | |
|---|---|---|---|---|---|---|
| | Bleeding group (*n* = 75) | Non-bleeding group (*n* = 1359) | SMD | Bleeding group (*n* = 72) | Non-bleeding group (*n* = 267) | SMD |
| Age at diagnosis, years (median, IQR) | 23 (18-32) | 23 (18-33) | 0.009 | 22 (18-31.5) | 23 (18-30) | 0.061 |
| Male, *n* (%) | 63 (84.0) | 1000 (73.6) | 0.257 | 63 (87.5) | 234 (87.6) | 0.004 |
| Disease location at diagnosis, *n* (%) | | | 0.391 | | | 0.020 |
| Ileum | 34 (45.3) | 377 (27.7) | | 31 (43.1) | 111 (41.6) | |
| Colon | 1 (1.3) | 81 (6.0) | | 1 (1.4) | 4 (1.5) | |
| Ileocolon | 40 (53.3) | 901 (66.3) | | 40 (55.6) | 152 (56.9) | |
| Disease behavior at diagnosis, *n* (%) | | | 0.301 | | | 0.030 |
| Non-stricturing, non-penetrating | 59 (78.7) | 1012 (74.5) | | 58 (80.6) | 219 (82.0) | |
| Stricturing | 11 (14.7) | 155 (11.4) | | 9 (12.5) | 32 (12.0) | |
| Penetrating | 5 (6.7) | 192 (14.1) | | 5 (6.9) | 16 (6.0) | |

SMD: Standardized mean difference; IQR: Interquartile range.

**Table 4 Comparison of the outcome parameters between patients with acute severe lower gastrointestinal bleeding and matched patients without during the period before the bleeding/index date**

| | Bleeding group (*n* = 72) | Non-bleeding group (*n* = 267) | Matched OR (95%CI) | *P* value |
|---|---|---|---|---|
| Use of medications, *n* (%) | | | | |
| Corticosteroids | 19/72 (26.4) | 78/267 (29.2) | 0.82 (0.43-1.57) | 0.55 |
| Thiopurines | 27/72 (37.5) | 108/267 (40.4) | 0.74 (0.36-1.51) | 0.40 |
| Anti-TNF agents | 6/72 (8.3) | 27/267 (10.1) | 0.68 (0.24-1.91) | 0.47 |
| Behavioral progression, *n* (%) | 10/58 (17.2) | 21/219 (9.6) | 1.96 (0.87-4.44) | 0.10 |
| Intestinal resection, *n* (%) | 13/72 (18.1) | 30/267 (11.2) | 1.83 (0.73-4.58) | 0.20 |

OR: Odds ratio; CI: Confidence interval; TNF: Tumor necrosis factor.

because anti-TNF agents were more commonly used in the bleeding group than in the matched non-bleeding group. In addition, the rate of all-cause hospitalization was higher in the bleeding group than in the matched non-bleeding group; this is an expected result considering the high recurrence rates (21.4%-41.4%) of acute severe LGIB reported in previous studies[1-5]. A higher need for anti-TNF agents and a higher rate of hospitalization in the bleeding group indicate that a history of acute severe LGIB may have some prognostic value. Regardless of whether acute severe LGIB in CD is a poor prognostic factor, it is a common practice to use anti-TNF agents to lower the risk of rebleeding in CD patients who develop acute severe LGIB[1,5,30-32], and major disease outcomes including behavioral progression and intestinal resection in the bleeding group are comparable with those in the non-bleeding group.

The strength of our present study is that we used a well-defined inception cohort, which enabled us to identify the patients' demographic and clinical characteristics at the time of or at an early stage of CD diagnosis that could predict the occurrence of acute severe LGIB. However, our study has some limitations. First, although our results suggested that male sex was associated with a higher risk of bleeding, we cannot exclude the possibility that this difference was derived from non-biological

**Table 5 Comparison of the hospitalization rate between patients with acute severe lower gastrointestinal bleeding and matched patients without**

| Hospitalization rate (*per* 100 patient-years) | Bleeding group (*n* = 72) | Non-bleeding group (*n* = 267) | Hospitalization rate ratio (95%CI) | *P* value |
|---|---|---|---|---|
| Before bleeding/index date (95%CI) | 21.0 (14.5-30.5) | 23.6 (19.0-29.2) | 0.89 (0.58-1.37) | 0.599 |
| After bleeding/index date (95%CI) | | | | |
| Excluded: Index hospitalization | 22.1 (16.5-29.4) | 13.2 (11.2-15.5) | 1.67 (1.20-2.33) | 0.012 |
| Excluded: Hospitalizations due to bleeding | 14.5 (10.3-20.4) | 13.2 (11.2-15.5) | 1.10 (0.75-1.60) | 0.631 |

CI: Confidence interval.

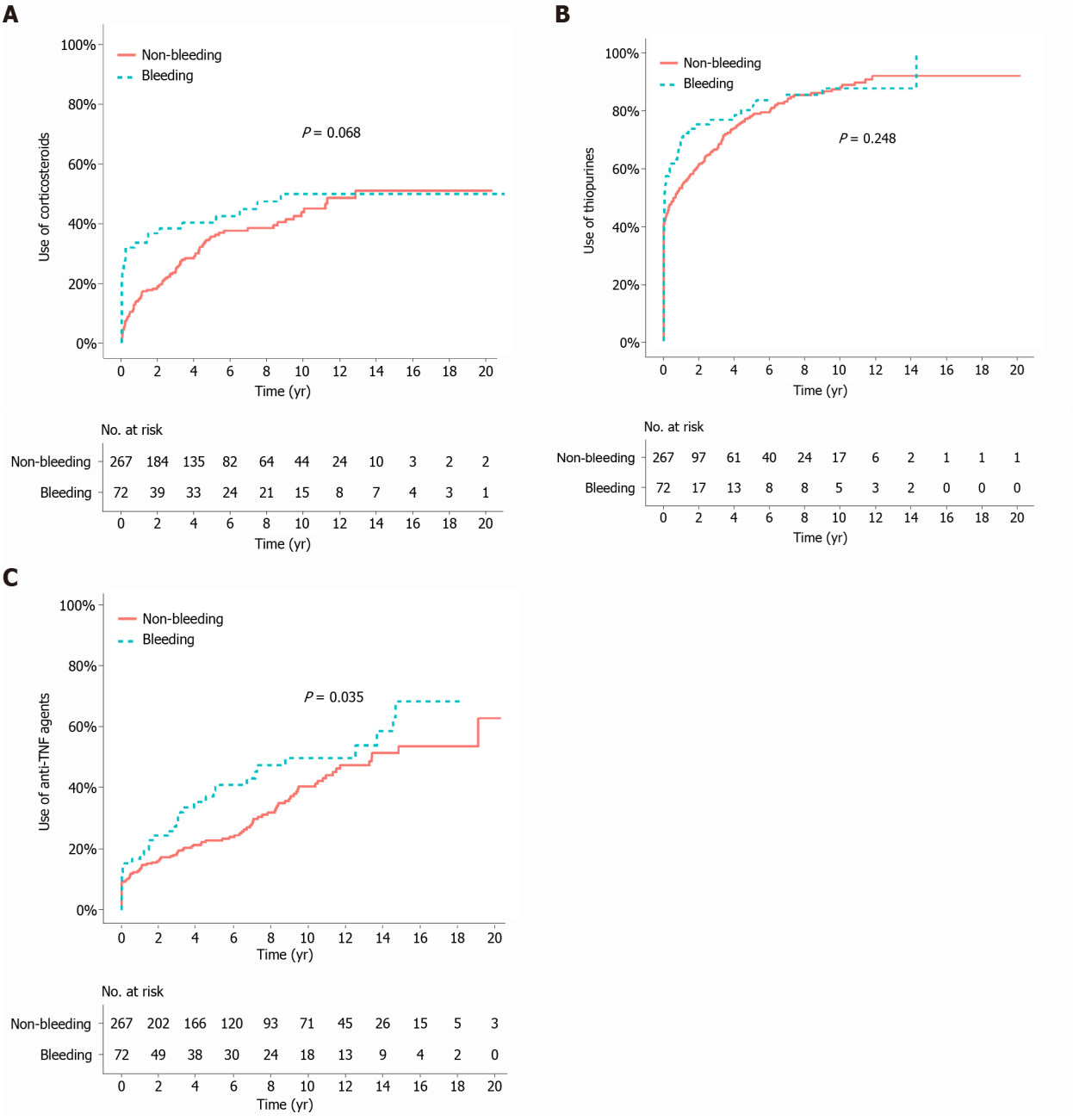



**Figure 2 Cumulative risks of receiving medications after the bleeding/index date according to bleeding.** A: Corticosteroids (*P* = 0.068); B: Thiopurines (*P* = 0.248); C: Anti-tumor necrosis factor agents (*P* = 0.035). TNF: Tumor necrosis factor.

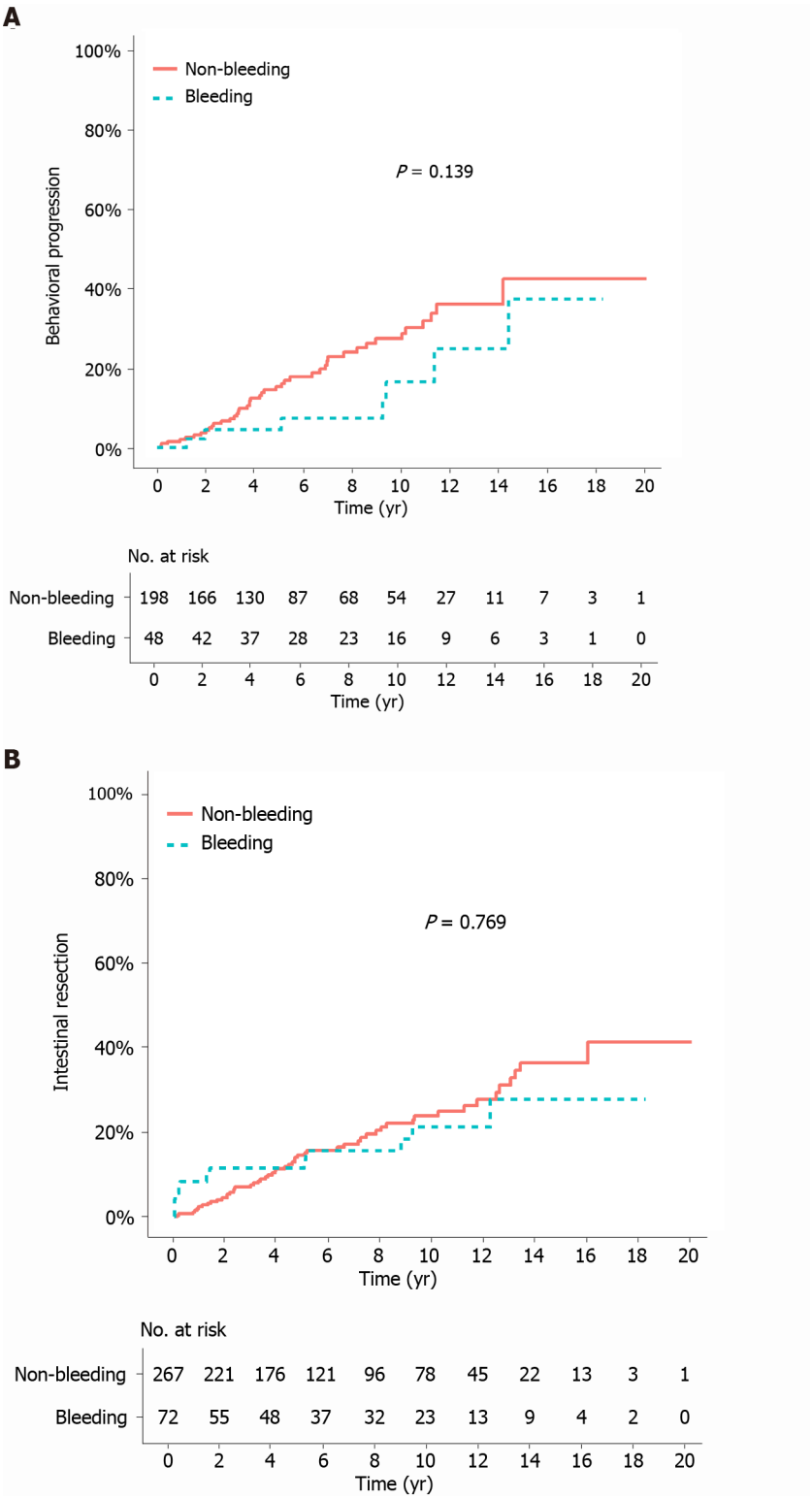



**Figure 3 Cumulative risks of behavioral progression and intestinal resection after the bleeding/index date according to bleeding.** A: Behavioral progression ($P$ = 0.139); B: Intestinal resection ($P$ = 0.769).

causes such as sex differences in access to health care or adherence to therapy in patients with IBD[33-35]. Second, our result that male sex is a risk factor for bleeding may not be generalized in Western patients considering the sex-related differences between Asian and Western patients with CD. For example, the male predominance in the incidence of CD is evident in Asian populations but not in Western populations [36].

## CONCLUSION

Our results suggest that early use of thiopurines may reduce the risk of acute severe LGIB. In addition, a history of acute severe LGIB may not have a significant impact on the subsequent clinical course of patients with CD in terms of behavioral progression, intestinal resection, and hospitalization due to non-bleeding causes. Further studies are needed to determine whether our results on the prognostic value of acute severe LGIB were biased by the differential use of anti-TNF agents between patients with acute severe LGIB and those without.

## ARTICLE HIGHLIGHTS

### *Research background*

Acute severe lower gastrointestinal bleeding (LGIB) is an uncommon but challenging complication of Crohn's disease (CD).

### *Research motivation*

Acute severe LGIB in CD has not been well-studied.

### *Research objectives*

To identify the predictors of acute severe LGIB and to evaluate the impact of acute severe LGIB on the subsequent clinical course in patients with CD.

### *Research methods*

A hospital-based retrospective inception cohort study was conducted. Multivariable analysis with Cox proportional hazard regression was performed to identify the predictors of acute severe LGIB. A matched analysis within the cohort was also conducted to investigate whether acute severe LGIB is a predictor of clinical outcomes of CD. Disease courses were compared using conditional logistic regression analysis.

### *Research results*

Early use of thiopurines and female sex were associated with a decreased risk of acute severe LGIB. The risks of behavioral progression, intestinal resection, and hospitalization due to non-bleeding causes did not significantly differ between the bleeding group and the matched non-bleeding group.

### *Research conclusions*

Early use of thiopurines may reduce the risk of acute severe LGIB. History of acute severe LGIB may not have a significant prognostic value in patients with CD.

### *Research perspectives*

Further studies are needed to investigate whether our findings on the possible null prognostic value of acute severe LGIB in CD was biased by the differential use of anti-tumor necrosis factor agents between patients with acute severe LGIB and those without.

## REFERENCES

1 **Kim KJ**, Han BJ, Yang SK, Na SY, Park SK, Boo SJ, Park SH, Yang DH, Park JH, Jeong KW, Ye BD, Byeon JS, Myung SJ, Kim JH. Risk factors and outcome of acute severe lower gastrointestinal bleeding in Crohn's disease. *Dig Liver Dis* 2012; **44**: 723-728 [PMID: 22497905 DOI: 10.1016/j.dld.2012.03.010]

2 **Belaiche J**, Louis E, D'Haens G, Cabooter M, Naegels S, De Vos M, Fontaine F, Schurmans P, Baert F, De Reuck M, Fiasse R, Holvoet J, Schmit A, Van Outryve M. Acute lower gastrointestinal bleeding in Crohn's disease: characteristics of a unique series of 34 patients. Belgian IBD Research Group. *Am J Gastroenterol* 1999; **94**: 2177-2181 [PMID: 10445546 DOI: 10.1111/j.1572-0241.1999.01291.x]

3 **Li G**, Ren J, Wang G, Wu Q, Gu G, Ren H, Liu S, Hong Z, Li R, Li Y, Guo K, Wu X, Li J. Prevalence and risk factors of acute lower gastrointestinal bleeding in Crohn disease. *Medicine (Baltimore)* 2015; **94**: e804 [PMID: 25984665 DOI: 10.1097/MD.0000000000000804]

4 **Pardi DS**, Loftus EV Jr, Tremaine WJ, Sandborn WJ, Alexander GL, Balm RK, Gostout CJ. Acute major gastrointestinal hemorrhage in inflammatory bowel disease. *Gastrointest Endosc* 1999; **49**:

153-157 [PMID: 9925691 DOI: 10.1016/s0016-5107(99)70479-7]
5 **Lee S**, Ye BD, Park SH, Lee KJ, Kim AY, Lee JS, Kim HJ, Yang SK. Diagnostic Value of Computed Tomography in Crohn's Disease Patients Presenting with Acute Severe Lower Gastrointestinal Bleeding. *Korean J Radiol* 2018; **19**: 1089-1098 [PMID: 30386140 DOI: 10.3348/kjr.2018.19.6.1089]
6 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16 [PMID: 2617184 DOI: 10.3109/00365528909091339]
7 **Ramadas AV**, Gunesh S, Thomas GA, Williams GT, Hawthorne AB. Natural history of Crohn's disease in a population-based cohort from Cardiff (1986-2003): a study of changes in medical treatment and surgical resection rates. *Gut* 2010; **59**: 1200-1206 [PMID: 20650924 DOI: 10.1136/gut.2009.202101]
8 **Cosnes J**, Bourrier A, Laharie D, Nahon S, Bouhnik Y, Carbonnel F, Allez M, Dupas JL, Reimund JM, Savoye G, Jouet P, Moreau J, Mary JY, Colombel JF; Groupe d'Etude Thérapeutique des Affections Inflammatoires du Tube Digestif (GETAID). Early administration of azathioprine *vs* conventional management of Crohn's Disease: a randomized controlled trial. *Gastroenterology* 2013; **145**: 758-65. quiz e14-5 [PMID: 23644079 DOI: 10.1053/j.gastro.2013.04.048]
9 **Burisch J**, Kiudelis G, Kupcinskas L, Kievit HAL, Andersen KW, Andersen V, Salupere R, Pedersen N, Kjeldsen J, D'Incà R, Valpiani D, Schwartz D, Odes S, Olsen J, Nielsen KR, Vegh Z, Lakatos PL, Toca A, Turcan S, Katsanos KH, Christodoulou DK, Fumery M, Gower-Rousseau C, Zammit SC, Ellul P, Eriksson C, Halfvarson J, Magro FJ, Duricova D, Bortlik M, Fernandez A, Hernández V, Myers S, Sebastian S, Oksanen P, Collin P, Goldis A, Misra R, Arebi N, Kaimakliotis IP, Nikuina I, Belousova E, Brinar M, Cukovic-Cavka S, Langholz E, Munkholm P; Epi-IBD group. Natural disease course of Crohn's disease during the first 5 years after diagnosis in a European population-based inception cohort: an Epi-IBD study. *Gut* 2019; **68**: 423-433 [PMID: 29363534 DOI: 10.1136/gutjnl-2017-315568]
10 **Lakatos PL**, Golovics PA, David G, Pandur T, Erdelyi Z, Horvath A, Mester G, Balogh M, Szipocs I, Molnar C, Komaromi E, Veres G, Lovasz BD, Szathmari M, Kiss LS, Lakatos L. Has there been a change in the natural history of Crohn's disease? *Am J Gastroenterol* 2012; **107**: 579-588 [PMID: 22233693 DOI: 10.1038/ajg.2011.448]
11 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus EV Jr, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5A-36A [PMID: 16151544 DOI: 10.1155/2005/269076]
12 **Yamamoto-Furusho JK**, Al Harbi O, Armuzzi A, Chan W, Ponce de Leon E, Qian J, Shapina M, Toruner M, Tu CH, Ye BD, Guennec M, Sison C, Demuth D, Fadeeva O, Khan QMR. Incidence of suboptimal response to tumor necrosis factor antagonist therapy in inflammatory bowel disease in newly industrialised countries: The EXPLORE study. *Dig Liver Dis* 2020; **52**: 869-877 [PMID: 32563721 DOI: 10.1016/j.dld.2020.05.031]
13 **Austin PC**. Using the standardized difference to compare the prevalence of a binary variable between two groups in observational research. *Commun Stat Simul Comput* 2009; **38**: 1228-1234 [DOI: 10.1080/03610910902859574]
14 **Maeda T**, Sakuraba H, Hiraga H, Yoshida S, Kakuta Y, Kikuchi H, Kawaguchi S, Hasui K, Tatsuta T, Chinda D, Mikami T, Fukuda S. Long-term efficacy and tolerability of dose-adjusted thiopurine treatment in maintaining remission in inflammatory bowel disease patients with NUDT15 heterozygosity. *Intest Res* 2021 [PMID: 33472343 DOI: 10.5217/ir.2020.00133]
15 **Chande N**, Patton PH, Tsoulis DJ, Thomas BS, MacDonald JK. Azathioprine or 6-mercaptopurine for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2015; CD000067 [PMID: 26517527 DOI: 10.1002/14651858.CD000067.pub3]
16 **Park SH**, Yang SK, Park SK, Kim JW, Yang DH, Jung KW, Kim KJ, Ye BD, Byeon JS, Myung SJ, Yoon YS, Yu CS, Kim JH. Long-term prognosis of crohn's disease and its temporal change between 1981 and 2012: a hospital-based cohort study from Korea. *Inflamm Bowel Dis* 2014; **20**: 488-494 [PMID: 24412992 DOI: 10.1097/01.MIB.0000441203.56196.46]
17 **Solberg IC**, Vatn MH, Høie O, Stray N, Sauar J, Jahnsen J, Moum B, Lygren I; IBSEN Study Group. Clinical course in Crohn's disease: results of a Norwegian population-based ten-year follow-up study. *Clin Gastroenterol Hepatol* 2007; **5**: 1430-1438 [PMID: 18054751 DOI: 10.1016/j.cgh.2007.09.002]
18 **Bernell O**, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. *Ann Surg* 2000; **231**: 38-45 [PMID: 10636100 DOI: 10.1097/00000658-200001000-00006]
19 **Torres J**, Caprioli F, Katsanos KH, Lobatón T, Micic D, Zerôncio M, Van Assche G, Lee JC, Lindsay JO, Rubin DT, Panaccione R, Colombel JF. Predicting Outcomes to Optimize Disease Management in Inflammatory Bowel Diseases. *J Crohns Colitis* 2016; **10**: 1385-1394 [PMID: 27282402 DOI: 10.1093/ecco-jcc/jjw116]
20 **Rustgi SD**, Kayal M, Shah SC. Sex-based differences in inflammatory bowel diseases: a review. *Therap Adv Gastroenterol* 2020; **13**: 1756284820915043 [PMID: 32523620 DOI: 10.1177/1756284820915043]
21 **Mazor Y**, Maza I, Kaufman E, Ben-Horin S, Karban A, Chowers Y, Eliakim R. Prediction of disease complication occurrence in Crohn's disease using phenotype and genotype parameters at diagnosis. *J Crohns Colitis* 2011; **5**: 592-597 [PMID: 22115380 DOI: 10.1016/j.crohns.2011.06.002]
22 **Severs M**, Spekhorst LM, Mangen MJ, Dijkstra G, Löwenberg M, Hoentjen F, van der Meulen-de

Jong AE, Pierik M, Ponsioen CY, Bouma G, van der Woude JC, van der Valk ME, Romberg-Camps MJL, Clemens CHM, van de Meeberg P, Mahmmod N, Jansen J, Jharap B, Weersma RK, Oldenburg B, Festen EAM, Fidder HH. Sex-Related Differences in Patients With Inflammatory Bowel Disease: Results of 2 Prospective Cohort Studies. *Inflamm Bowel Dis* 2018; **24**: 1298-1306 [PMID: 29688413 DOI: 10.1093/ibd/izy004]

23 **Peyrin-Biroulet L**, Harmsen WS, Tremaine WJ, Zinsmeister AR, Sandborn WJ, Loftus EV Jr. Surgery in a population-based cohort of Crohn's disease from Olmsted County, Minnesota (1970-2004). *Am J Gastroenterol* 2012; **107**: 1693-1701 [PMID: 22945286 DOI: 10.1038/ajg.2012.298]

24 **Hofer B**, Böttger T, Hernandez-Richter T, Seifert JK, Junginger T. The impact of clinical types of disease manifestation on the risk of early postoperative recurrence in Crohn's disease. *Hepatogastroenterology* 2001; **48**: 152-155 [PMID: 11268954]

25 **Thia KT**, Sandborn WJ, Harmsen WS, Zinsmeister AR, Loftus EV Jr. Risk factors associated with progression to intestinal complications of Crohn's disease in a population-based cohort. *Gastroenterology* 2010; **139**: 1147-1155 [PMID: 20637205 DOI: 10.1053/j.gastro.2010.06.070]

26 **Magro F**, Portela F, Lago P, Ramos de Deus J, Vieira A, Peixe P, Cremers I, Cotter J, Cravo M, Tavares L, Reis J, Gonçalves R, Lopes H, Caldeira P, Ministro P, Carvalho L, Azevedo L, da Costa-Pereira A; GEDII. Crohn's disease in a southern European country: Montreal classification and clinical activity. *Inflamm Bowel Dis* 2009; **15**: 1343-1350 [PMID: 19235885 DOI: 10.1002/ibd.20901]

27 **Watanabe K**. Clinical management for small bowel of Crohn's disease in the treat-to-target era: now is the time to optimize treatment based on the dominant lesion. *Intest Res* 2020; **18**: 347-354 [PMID: 33131231 DOI: 10.5217/ir.2020.00032]

28 **Sands BE**, Arsenault JE, Rosen MJ, Alsahli M, Bailen L, Banks P, Bensen S, Bousvaros A, Cave D, Cooley JS, Cooper HL, Edwards ST, Farrell RJ, Griffin MJ, Hay DW, John A, Lidofsky S, Olans LB, Peppercorn MA, Rothstein RI, Roy MA, Saletta MJ, Shah SA, Warner AS, Wolf JL, Vecchio J, Winter HS, Zawacki JK. Risk of early surgery for Crohn's disease: implications for early treatment strategies. *Am J Gastroenterol* 2003; **98**: 2712-2718 [PMID: 14687822 DOI: 10.1111/j.1572-0241.2003.08674.x]

29 **Robert JR**, Sachar DB, Greenstein AJ. Severe gastrointestinal hemorrhage in Crohn's disease. *Ann Surg* 1991; **213**: 207-211 [PMID: 1998401 DOI: 10.1097/00000658-199103000-00004]

30 **Tsujikawa T**, Nezu R, Andoh A, Saotome T, Araki Y, Ishizuka Y, Sasaki M, Koyama S, Fujiyama Y. Infliximab as a possible treatment for the hemorrhagic type of Crohn's disease. *J Gastroenterol* 2004; **39**: 284-287 [PMID: 15065007 DOI: 10.1007/s00535-003-1290-9]

31 **Belaiche J**, Louis E. Severe lower gastrointestinal bleeding in Crohn's disease: successful control with infliximab. *Am J Gastroenterol* 2002; **97**: 3210-3211 [PMID: 12492221 DOI: 10.1111/j.1572-0241.2002.07143.x]

32 **Meyer MM**, Levine EJ. Acute hemorrhagic Crohn's disease controlled with infliximab. *Inflamm Bowel Dis* 2009; **15**: 1456-1457 [PMID: 19107774 DOI: 10.1002/ibd.20840]

33 **Zelante A**, De Giorgi A, Borgoni R, Trevisani L, Gallerani M. Adherence to medical treatment in inflammatory bowel disease patients. *Minerva Gastroenterol Dietol* 2014; **60**: 269-274 [PMID: 25384805]

34 **Lopez A**, Billioud V, Peyrin-Biroulet C, Peyrin-Biroulet L. Adherence to anti-TNF therapy in inflammatory bowel diseases: a systematic review. *Inflamm Bowel Dis* 2013; **19**: 1528-1533 [PMID: 23518810 DOI: 10.1097/MIB.0b013e31828132cb]

35 **Blumenstein I**, Bock H, Zosel C, Dignass AU, Hartmann F, Zeuzem S, Stein JM, Schroeder O. [Are there gender-related differences in the therapeutic management of patients suffering from inflammatory bowel disease? *Z Gastroenterol* 2009; **47**: 1045-1051 [PMID: 19809954 DOI: 10.1055/s-0028-1109647]

36 **Shah SC**, Khalili H, Chen CY, Ahn HS, Ng SC, Burisch J, Colombel JF. Sex-based differences in the incidence of inflammatory bowel diseases-pooled analysis of population-based studies from the Asia-Pacific region. *Aliment Pharmacol Ther* 2019; **49**: 904-911 [PMID: 30773656 DOI: 10.1111/apt.15178]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2366-2375

DOI: 10.3748/wjg.v27.i19.2366

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

ORIGINAL ARTICLE

Retrospective Study

# Changes in the nutritional status of nine vitamins in patients with esophageal cancer during chemotherapy

Lan-Qing Liang, Ling-Ling Meng, Bo-Ning Cai, Ze-Ping Cui, Na Ma, Le-Hui Du, Wei Yu, Bao-Lin Qu, Sheng-Qiang Feng, Fang Liu

**ORCID number:** Lan-Qing Liang 0000-0003-4840-6117; Ling-Ling Meng 0000-0003-4674-5297; Bo-Ning Cai 0000-0003-2358-4806; Ze-Ping Cui 0000-0002-4589-7187; Na Ma 0000-0002-7045-6623; Le-Hui Du 0000-0002-6865-8461; Wei Yu 0000-0002-5024-8675; Bao-Lin Qu 0000-0002-8911-3460; Sheng-Qiang Feng 0000-0002-3996-0872; Fang Liu 0000-0002-8563-4664.

**Author contributions:** Liang LQ and Meng LL contributed equally to this work; Liang LQ and Meng LL designed and performed the research and wrote the paper; Liu F designed the research and supervised the report; Cui ZP and Ma N designed the research and contributed to the analysis; Du LH, Yu W and Feng SQ provided clinical advice; Qu BL and Cai BN supervised the report.

**Supported by** Health Bureau of the Department of Logistics and Security of the Central Military Commission of China, No. 17BJZ47.

**Institutional review board statement:** The study was reviewed and approved by the Medical Ethics Committee of the General Hospital of the Chinese People's Liberation Army, No. S2019-198-02.

**Lan-Qing Liang, Ling-Ling Meng, Bo-Ning Cai, Ze-Ping Cui, Na Ma, Le-Hui Du, Wei Yu, Bao-Lin Qu, Fang Liu,** Department of Radiotherapy, Chinese PLA General Hospital, Beijing 100853, China

**Sheng-Qiang Feng,** Department of Health Service, The Guard Bureau of Joint Staff Department of Chinese PLA, Beijing 100017, China

**Corresponding author:** Fang Liu, MD, Associate Chief Physician, Department of Radiotherapy, Chinese PLA General Hospital, No. 28 Fuxing Road, Haidian District, Beijing 100853, China. liufangfsq@163.com

## Abstract

### *BACKGROUND*

Many studies have investigated the relationships between vitamins and esophageal cancer (EC). Most of these studies focused on the roles of vitamins in the prevention and treatment of EC, and few studies have examined the changes in vitamin nutritional status and their influencing factors before and after chemotherapy for EC. Chemotherapy may have a considerable effect on EC patients' vitamin levels and hematological indicators.

### *AIM*

To research the nutritional status of multiple vitamins in EC patients during chemotherapy and to assess its clinical significance.

### *METHODS*

EC patients admitted to our center from July 2017 to September 2020 were enrolled in this study. Serum concentrations of nine vitamins (A, D, E, $B_9$, $B_{12}$, $B_1$, C, $B_2$ and $B_6$), hemoglobin, total protein, albumin, blood calcium, blood phosphorus concentrations and body mass index (BMI) were measured in all EC patients. The changes in nine vitamins, hematological indicators and BMI were compared before and after two cycles of chemotherapy. The possible influential factors were analyzed.

### *RESULTS*

In total, 203 EC patients receiving chemotherapy were enrolled in this study. Varying degrees of vitamin A, D, C and $B_2$ deficiency and weight loss were found in these patients, and the proportions of vitamin $B_2$ and vitamin C deficiencies increased significantly after chemotherapy (both $P < 0.05$). Serum concentrations

of vitamins A, C, $B_2$ and $B_6$ and BMI before and after chemotherapy were statistically significant (all $P < 0.05$). Multivariate analysis showed that vitamin A levels significantly differed between male and female EC patients, whereas vitamin D concentration significantly differed in EC patients in different stages (all $P < 0.05$). Correlations were observed between the changes in serum concentrations of vitamin A and C before and after two cycles chemotherapy and the change in BMI ($P < 0.05$). Hemoglobin, total protein, serum albumin and blood calcium concentrations significantly decreased in EC patients after chemotherapy (all $P < 0.05$), while the blood phosphorus level significantly increased after chemotherapy ($P < 0.05$). Using the difference in vitamin concentrations as the independent variables and the difference in BMI as the dependent variable, logistic regression analysis revealed statistically significant differences for vitamin A, vitamin D and vitamin C ($F = 5.082$, $P = 0.002$).

*CONCLUSION*

Vitamin A, D, C and $B_2$ were mainly deficient in patients with EC during chemotherapy. Multivitamin supplementation may help to improve the nutritional status, chemotherapy tolerance and efficacy.

**Key Words:** Esophageal cancer; Chemotherapy; Vitamins; Nutritional status; Body mass index

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** This retrospective study investigated the changes in serum vitamins before and after chemotherapy for esophageal cancer. Vitamin deficiencies are common in esophageal cancer patients during chemotherapy and may be associated with the change in body mass index. There were correlations between the changes in vitamin A and C concentrations and the change in body mass index during chemotherapy. Vitamin A level after chemotherapy showed a significant difference between males and females, and the vitamin D level after chemotherapy showed a significant difference among different stages. Vitamin supplementation may reduce the adverse effects of chemotherapy and improve the nutritional status.

**Citation:** Liang LQ, Meng LL, Cai BN, Cui ZP, Ma N, Du LH, Yu W, Qu BL, Feng SQ, Liu F. Changes in the nutritional status of nine vitamins in patients with esophageal cancer during chemotherapy. *World J Gastroenterol* 2021; 27(19): 2366-2375
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2366.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2366

## INTRODUCTION

Esophageal cancer (EC) is the ninth most commonly diagnosed cancer and the sixth leading cause of cancer death worldwide[1]. The incidence and mortality rates of EC are highly heterogeneous in terms of gender, histological type, geographic distribution and race[2]. The morbidity and mortality of EC in China are higher than the global averages[3]. Chemoradiotherapy remains the mainstay of treatment for patients with advanced EC. The most common complications during chemoradiotherapy in EC patients include weight loss, malnutrition, bone marrow suppression, electrolyte disturbances, hypoproteinemia and decreased quality of life[4-7]. Multiple vitamins are involved in the pathogenesis, progression and prognosis of tumors and are closely related to the tumor microenvironment. Vitamin testing is valuable in tumor patients as it can identify whether there is a specific vitamin deficiency and/or justify vitamin therapy. Most clinical studies have shown that vitamin nutritional status varies greatly in tumors of different systems[8]. Although many studies have investigated the relationships between vitamins B, A, D and C with EC, most of these studies focused on the roles of vitamins in the prevention and treatment of EC. Few studies have examined the changes in vitamin nutritional status and their influencing factors before and after chemotherapy for EC.

**Informed consent statement:** Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

**Conflict-of-interest statement:** All authors hereby declare that there is no personal conflict of interest that may cause impact or bias to the results of this study. All authors read and approved the final manuscript.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** China

**Peer-review report's scientific quality classification**

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

**Received:** February 2, 2021
**Peer-review started:** February 2, 2021
**First decision:** March 6, 2021
**Revised:** March 18, 2021
**Accepted:** April 13, 2021
**Article in press:** April 13, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Kumar N, Levick C
**S-Editor:** Fan JR
**L-Editor:** Filipodia
**P-Editor:** Ma YJ

Many vitamins have antitumor effects and are closely related to the occurrence, progression, prognosis and recovery of tumors. Vitamin supplementation can lower the risk of tumorigenesis and prevent abnormal DNA methylation changes in tumor cells[9]. With the advances in nutritional therapy in China, nutritional screenings, assessments and interventions have increasingly been applied in tumor patients. The standardized nutritional therapies involve three major macronutrients including carbohydrates, proteins and fats and have specific requirements on micronutrients. Nutritional therapy for tumor patients provides nutrients and energy and focuses on the metabolism-regulating roles of nutrients[10]. Vitamin supplementation is a common nutritional therapy for tumor patients in clinical practice. Serum vitamin levels vary among patients with different tumors. Zhang *et al*[11] analyzed the vitamin nutritional status of approximately 1000 hospitalized tumor patients and found that vitamin B1 concentrations were low in patients with digestive system tumors such as EC and gastric cancer. A study on postoperative nutritional deficiencies in patients with EC or gastric cancer revealed that the incidence of ferritin, folic acid, vitamin $B_{12}$ and vitamin D deficiencies was 42.86%, 9.52%, 6.35% and 36.67%, respectively, and the vitamin levels were significantly improved after nutritional interventions[12]. In another study[13], most patients with advanced tumors had vitamin (particularly vitamins D, $B_6$ and C) deficiency symptoms during palliative care, and further analysis revealed a correlation between the degree of vitamin deficiency and clinical discomfort in these patients. Most vitamins were found to be negatively associated with the risk of colorectal and gastric cancer in addition to EC in an observational study, yet interventional treatment failed to demonstrate a clear preventive effect in these malignancies [14].

In the current study, we analyzed the effects of chemotherapeutic drugs on EC patients' vitamin levels and hematological indicators by detecting the changes in nine vitamins and hematological indicators (*e.g.*, hemoglobin, total protein, serum albumin and electrolytes) before and after chemotherapy, with an attempt to provide evidence for clinical vitamin supplementation and nutritional therapies. Our findings may be valuable for the implementation of tailored nutritional interventions, which will help to reduce chemotherapy-related complications, alleviate treatment resistance and improve chemotherapy efficacy.

## MATERIALS AND METHODS

### Subjects

In total, 203 EC patients (181 men and 22 women) aged 37-78 yrs (mean: 60.03 ± 7.95 years) who were receiving chemotherapy in our center between July 2017 and September 2020 were enrolled in this study. The vast majority of subjects had esophageal squamous cell carcinoma ($n$ = 192, 94.58%), and 166 patients (81.77%) were in stage III-IV (Table 1). The inclusion criteria were as follows: (1) pathologically confirmed esophageal malignancies, with indications for chemotherapy, regardless of gender; (2) aged 18-80 years; (3) an expected survival of > 6 mo; and (4) a Karnofsky performance status score of ≥ 70 points. The exclusion criteria included: (1) contraindications to chemotherapy; (2) coexisting tumors in other systems; (3) coexisting primary blood diseases or endocrine diseases; (4) coexisting cardiopulmonary dysfunction; and (5) coexisting psychiatric disorders.

### Methods

**Determination of vitamin concentrations and hematological indicators:** Vitamin levels were measured using electrochemiluminescence with an LK3000VI vitamin detector (Tianjin Lanbiao Electronic Technology Development Co., Ltd., Tianjin, China) pre- and postchemotherapy in EC patients. The normal thresholds were as follows: hemoglobin: 137-179 g/L in males and 116-155 g/L in females; total protein: 55-88 g/L; serum albumin: 35-50 g/L; blood calcium: 2.09-2.54 mmol/L; blood phosphorus: 0.89-1.60 mmol/L; vitamin A: 0.52-2.20 mmol/L; vitamin D: 25-200 nmol/L; vitamin E: 10-15 mg/mL; vitamin $B_9$: 6.8-36.3 nmol/L; vitamin $B_{12}$: 200-900 pg/mL; vitamin $B_1$: 50-150 nmol/L; vitamin C: 34-114 mmol/L; vitamin $B_2$: 4.26-18.42 mg/L; and vitamin $B_6$: 14.6-72.9 nmol/L.

**Chemotherapy regimens:** Of these 203 EC patients, 168 were treated with induction chemotherapy, 15 with postoperative adjuvant chemotherapy and 20 with postrelapse chemotherapy. The specific regimens were as follows: (1) squamous cell carcinoma: paclitaxel + platinum; (2) adenocarcinoma: oxaliplatin + fluorouracil; and (3) small cell

**Table 1 Baseline data of esophageal cancer patients (*n* = 203)**

| Clinical features | *n* | (%) |
|---|---|---|
| Gender | | |
| Males | 181 | 89.16 |
| Females | 22 | 10.84 |
| Age | | |
| < 60 yr | 94 | 46.31 |
| ≥ 60 yr | 109 | 53.69 |
| Tumor stage | | |
| II | 16 | 7.88 |
| III | 98 | 48.28 |
| IV | 68 | 33.50 |
| Uncertain | 21 | 10.34 |
| Pathologic type | | |
| Squamous cell carcinoma | 192 | 94.58 |
| Nonsquamous cell carcinoma | 11 | 5.42 |
| Treatment | | |
| Induction chemotherapy | 168 | 82.76 |
| Postoperative adjuvant chemotherapy | 15 | 7.39 |
| Postrelapse chemotherapy | 20 | 9.85 |
| Primary tumor site | | |
| Cervical | 14 | 6.90 |
| Upper thoracic | 28 | 13.79 |
| Middle thoracic | 77 | 37.93 |
| Lower thoracic | 84 | 41.38 |

carcinoma: etoposide + platinum. All of these regimens were repeated every 3 wk.

### Statistical methods

SPSS 24.0 statistical software was used for analyzing the data. Normally distributed data were expressed as mean ± SD, and intergroup comparisons were based on the paired-sample *t*-test. Non-normally distributed data were expressed as quartiles and medians, and the rank sum test was applied for comparisons between two groups. The Spearman method was used to test for correlations between the changes in multiple vitamin serum concentrations (the independent variables) and body mass index (BMI) (the dependent variable) during chemotherapy using. $P$ value < 0.05 was regarded as statistically significant.

## RESULTS

### Variation in BMI and hematological parameters in EC patients during chemotherapy

BMI declined after chemotherapy in 133 cases (65.52%). The number of patients with anemia increased from 119 before chemotherapy to 182 after chemotherapy. The differences in these proportions were statistically significant ($P$ < 0.05) (Table 2).

### Vitamin levels in EC patients pre- and postchemotherapy

Vitamin A, C, $B_2$ and $B_6$ concentrations significantly differed before and after chemotherapy (all $P$ < 0.05) (Table 3).

**Table 2 Changes in body mass index and hematological indicators in esophageal cancer patients before and after chemotherapy [*n* (%)]**

| Item | Before chemotherapy | After chemotherapy | $\chi^2$/*Z* | *P* value |
|---|---|---|---|---|
| Body mass index (kg/m$^2$) | | | -2.646 | 0.008 |
| < 18.5 | 25 (12.32) | 22 (10.84) | | |
| 18.5-23.9 | 103 (50.74) | 123 (60.59) | | |
| > 23.9 | 75 (36.95) | 58 (28.57) | | |
| Hemoglobin (g/L) | | | 55.710 | < 0.001 |
| Decreased | 119 (58.62) | 182 (89.66) | | |
| Normal | 84 (41.38) | 21 (10.34) | | |
| Serum albumin (g/L) | | | 2.717 | 0.099 |
| Decreased | 34 (16.75) | 47 (23.15) | | |
| Normal | 169 (83.25) | 156 (76.85) | | |
| Serum calcium (mmol/L) | | | 4.780 | 0.029 |
| Decreased | 21 (10.34) | 36 (17.73) | | |
| Normal | 182 (89.66) | 167 (82.27) | | |
| Blood phosphorus (mmol/L) | | | 2.382 | 0.123 |
| Decreased | 23 (11.33) | 13 (6.40) | | |
| Normal | 180 (88.67) | 190 (93.60) | | |

**Table 3 Vitamin concentrations in esophageal cancer patients before and after chemotherapy**

| Item | Before chemotherapy (Q1-Q3) median | After chemotherapy (Q1-Q3) median | *Z* | *P* value |
|---|---|---|---|---|
| Vitamin A (μmol/L) | (0.561-0.980) 0.741 | (0.528-0.858) 0.678 | -3.465 | 0.001 |
| Vitamin D (nmol/L) | (33.618-49.939) 41.383 | (33.235-46.473) 38.832 | -1.599 | 0.110 |
| Vitamin E (μg/mL) | (10.898-11.673) 11.267 | (10.905-11.867) 11.298 | -1.678 | 0.093 |
| Vitamin $B_9$ (nmol/L) | (14.529-23.014) 18.718 | (13.859-22.037) 17.539 | -1.578 | 0.115 |
| Vitamin $B_{12}$ (pg/mL) | (491.023-598.303) 557.219 | (491.112-590.338) 546.789 | -0.326 | 0.745 |
| Vitamin $B_1$ (nmol/L) | (75.056-97.909) 84.070 | (75.826-93.322) 83.095 | -1.477 | 0.140 |
| Vitamin C (μmol/L) | (34.324-38.543) 36.075 | (33.299-37.849) 35.259 | -3.824 | < 0.001 |
| Vitamin $B_2$ (μg/L) | (4.182-5.050) 4.501 | (4.105-4.761) 4.284 | -2.631 | 0.009 |
| Vitamin $B_6$ (nmol/L) | (29.702-33.645) 31.747 | (28.875-32.868) 31.363 | -2.351 | 0.019 |

Q1-Q3: Quartiles 1-3.

### *Vitamin deficiencies in EC patients undergoing chemotherapy*

Deficiencies of vitamin A, D, C and $B_2$ were detected pre- and postchemotherapy, and the proportion of each of these four vitamin deficiencies increased after chemotherapy. In particular the proportions of vitamin C and $B_2$ deficiencies (24.46% *vs* 38.85%) increased significantly (both $P < 0.05$) (Table 4). Three EC patients had excessively high vitamin A concentrations before chemotherapy, which decreased to normal levels in 2 cases and to vitamin A deficiency in 1 case after chemotherapy. None of the other five vitamin (E, $B_9$, $B_{12}$, $B_1$, $B_6$) deficiencies were detected during chemotherapy.

### *Changes in hematological indicators in EC patients pre- and postchemotherapy*

Hemoglobin, total protein, serum albumin and blood calcium concentrations significantly decreased, and blood phosphorus concentrations significantly increased after chemotherapy (all $P < 0.05$) (Table 5).

**Table 4 Vitamin deficiencies in esophageal cancer patients before and after chemotherapy, *n* (%)**

| Item | Before chemotherapy | | After chemotherapy | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|---|
| | Normal | Deficiency | Normal | Deficiency | | |
| Vitamin A (μmol/L) | 163 (81.50) | 37 (18.50) | 158 (79.00) | 42 (21.00) | 0.485 | 0.486 |
| Vitamin D (nmol/L) | 194 (95.57) | 9 (4.43) | 191 (94.09) | 12 (5.91) | 0.235 | 0.629 |
| Vitamin C (μmol/L) | 155 (76.35) | 48 (23.65) | 133 (65.52) | 70 (34.48) | 6.682 | 0.010 |
| Vitamin $B_2$ (μg/L) | 139 (68.47) | 64 (31.53) | 108 (53.20) | 95 (46.80) | 9.677 | 0.002 |

**Table 5 Changes in hematological indicators in esophageal cancer patients before and after chemotherapy**

| Item | Before chemotherapy (mean ± SD) | After chemotherapy (mean ± SD) | *t* | *P* value |
|---|---|---|---|---|
| Hemoglobin (g/L) | 130.070 ± 16.484 | 115.010 ± 14.584 | 15.342 | < 0.001 |
| Total protein (g/L) | 66.572 ± 5.726 | 64.499 ± 5.528 | 5.032 | < 0.001 |
| Serum albumin (g/L) | 38.880 ± 4.138 | 37.546 ± 3.719 | 4.355 | < 0.001 |
| Blood calcium (mmol/L) | 2.240 ± 0.128 | 2.207 ± 0.132 | 2.835 | 0.005 |
| Blood phosphorus (mmol/L) | 1.130 ± 0.200 | 1.180 ± 0.184 | -2.818 | 0.005 |

SD: Standard deviation.

### *Factors affecting vitamins and hematological indicators in EC patients*

After adjustment for covariates of each indicator before chemotherapy, vitamin A levels after chemotherapy showed a significant difference between males and females. Vitamin D levels after chemotherapy showed a significant difference among different tumor grades (both $P < 0.05$). No statistically significant differences were shown for the other baseline data (all $P > 0.05$) (Table 6).

### *Correlation between vitamin concentrations and BMI during chemotherapy in EC patients*

There were correlations between the changes in serum vitamin A and C concentrations and the change in BMI before and after chemotherapy ($P < 0.05$) (Table 7).

### *Regression analysis of vitamins and BMI in EC patients*

Using the difference in vitamin concentrations as the independent variables and the difference in BMI as the dependent variable, logistic regression analysis revealed statistically significant differences for three vitamins ($F = 5.082$, $P = 0.002$) (Table 8).

## DISCUSSION

It was found that EC patients had different degrees of vitamin deficiency during chemotherapy, and the hemoglobin, total protein, serum albumin concentration and blood calcium concentration significantly decreased after chemotherapy. In addition, the proportions of patients with weight loss, anemia and hypoproteinemia also significantly increased. Our subjects were most deficient in vitamin $B_2$ and vitamin C followed by vitamins A and D.

In the current study, EC patients undergoing chemotherapy were most deficient in vitamin $B_2$ (31.53% and 46.80% before and after chemotherapy, respectively), and vitamin $B_2$ concentration significantly decreased after chemotherapy ($P < 0.05$). Vitamin $B_1$, $B_9$, $B_{12}$ and $B_6$ deficiency or excess was not found in any of our EC patients. A comparison of vitamin levels before and after chemotherapy suggested that vitamin $B_6$ concentrations decreased significantly after chemotherapy but were still within the normal range. It has been reported that B vitamin intake is correlated with the risk of EC. Appropriate supplementation with vitamins $B_6$ and $B_9$ (also known as folate) can reduce the risk, while higher intake of vitamin $B_{12}$ may increase the risk[15,16]. A study of residents in Yanting County, Sichuan Province, a high-incidence area of EC in

**Table 6 Factors affecting various indicators in esophageal cancer patients during chemotherapy**

| Item | Gender (Males/females) | | Age (≥ 60 yr/< 60 yr) | | Tumor grades (grades II/III/IV) | | Tumor location (cervical/upper, middle, lower thoracic) | |
|---|---|---|---|---|---|---|---|---|
| | F | *P* value | F | *P* value | F | *P* value | F | *P* value |
| Body mass index | 0.014 | 0.904 | 0.042 | 0.837 | 0.672 | 0.512 | 0.718 | 0.542 |
| Hemoglobin | 0.044 | 0.834 | 0.757 | 0.385 | 0.747 | 0.475 | 0.195 | 0.900 |
| Serum albumin | 0.707 | 0.402 | 3.387 | 0.067 | 0.210 | 0.811 | 2.554 | 0.057 |
| Vitamin A | 5.407 | 0.021 | 0.059 | 0.809 | 0.151 | 0.860 | 0.692 | 0.558 |
| Vitamin D | 0.594 | 0.442 | 0.326 | 0.568 | 6.899 | 0.001 | 0.324 | 0.808 |
| Vitamin C | 3.774 | 0.053 | 0.537 | 0.464 | 2.633 | 0.075 | 2.261 | 0.083 |
| Vitamin $B_2$ | 0.178 | 0.674 | 0.863 | 0.354 | 0.381 | 0.684 | 2.273 | 0.081 |

The descriptive statistics of each indicator before and after chemotherapy are shown in Tables 3 and 5.

**Table 7 Relationships between the differences in vitamins before and after chemotherapy and the difference in body mass index before and after chemotherapy in esophageal cancer patients**

| Difference in vitamin concentration | Difference in BMI | |
|---|---|---|
| | *r* | *P* value |
| Vitamin A | 0.240 | 0.001 |
| Vitamin D | -0.080 | 0.259 |
| Vitamin E | -0.095 | 0.177 |
| Vitamin $B_9$ | -0.016 | 0.824 |
| Vitamin $B_{12}$ | -0.053 | 0.449 |
| Vitamin $B_1$ | 0.021 | 0.771 |
| Vitamin C | 0.188 | 0.007 |
| Vitamin $B_2$ | -0.102 | 0.149 |
| Vitamin $B_6$ | -0.078 | 0.269 |

BMI: Body mass index.

**Table 8 Regression analysis of the relationships between the concentrations of various vitamins and body mass index in 203 esophageal cancer patients**

| Independent variables (differences) | Dependent variable (difference in BMI) | | |
|---|---|---|---|
| | B (coefficient) | *t* | *P* value |
| Constant | 0.365 | 4.978 | < 0.001 |
| Vitamin A | 0.304 | 1.877 | 0.062 |
| Vitamin D | -0.009 | -2.355 | 0.020 |
| Vitamin C | 0.026 | 1.793 | 0.074 |

BMI: Body mass index.

China, found that riboflavin intake was markedly deficient, and riboflavin supplements in high-risk groups reduced the incidence of EC[17]. In a multicenter study in China, whole blood riboflavin was tested in 764 EC patients (and in controls), and the analysis revealed that whole blood riboflavin levels were not significantly correlated with the prevalence of EC. However, high whole blood riboflavin level was more

favorable for the survival of elderly EC patients aged 50-70 yrs[18]. Therefore, B vitamin supplementation in EC patients undergoing chemotherapy is beneficial to improve vitamin nutritional status and reduce complications.

In our research, the proportion of vitamin C deficiency cases was 23.65% and 34.48%, before and after chemotherapy, respectively. The difference was statistically significant, and vitamin C was the most deficient vitamin after chemotherapy. Thus, chemotherapy may have a considerable effect on vitamin C levels in EC patients. A meta-analysis showed a negative correlation between dietary vitamin C intake and EC risk and concluded that a high level of vitamin C may prevent EC[19]. Vitamin C supplementation was found to downregulate nuclear factor kappa B activity and significantly decrease proinflammatory cytokine levels in EC patients treated with neoadjuvant radiotherapy[20].

In the current study, vitamin A and D deficiencies were found in EC patients both before and after chemotherapy, and their proportions increased following chemotherapy. However, the changes in the proportions were not statistically significant after chemotherapy. Further stratified analysis of the baseline data showed that the change in vitamin A concentration during chemotherapy might be associated with gender, while vitamin D might differ among different tumor stages. Vitamins A and D are mainly derived from food sources. EC itself can affect the intake and absorption of food, and chemotherapy drugs further damage the nutritional status of patients, resulting in more significant vitamin deficiencies in patients after treatment. In the current study, we further analyzed the difference between vitamin concentrations and BMI in EC patients before and after chemotherapy and found that vitamins A and C were significantly correlated with the change in BMI. Regression analysis of multiple vitamins and BMI revealed a statistically significant overall model for three vitamins (A, D and C). Therefore, body weight, vitamin levels and hematological indicators interact with each other during chemotherapy in EC patients, and the supplementation of macronutrients and micronutrients are equally important. Developing holistic care is the key to nutritional therapy for tumor patients.

There were different degrees of vitamin A and D deficiencies in EC patients treated with chemotherapy, and a higher proportion of vitamin A deficiency than vitamin D deficiency and no vitamin E deficiency was detected during our observation. A meta-analysis suggested that vitamin A levels were negatively correlated with EC risk[21], and further studies are needed to confirm whether it affects the prognosis of EC patients. Vitamin D has been found to inhibit tumor cell proliferation, induce cell differentiation, promote apoptosis and suppress angiogenesis[22]. A meta-analysis did not observe an association between vitamin D levels and the development of esophageal lesions[23]. Another study reported that appropriate vitamin D supplementation in postoperative EC patients improved quality of life and disease-free survival, and further multivariate analysis found that vitamin D supplementation was an independent prognostic factor for disease-free survival but was not associated with overall survival[24].

This study had some limitations: (1) the sample size was not large due to the single center retrospective design of the study; (2) a control group was not included, and it is unclear whether vitamin supplementation is beneficial in EC patients undergoing chemotherapy; (3) the results might be biased due to different disease states and chemotherapy regimens; and (4) some patients might have received targeted therapy or immunotherapy during chemotherapy, which may have had an impact on the study results. In addition, no data on response rate or overall survival were included in our analysis, and the relationship between vitamin nutritional status and prognosis in EC patients requires further investigation in multicenter prospective studies.

## CONCLUSION

Vitamin deficiencies (mainly vitamins A, D, C and $B_2$ deficiencies) are common in EC patients during chemotherapy and may be associated with the change in BMI. In addition, chemotherapy drugs decrease hematological indicators such as hemoglobin and albumin. Appropriate nutritional interventions and vitamin supplementation can reduce the adverse effects of chemotherapy, improve overall nutritional status of patients and improve drug tolerability and quality of life.

## ARTICLE HIGHLIGHTS

### *Research background*

Few studies have examined the changes in vitamin nutritional status and their influencing factors during chemotherapy for esophageal cancer (EC). Most vitamins were found to be negatively associated with the risk of colorectal and gastric cancer in addition to EC, yet interventional treatment failed to demonstrate a clear preventive effect in these malignancies. In our study, the effects of chemotherapeutic drugs on EC patients' vitamin levels and hematological indicators were analyzed.

### *Research motivation*

We analyzed the effects of chemotherapeutic drugs on EC patients' vitamin levels and hematological indicators by detecting the changes in nine vitamins and hematological indicators before and after chemotherapy, with an attempt to provide evidence for vitamin supplementation. Many oncologists believe that vitamin testing is valuable in tumor patients as it can identify whether there is a specific vitamin deficiency and/or justify vitamin therapy. Our findings may be valuable for the implementation of tailored nutritional interventions.

### *Research objectives*

To explore multiple vitamin levels and the possible influential factors in EC patients treated with chemotherapy. Varying degrees of vitamin deficiency and weight loss were found in these patients. Vitamin supplementation may reduce the adverse effects of chemotherapy.

### *Research methods*

Vitamin nutritional status was measured using the electrochemiluminescence method with an LK3000VI vitamin detector before and after two cycles of chemotherapy in EC patients. Statistical analysis was performed using the SPSS 24.0 software package. The latent correlations between multiple vitamin levels (the independent variables) and body mass index (the dependent variable) during chemotherapy were analyzed using the Spearman method.

### *Research results*

Varying degrees of vitamin A, D, C and $B_2$ deficiency and weight loss were found in EC patients. Statistically significant differences were shown in vitamins A, C, $B_2$ and $B_6$ levels and body mass index before and after chemotherapy. Multivariate analysis showed that vitamin A levels significantly differed between male and female EC patients, whereas vitamin D concentrations significantly differed in EC patients in different stages. Correlations were observed between the changes in serum vitamin A and C levels pre- and postchemotherapy and the variation in body mass index.

### *Research conclusions*

Varying degrees of vitamin deficiency and weight loss were found in EC patients undergoing chemotherapy. Vitamin supplementation may help to improve the nutritional status, chemotherapy tolerance and efficacy. To detect the concentrations of vitamins is valuable for EC patients.

### *Research perspectives*

A multicenter prospective study should be performed to reveal the suitable vitamin replenishment programs and potential effects on treatment outcomes and the adverse effects of chemotherapy in EC patients. Thus, randomized control studies and intervention are needed to verify our finding.

## REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Chen R**, Zheng RS, Zhang SW, Zeng HM, Wang SM, Sun KX, Gu XY, Wei WW, He J. [Analysis of incidence and mortality of esophageal cancer in China, 2015]. *Zhonghua Yu Fang Yi Xue Za Zhi* 2019; **53**: 1094-1097 [PMID: 31683393 DOI: 10.3760/cma.j.issn.0253-9624.2019.11.004]

3 **Malhotra GK**, Yanala U, Ravipati A, Follet M, Vijayakumar M, Are C. Global trends in esophageal

cancer. *J Surg Oncol* 2017; **115**: 564-579 [PMID: 28320055 DOI: 10.1002/jso.24592]
4 **Ohnuma H**, Sato Y, Hayasaka N, Matsuno T, Fujita C, Sato M, Osuga T, Hirakawa M, Miyanishi K, Sagawa T, Fujikawa K, Ohi M, Okagawa Y, Tsuji Y, Hirayama M, Ito T, Nobuoka T, Takemasa I, Kobune M, Kato J. Neoadjuvant chemotherapy with docetaxel, nedaplatin, and fluorouracil for resectable esophageal cancer: A phase II study. *Cancer Sci* 2018; **109**: 3554-3563 [PMID: 30137686 DOI: 10.1111/cas.13772]
5 **Ma L**, Luo GY, Ren YF, Qiu B, Yang H, Xie CX, Liu SR, Liu SL, Chen ZL, Li Q, Fu JH, Liu MZ, Hu YH, Ye WF, Liu H. Concurrent chemoradiotherapy combined with enteral nutrition support: a radical treatment strategy for esophageal squamous cell carcinoma patients with malignant fistulae. *Chin J Cancer* 2017; **36**: 8 [PMID: 28077159 DOI: 10.1186/s40880-016-0171-6]
6 **Verzicco I**, Regolisti G, Quaini F, Bocchi P, Brusasco I, Ferrari M, Passeri G, Cannone V, Coghi P, Fiaccadori E, Vignali A, Volpi R, Cabassi A. Electrolyte Disorders Induced by Antineoplastic Drugs. *Front Oncol* 2020; **10**: 779 [PMID: 32509580 DOI: 10.3389/fonc.2020.00779]
7 **Smyth EC**, Lagergren J, Fitzgerald RC, Lordick F, Shah MA, Lagergren P, Cunningham D. Oesophageal cancer. *Nat Rev Dis Primers* 2017; **3**: 17048 [PMID: 28748917 DOI: 10.1038/nrdp.2017.48]
8 **Jain A**, Tiwari A, Verma A, Jain SK. Vitamins for Cancer Prevention and Treatment: An Insight. *Curr Mol Med* 2017; **17**: 321-340 [PMID: 29210648 DOI: 10.2174/1566524018666171205113329]
9 **Nasir A**, Bullo MMH, Ahmed Z, Imtiaz A, Yaqoob E, Jadoon M, Ahmed H, Afreen A, Yaqoob S. Nutrigenomics: Epigenetics and cancer prevention: A comprehensive review. *Crit Rev Food Sci Nutr* 2020; **60**: 1375-1387 [PMID: 30729798 DOI: 10.1080/10408398.2019.1571480]
10 **Shi HP**. Cancer is a metabolic disease. *Zhongliu Daixie Yu Yingyang Dianzi Zazhi* 2018; **5**: 111-116 [DOI: 10.16689/j.cnki.cn11-9349/r.2018.02.001]
11 **Zhang XS**, Liu ZH, Peng Y, Yang XY, Zhang Y, Xu Q, Liu YH. Analysis of vitamin nutritional status in hospitalized cancer patients. *Zhongguo Zhongliu Linchuang Yu Kangfu* 2018; **25**: 1448-1451 [DOI: 10.13455/j.cnki.cjcor.2018.12.11]
12 **Veeralakshmanan P**, Tham JC, Wright A, Bolter M, Wadhawan H, Humphreys LM, Sanders G, Wheatley T, Berrisford RJ, Ariyarathenam A. Nutritional deficiency post esophageal and gastric cancer surgery: A quality improvement study. *Ann Med Surg (Lond)* 2020; **56**: 19-22 [PMID: 32566222 DOI: 10.1016/j.amsu.2020.05.032]
13 **Zhang X**. Analysis of vitamin deficiency in palliative care cancer patients. *Heilongjiang Yixue* 2020; **44**: 437-438
14 **Masri OA**, Chalhoub JM, Sharara AI. Role of vitamins in gastrointestinal diseases. *World J Gastroenterol* 2015; **21**: 5191-5209 [PMID: 25954093 DOI: 10.3748/wjg.v21.i17.5191]
15 **Ma JL**, Zhao Y, Guo CY, Hu HT, Zheng L, Zhao EJ, Li HL. Dietary vitamin B intake and the risk of esophageal cancer: a meta-analysis. *Cancer Manag Res* 2018; **10**: 5395-5410 [PMID: 30464635 DOI: 10.2147/CMAR.S168413]
16 **Qiang Y**, Li Q, Xin Y, Fang X, Tian Y, Ma J, Wang J, Wang Q, Zhang R, Wang F. Intake of Dietary One-Carbon Metabolism-Related B Vitamins and the Risk of Esophageal Cancer: A Dose-Response Meta-Analysis. *Nutrients* 2018; **10** [PMID: 29954131 DOI: 10.3390/nu10070835]
17 **Li JB**, Zou XN, Wang HY, Tao DM, Qiao YL, Gu YK, Zheng SF. Effect of riboflavin-fortified-salt intervention on esophageal preancerous lesions among population with high risk in Yanting County. *Zhongguo Zhongliu Fangzhi Zazhi* 2009; **16**: 325-328 [DOI: 10.16073/j.cnki.cjcpt.2009.05.002]
18 **Li SS**, Tan HZ, Xu YW, Wu ZY, Wu JY, Zhao XK, Wang LD, Long L, Li EM, Xu LY, Zhang JJ. [The association between the whole blood riboflavin level and the occurrence, development and prognosis of esophageal squamous cell carcinoma]. *Zhonghua Yu Fang Yi Xue Za Zhi* 2019; **53**: 1124-1129 [PMID: 31683399 DOI: 10.3760/cma.j.issn.0253-9624.2019.11.010]
19 **Bo Y**, Lu Y, Zhao Y, Zhao E, Yuan L, Lu W, Cui L, Lu Q. Association between dietary vitamin C intake and risk of esophageal cancer: A dose-response meta-analysis. *Int J Cancer* 2016; **138**: 1843-1850 [PMID: 26355388 DOI: 10.1002/ijc.29838]
20 **Abdel-Latif MMM**, Babar M, Kelleher D, Reynolds JV. A pilot study of the impact of Vitamin C supplementation with neoadjuvant chemoradiation on regulators of inflammation and carcinogenesis in esophageal cancer patients. *J Cancer Res Ther* 2019; **15**: 185-191 [PMID: 30880777 DOI: 10.4103/jcrt.JCRT_763_16]
21 **Li K**, Zhang B. The association of dietary β-carotene and vitamin A intake on the risk of esophageal cancer: a meta-analysis. *Rev Esp Enferm Dig* 2020; **112**: 620-626 [PMID: 32543872 DOI: 10.17235/reed.2020.6699/2019]
22 **Rouphael C**, Kamal A, Sanaka MR, Thota PN. Vitamin D in esophageal cancer: Is there a role for chemoprevention? *World J Gastrointest Oncol* 2018; **10**: 23-30 [PMID: 29375745 DOI: 10.4251/wjgo.v10.i1.23]
23 **Zgaga L**, O'Sullivan F, Cantwell MM, Murray LJ, Thota PN, Coleman HG. Markers of Vitamin D Exposure and Esophageal Cancer Risk: A Systematic Review and Meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2016; **25**: 877-886 [PMID: 27030602 DOI: 10.1158/1055-9965.EPI-15-1162]
24 **Wang L**, Wang C, Wang J, Huang X, Cheng Y. Longitudinal, observational study on associations between postoperative nutritional vitamin D supplementation and clinical outcomes in esophageal cancer patients undergoing esophagectomy. *Sci Rep* 2016; **6**: 38962 [PMID: 27958342 DOI: 10.1038/srep38962]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2376-2393

DOI: 10.3748/wjg.v27.i19.2376

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

ORIGINAL ARTICLE

Observational Study

# Effects of sepsis and its treatment measures on intestinal flora structure in critical care patients

Xiao-Juan Yang, Dan Liu, Hong-Yan Ren, Xiao-Ya Zhang, Jun Zhang, Xiao-Jun Yang

**ORCID number:** Xiao-Juan Yang 0000-0002-7759-2417; Dan Liu 0000-0003-4189-4402; Hong-Yan Ren 0000-0002-0552-9005; Xiao-Ya Zhang 0000-0003-1714-6797; Jun Zhang 0000-0002-2540-6352; Xiao-Jun Yang 0000-0002-9659-8662.

**Author contributions:** Yang XJ was the guarantor and designed the study; Yang XJ participated in the acquisition, analysis, and interpretation of the data, and drafted the initial manuscript; Liu D, Zhang XY, Ren HY, and Zhang J revised the article critically for important intellectual content.

**Supported by** the National Key Research and Development Program of China, No. 2016YFD0400605.

**Institutional review board statement:** This study was approved by the Ethics Committee of the General Hospital of Ningxia Medical University (Ethical Approval No. 2016-258).

**Informed consent statement:** Informed consent was obtained from all patients or their immediate family members.

**Conflict-of-interest statement:** Yang XJ reports grants from Ministry of Science and Technology of the People's Republic of China, during

**Xiao-Juan Yang, Xiao-Ya Zhang, Jun Zhang, Xiao-Jun Yang,** Department of Critical Care Medicine, General Hospital of Ningxia Medical University, Yinchuan 750004, Ningxia Hui Autonomous Region, China

**Dan Liu,** Department of Critical Care Medicine, Taihe Hospital, Hubei University of Medicine, Shiyan 442000, Hubei Province, China

**Hong-Yan Ren,** Shanghai Mobio Biomedical Technology Co., Shanghai 201318, China

**Corresponding author:** Xiao-Jun Yang, MM, Chief Doctor, Professor, Department of Critical Care Medicine, General Hospital of Ningxia Medical University, No. 804 Shengli South Street, Xingqing District, Yinchuan 750004, Ningxia Hui Autonomous Region, China.
yxjicu@163.com

## Abstract

### *BACKGROUND*

Sepsis is a common disease in intensive care units, with high morbidity and mortality. Intestinal microecology plays a vital part in the development and progression of this disease, possibly because sepsis and its treatment cause specific changes in the composition of the intestinal flora.

### *AIM*

To investigate the characteristics of intestinal flora disturbance in sepsis patients treated with antibiotics.

### *METHODS*

In this prospective comparative study, we enrolled ten patients with sepsis (sepsis group), hospitalized in the Department of Critical Care Medicine of the General Hospital, Ningxia Medical University, China (a class IIIa general hospital) from February 2017 to June 2017; ten patients without sepsis hospitalized in the same period (non-sepsis group) and ten healthy individuals (control group) were also enrolled. Fecal samples collected from the three groups were subjected to *16S rRNA* gene sequencing and the intestinal flora diversity, structure, and composition were determined. Additionally, the dynamics of the intestinal flora diversity, structure, and composition in sepsis patients were investigated *via 16S rRNA* gene sequencing of samples collected 0 d, 3 d, and 7 d after admittance to the intensive care unit. Correlations between the serum levels of procalcitonin, endotoxin, diamine oxidase, and D-lactic acid and the intestinal flora composition

the conduct of the study. Other authors have nothing to disclose.

**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. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Specialty type:** Infectious diseases

**Country/Territory of origin:** China

**Peer-review report's scientific quality classification**

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

**Received:** January 16, 2021
**Peer-review started:** January 16, 2021
**First decision:** February 10, 2021
**Revised:** February 23, 2021
**Accepted:** April 22, 2021
**Article in press:** April 22, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Zhang Z
**S-Editor:** Liu M
**L-Editor:** Wang TQ
**P-Editor:** Liu JH

of sepsis patients were also investigated.

*RESULTS*

Compared with the healthy control group, sepsis and non-sepsis patients showed reduced intestinal flora α-diversity and a distinct flora structure, with Firmicutes as the dominant phylum, and significantly decreased proportions of Bacteroidetes, as well as *Prevotella* and *Lachnospira*, among other genera. Of note, the proportion of *Enterococcus* was significantly increased in the intestinal tract of sepsis patients. Interestingly, the α-diversity in the sepsis group decreased gradually, from days 1 to 7 of treatment. However, pairwise comparisons showed that both the diversity and structure of the intestinal flora were not significantly different considering the three different time points studied. Curiously, the serum levels of procalcitonin, endotoxin, diamine oxidase, and D-lactic acid in sepsis patients correlated with the prevalence of various bacterial genera. For example, the prevalence of *Ruminococcus* was positively correlated with serum procalcitonin, endotoxins, and diamine oxidase; similarly, the prevalence of *Roseburia* was positively correlated with serum procalcitonin, endotoxins, and D-lactic acid.

*CONCLUSION*

Sepsis patients in intensive care units show dysbiosis, lasting for at least 1 wk.

**Key Words:** Sepsis; Intestinal flora; *16S rRNA* gene sequencing; Dynamic changes; Intestinal barrier index; Procalcitonin

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** As the largest reservoir of bacteria and endotoxins in the body, the intestinal tract is regarded as the "engine" of sepsis and multiple organ dysfunction. Through *16S rRNA* gene sequencing, we observed that intestinal flora disturbance occurs in sepsis patients. Notably, here, we revealed for the first time the intestinal flora dynamic changes in sepsis patients during treatment. We found that the abundance of some intestinal bacteria in sepsis patients significantly correlated with infection- and intestinal barrier-related clinical indicators. These findings add to the understanding of the intestinal flora in sepsis, providing a basis for the reversal of dysbiosis.

**Citation:** Yang XJ, Liu D, Ren HY, Zhang XY, Zhang J, Yang XJ. Effects of sepsis and its treatment measures on intestinal flora structure in critical care patients. *World J Gastroenterol* 2021; 27(19): 2376-2393
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2376.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2376

## INTRODUCTION

Sepsis refers to the life-threatening organ dysfunction caused by imbalanced responses to infection[1]. As the largest reservoir of bacteria and endotoxins in the body, the intestinal tract is regarded as the "engine" of sepsis and multiple organ dysfunction syndrome. Under normal circumstances, the intestinal flora plays beneficial roles in the context of human physiology and immunity[2-3], existing in a homeostatic state important for the maintenance of human health. However, in the context of critical illness, homeostasis is interrupted, leading to abnormal changes in the types, quantities, proportions, and locations of microorganisms in the intestinal tract[4], thus increasing susceptibility to sepsis. In fact, studies have shown that there is a dose–response relationship between the degree of intestinal microecological disturbance and the incidence of subsequent severe sepsis[5]. Therefore, an improved understanding of the status and degree of intestinal flora disturbances in sepsis patients is of great significance; for instance, the establishment of strong intestinal microecology-based biomarkers will allow for the accurate prediction of prognosis of sepsis patients and the consequent adoption of adequate treatment measures. However, there are few reports on the sepsis-related intestinal flora and its dynamic

changes.

High-throughput *16S rRNA* gene sequencing allows for a comprehensive understanding of the intestinal flora structure, and it is the standard technology used for human intestinal flora analysis[6]. In this study, the diversity and composition of the intestinal flora in sepsis patients, non-sepsis patients, and healthy individuals were analyzed and compared. The dynamic changes in the intestinal flora α-diversity and structure throughout a 1 wk period [1, 3, and 7 d after admittance to the intensive care unit (ICU)] were also investigated to disclose the transformation of the intestinal flora in the context of antibiotic treatment. In addition, the potential relationships between the intestinal flora imbalance and clinical indicators in sepsis were investigated *via* correlation analysis.

## MATERIALS AND METHODS

### General information

This is a prospective observational study, including ten sepsis patients (sepsis group) admitted to the ICU of the General Hospital, Ningxia Medical University, China from February 2017 to June 2017, ten patients without sepsis (non-sepsis group) admitted to the same ICU during the same time period, and ten local healthy individuals (control group). The inclusion criteria for sepsis patients were as follows: 18-75 years old; sepsis patients meeting the latest definition of sepsis in "Sepsis-3.0" issued by the Society of Critical Care Medicine (SCCM) in 2016[7]; and estimated time of more than 2 d spent in the ICU after enrollment. The inclusion criteria for the non-sepsis patients were as follows: 18-75 years old; and patients admitted to the ICU at the same period owing to diseases other than sepsis, such as multiple injuries and high-risk operations. Of note, we tried to match the age, underlying disease, and surgical site with those in patients in the sepsis group. Because there was no definite infection, the non-sepsis group was not given antibiotics before the fecal samples were collected. The exclusion criteria were as follows: Not meeting the inclusion criteria; perianal infection; patients subjected to enterostomy; and patients with chronic gastrointestinal diseases. Additionally, the requirements for the healthy control group were as follows: Matched age with the aforementioned two groups of patients; good health; no history of chronic or metabolic diseases (*e.g.*, hypertension, coronary heart disease, diabetes, hepatitis, and hyperthyroidism); no history of digestive tract diseases or digestive tract surgery; and not using antibiotics, probiotics, enteral nutrients, or other drugs within 3 mo before enrollment. Informed consent was obtained from all patients or their immediate family members, and this study was approved by the hospital ethics committee (ethics approval number: 2016-258).

### Research methods

The general clinical data of patients in the sepsis and non-sepsis groups were recorded, including age, main diagnosis, operation type (abdominal cavity organ operation and non-abdominal cavity organ operation), clinical infection site, pathogenic bacterial agent (based on qualitative culture), and use of antibiotics (Tables 1-3). The acute physiology and chronic health (APACHE II) score and sequential organ failure assessment (SOFA) score of patients on the day of ICU admission were recorded (Table 1). The primary infection site, surgical site, and use of antibiotics in the sepsis group were also recorded (Table 2), as were the positive results of blood, urine, sputum, and other body fluids collected from patients with sepsis (Table 3). Additionally, venous blood samples were collected from patients with sepsis on days 1, 3, and 7 after admission to the ICU, and procalcitonin (PCT) was detected by immunochromatography [PCT-Q detection card produced by BRAHMS GmbH in Germany, provided by Thermo Fisher Scientific (China) Co., Ltd.], while the levels of serum D-lactic acid (D-Lac), bacterial endotoxin (endotoxin), and diamine oxidase (DAO) were determined using the JY-DLT intestinal barrier function biochemical index analysis system (Beijing Zhongsheng Jinyu Diagnosis Technology Co., Ltd., China.) within 0.5 h after blood collection.

### Collection of stool samples and sequencing

Stool samples were collected from sepsis patients on days 1, 3, and 7 after admission to the ICU, from non-sepsis patients on day 1 after admission to the ICU, and from healthy controls. Samples from patients were taken from a deep part of the fresh stool of patients by using a sampling spoon, and the stool samples were quickly placed in a special stool sample box. The stool samples of healthy control subjects were collected

**Table 1 Comparison of the general information among the three groups of individuals**

| Indicator | Control group (*n* = 10) | Non-sepsis group (*n* = 10) | Sepsis group (*n* = 10) | *H*/*F*/$\chi^2$/*Z*/*t* value | *P* value |
|---|---|---|---|---|---|
| Age (yr, mean ± SD) | 55 (48.75, 55.25) | 59 (50.00, 74.00)[a] | 63.50 (44.75, 76.25)[a,b] | 2.05 | 0.36 |
| BMI (kg/m$^2$, mean ± S) | 23.95 ± 2.04 | 27.19 ± 4.40[a] | 23.91 ± 4.09[a,b] | 2.64 | 0.09 |
| Underlying disease (cases) | | | | 0.84 | 0.65 |
| Multiple injuries | | 3 | 4 | | |
| Malignant tumor | | 5 | 3 | | |
| Other diseases | | 2 | 3 | | |
| Type of operation (cases) | | | | 0.00 | 1.00 |
| Surgery on abdominal hollow viscera | - | 5 | 5 | | |
| Surgery on non-abdominal hollow viscera | - | 5 | 5 | | |
| APACHE II score on day 1 [points, *M* ($P_{25}$, $P_{75}$)] | - | 10.00 (7.00, 17.00) | 19.00 (15.25, 21.50) | −2.40 | 0.02 |
| SOFA score on day 1 (points, mean ± SD) | - | 3.30 ± 1.42 | 10.80 ± 2.97 | −7.20 | 0.00 |

APACHE II score: Acute Physiologic Assessment and Chronic Health Evaluation II Scoring System; SOFA score: Sequential Organ Failure Assessment Score; BMI: Body mass index.
[a]$P < 0.05$.
[b]$P < 0.01$.

**Table 2 General clinical information of the patients in the sepsis group**

| Patient No. | Primary infection site | Surgical site | Categories of antibiotics used within 7 d after admission to the ICU |
|---|---|---|---|
| S1 | Lungs | - | Broad-spectrum penicillins |
| S2 | Abdominal cavity | Hollow viscera | Carbapenems + glycopeptides |
| S3 | Lungs | Hollow viscera | Carbapenems + oxazolidinones |
| S4 | Lungs | Joint | Broad-spectrum penicillins + carbapenems |
| S5 | Abdomen | Hollow viscera | Broad-spectrum penicillins + glycopeptides |
| S6 | Lungs | - | Broad-spectrum penicillins + tetracyclines |
| S7 | Lungs | - | Carbapenems + broad-spectrum penicillins |
| S8 | Abdominal cavity | - | Carbapenems |
| S9 | Abdominal cavity | Hollow viscera | Carbapenems |
| S10 | Abdominal cavity | Hollow viscera | Carbapenems + glycopeptides |

Note: S*n* represents the sepsis patient number *n* in the sepsis group; *e.g.*, S1 represents sepsis patient No. 1. ICU: Intensive care unit.

in a special stool sample box after normal defecation. Then, the small stool boxes filled with samples were sealed, labeled, placed in a liquid nitrogen tank, and transferred to a -80 °C freezer for storage.

Each stool sample was added to 790 μL of lysis buffer [4 M guanidine thiocyanate, 250 μL; 10% N-lauroyl sarcosine, 40 μL; 5% N-lauroyl sarcosine-0.1 M phosphate buffer (pH 8.0), 500 μL] together with 1 g glass beads (0.1 mm, Biospec Products, Inc., United States). After sufficient vortex-based homogenization, bead beating was performed for 10 min at full speed. The subsequent extraction was carried out according to the instructions of the extraction kit manufacturer (E.Z.N.A.®Stool DNA Kit, Omega Bio-tek, Inc., GA). The V3–V4 region of the *16S rRNA* gene was amplified with the primers 341F/805R (341F: 5′-CCTACGGGNGGCWGCAG-3′; 805R: 5′-GACTACHVGGGTATCTAATCC-3′), and the PCR products were sequenced on an Illumina MiSeq 2*300 bp platform. The raw sequencing data and accompanying information are available in the Sequence Read Archive database under the accession

**Table 3 Pathogenic bacteria isolated in the sepsis group**

| Pathogenic bacteria | Cultured sample | Patient No. | Corresponding sample collection time (day *n* after admission to the ICU) |
|---|---|---|---|
| *Acinetobacter baumannii* | Sputum | S5, S6, S8 | 4, 13, 23 |
| *Stenotrophomonas maltophilia* | Sputum | S6, S7 | 1, 8 |
| *Enterococcus* | Sputum | S7 | 23 |
| *Escherichia coli* | Blood | S7 | 1 |

S*n* represents the sepsis patient number *n* in the sepsis group; *e.g.*, S5 represents sepsis patient No. 5. In the table, sputum and blood samples from patients in the sepsis group were subjected to qualitative cultures, and bacterial colonization and contamination were ruled out. ICU: Intensive care unit.

number PRJNA691455.

### Bioinformatics and statistical analysis

The clinical data were processed and analyzed using SPSS 19.0 software. All data were first tested for normality and homogeneity of variance. Data conforming to a normal distribution and homogeneity of variance are expressed as the mean ± SD, and the *t*-test or analysis of variance were used for statistical analysis. Data not conforming to a normal distribution are expressed as medians ($P_{25}$, $P_{75}$), and the rank-sum test was used for statistical analysis. The $\chi^2$ test was used to compare enumeration data, and $P < 0.05$ was considered statistically significant.

USEARCH 8.0 was used to process the raw sequencing data, and operational taxonomic units (OTUs) were classified according to 97% sequence similarity. The OTU representative sequences were compared against the SILVA database (SSU123; http://www.arb-silva.de) with a 70% confidence threshold, and the taxonomic status of each *16S rDNA* sequence was obtained. The α-diversity of each sample was evaluated using the Shannon–Wiener and Simpson's diversity indexes, and the significance of differences was tested using either the nonparametric Mann–Whitney *U* test or the Kruskal–Wallis rank-sum test. Principal coordinates analysis (PCoA) and the calculation of the linear discriminant analysis effect size (LEfSe) were performed using the R software package (http://www.R-project.org/); heatmaps were also obtained using the R software. Spearman correlation analysis was also carried out to assess the relationships between relevant parameters.

## RESULTS

### Characteristics of the participants

A total of ten sepsis patients, ten non-sepsis patients, and ten healthy individuals were included in this study. Table 1 shows that there were no significant differences with respect to age or body mass index among the groups, as revealed by variance analysis ($P > 0.05$). The underlying diseases of the patients in the sepsis and non-sepsis groups were divided into three categories: Multiple injuries, malignant tumors, and other diseases (including benign tumors and intestinal obstruction). Of note, there were no significant differences in the underlying diseases and operation types between the sepsis and non-sepsis groups as revealed by the $\chi^2$ test ($P > 0.05$), indicating that the groups were matched (and accurate comparisons were possible). The APACHE II and SOFA scores in the sepsis group were significantly higher than those in the non-sepsis group on day 1 of ICU admission ($P < 0.05$), suggesting that the patients in the sepsis group had worse pathology (with acute organ dysfunction) compared with those in the non-sepsis group.

### Comparison of intestinal flora structure in healthy individuals and non-sepsis and sepsis patients on day 1 after ICU admittance

A total of 49 fecal samples were included for analysis. Of note, one stool sample in the sepsis group (patient S3, day 3) was missing owing to a problem in specimen collection. After quality control procedures, 629665 high-quality sequences were obtained, and the qualified reads were clustered into 440 species-level OTUs using 97% as the similarity cutoff. In this study, the number of qualified reads of inpatients

in the ICU was generally much lower than that of healthy controls. To understand whether these differences were due to low bacterial numbers, we randomly selected five specimens from each group to quantify the total bacteria in the feces. Regardless of whether bacteria were counted as bacterial copy number per gram fecal samples (mean bacterial copy numbers in sepsis, non-sepsis, and healthy control groups, 5.07E + 11, 1.36E + 12, and 9.27E + 12, respectively) or per nanogram bacterial DNA (9.02E + 06, 3.54E + 07, and 4.90E + 07, respectively), there was a significant overall decreasing trend in both the sepsis and non-sepsis groups relative to that in healthy controls. Therefore, altogether, these results suggest that the low number of qualified reads in some samples in the sepsis and non-sepsis group is secondary to the significantly reduced number of intestinal bacteria, probably because of the use of large amounts of antibiotics in the ICU.

**α-diversity:** The Mann–Whitney test showed that the α-diversity in the non-sepsis and sepsis groups on day 1 after ICU admittance was significantly lower than that in the control group ($P < 0.05$). Moreover, the diversity of the intestinal flora in sepsis patients was lower than that in non-sepsis patients; however, this difference was not significant (Figure 1 and Table 4).

**Overall structural comparison of the intestinal flora:** The PCoA plot based on the Bray–Curtis dissimilarity between samples revealed that the flora composition in sepsis patients was similar to that in non-sepsis patients on day 1 after ICU admittance (Figure 2). In line with these results, Adonis analysis showed no significant difference in the composition of the intestinal flora between sepsis and non-sepsis patients on day 1 after ICU admittance ($P > 0.05$). However, the difference in the flora composition between these groups and normal controls was extremely significant (Adonis $P < 0.05$).

**Comparison of intestinal flora compositions at the phylum level:** The flora composition results revealed that Firmicutes and Bacteroidetes were the dominant phyla in healthy individuals, accounting for more than 70% of the total bacteria. Meanwhile, in sepsis and non-sepsis patients, Firmicutes was the dominant phylum in the intestinal flora (Figure 3A); the proportion of Bacteroidetes decreased significantly compared to that in healthy individuals ($P < 0.05$), and the relative abundance of Proteobacteria increased compared to that in normal controls, although the difference was not significant. Of note, the proportion of Fusobacteria in the intestinal tract of non-sepsis patients was significantly higher than that in both healthy controls and sepsis patients ($P < 0.05$; Figure 3B).

**Comparison of intestinal flora compositions at the genus and OTU levels:** The results of the Kruskal–Wallis test showed that the proportions of *Prevotella*, *Subdoligranulum*, *Lachnospira*, *Phascolarctobacterium*, *Alloprevotella*, *Megamonas*, and *Parasutterella* in the intestinal tract of patients in the non-sepsis and sepsis groups decreased significantly compared to those in the healthy control group ($P < 0.05$). Furthermore, the abundance of *Enterococcus* was much higher in the intestinal tract of sepsis patients than in non-sepsis patients and healthy controls. In contrast, the abundance of *Fusobacterium*, *Anaerococcus*, and *Peptostreptococcus* was significantly higher in the intestinal tract of non-sepsis patients than in healthy controls and sepsis patients (Figure 4A).

Additionally, as per the heatmap analysis (random forest-based), 64 key OTUs were different among the three groups (Figure 4B). Among these, the relative abundance of OTUs assigned to the genera *Subdoligranulum*, *Alistipes*, *Megamonas*, *Blautia, Bacteroides*, *Dialister*, *Anaerostipes*, *Lachnospira*,*Roseburia*, *Pseudobutyrivibrio, Parasutterella, Prevotella*, *Coprococcus*, *Parabacteroides*, *Paraprevotella*, and *Bifidobacterium* was lower in the non-sepsis and sepsis groups than in the normal control group, whereas the abundance of OTUs assigned to the genera *Peptostreptococcus*, *Enterococcus*, *Thermoanaerobacterium*, and *Anoxybacillus* was decreased in the intestinal microbiota of the normal control group. In addition, the relative abundance of OTUs assigned to the genera *Granulicatella* and *Streptococcus* was lower in the sepsis group than in the non-sepsis group.

### *Dynamic changes in intestinal flora structure in sepsis patients undergoing treatment*

**α-diversity:** The Mann–Whitney test showed that the α-diversity in the sepsis group decreased significantly within 1 wk (from days 1 to 7 after the initiation of ICU treatment) compared to that in the control group; importantly, these differences were significant ($P < 0.05$). Of note, on days 1, 3, and 7 of treatment, the α-diversity of the intestinal flora decreased gradually, but pairwise comparisons did not show

**Table 4 Statistical comparison of the α-diversity indexes of the intestinal flora in the three groups of individuals**

| α-diversity index | *P* value | | |
|---|---|---|---|
| | Control-median *vs* non-sepsis-median | Control-median *vs* sepsis-median | Non-sepsis-median *vs* sepsis-median |
| **Ace** | 0.003 | 0.002 | 0.247 |
| **Chao** | 0.002 | 0.003 | 0.393 |
| **Shannon** | 0.023 | 0.035 | 0.795 |
| **Simpson** | 0.035 | 0.023 | 0.739 |
| **Observed OTUs** | 0.001 | 0.002 | 0.623 |

The Ace and Chao indexes reflect the microbial abundances in samples. Larger values indicate higher microbial abundance. The Shannon and Simpson indexes reflect the microbial diversity of samples. A larger Shannon index indicates a higher diversity, whereas a larger Simpson index indicates a lower diversity. $P < 0.05$ was considered statistically significant. OTUs: Operational taxonomic units.

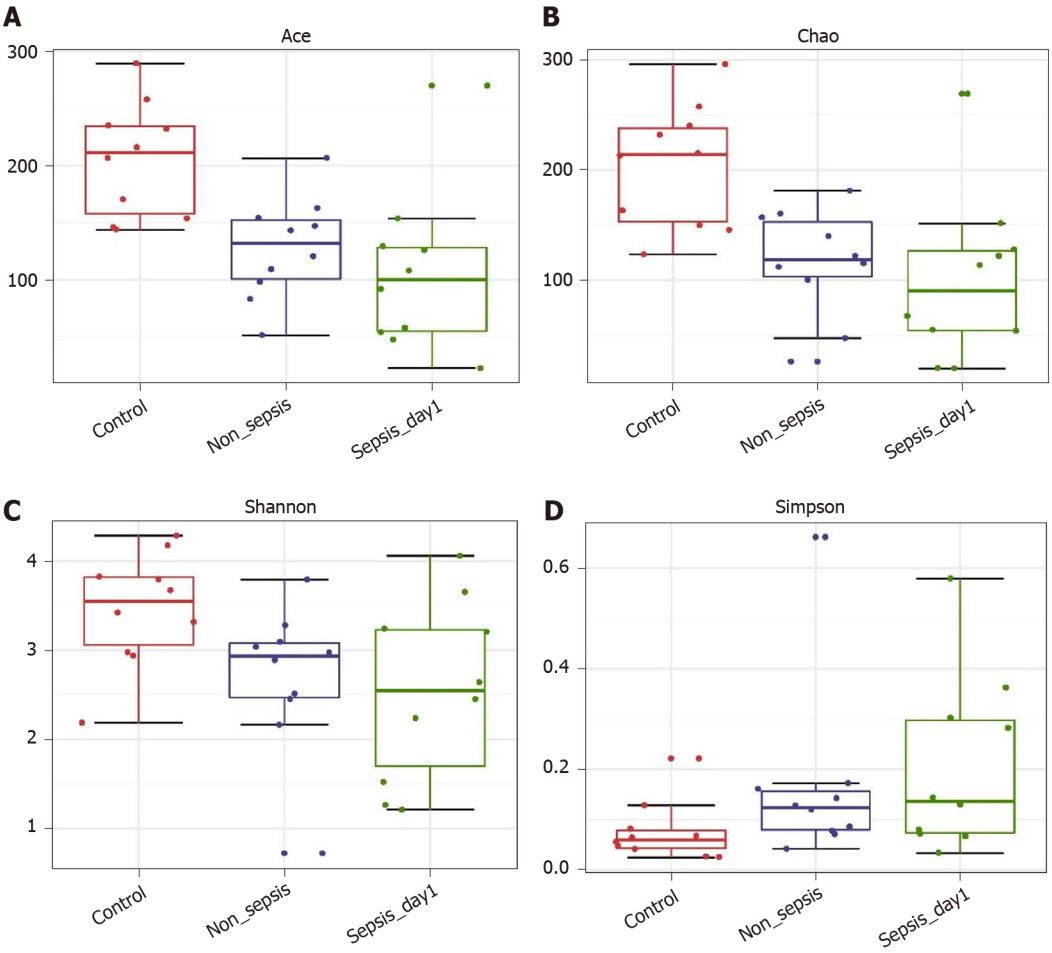



**Figure 1 α-diversity indexes of fecal microbiota in normal individuals, non-sepsis patients, and sepsis patients.** A: ACE estimator; B: Chao 1 estimator; C: Shannon index; D: Simpson index.

significant differences. This suggests that antibiotics killed many intestinal bacteria, and that the diversity of the intestinal flora not only did not recover but also showed a downward trend when antibiotics were continuously used (Figure 5 and Table 5).

**Overall intestinal flora composition changes in sepsis patients within 1 wk after treatment:** The PCoA plot based on Bray–Curtis dissimilarities between samples showed that the intestinal flora composition in sepsis patients within 1 wk of treatment (from days 1 to 7 after ICU admittance) was different from that in normal controls (Figure 6); Adonis analysis showed that the difference between sepsis patients

**Table 5 Variation in the α-diversity indexes of the intestinal flora in patients with sepsis within 1 wk of treatment (days 1, 3, and 7 after intensive care unit admittance)**

| α-diversity index | *P* value | | |
|---|---|---|---|
| | Day 1 *vs* day 3 | Day 1 *vs* day 7 | Day 3 *vs* day 7 |
| **Ace** | 0.066 | 0.121 | 0.347 |
| **Chao** | 0.205 | 0.384 | 0.595 |
| **Shannon** | 0.653 | 0.307 | 0.487 |
| **Simpson** | 0.595 | 0.241 | 0.347 |
| **Observed OTUs** | 0.177 | 0.384 | 0.623 |

OTUs: Operational taxonomic units.

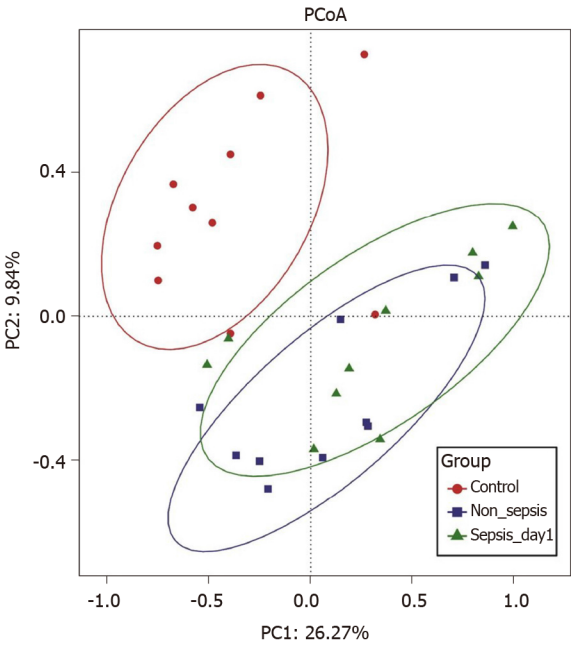



**Figure 2 Principal coordinate analysis of intestinal flora among normal individuals, sepsis patients, and non-sepsis patients.** Principal coordinate analysis was plotted based on the Bray–Curtis dissimilarity between samples. The ellipses highlight the clustering of the fecal microbiomes according to groups (red: Healthy control group; blue: Non-sepsis group; green: Day 1 of sepsis).

and normal controls was extremely significant ($P < 0.05$). However, the structure of the intestinal flora of sepsis patients did not differ significantly with respect to the different days after ICU admittance in the sepsis group.

**Identification of differential bacterial taxa between sepsis patients 1 wk after treatment and normal individuals:** To screen for the bacterial genera associated with significant differences in the intestinal flora of sepsis patients within 1 wk of admission to the ICU, we reduced the dimensions *via* the calculation of LEfSe to obtain the linear discriminant analysis scores. Compared to normal controls, in sepsis patients, some harmful bacteria such as *Coprococcus* disappeared from the intestinal flora within 1 wk of ICU treatment, indicating the efficacy of drugs against pathogenic bacteria (Figure 7). At the same time, the abundance of most of the beneficial bacteria, such as *Prevotella* and *Bifidobacterium*, also decreased, indicating that the effect of drug treatment, especially antibiotics, was not limited to harmful bacteria. However, the abundance of some bacteria, such as *Enterococcus* and *Hemophilus*, still increased despite the action of antibiotics, indicating that these bacteria were resistant to the antibiotics used.

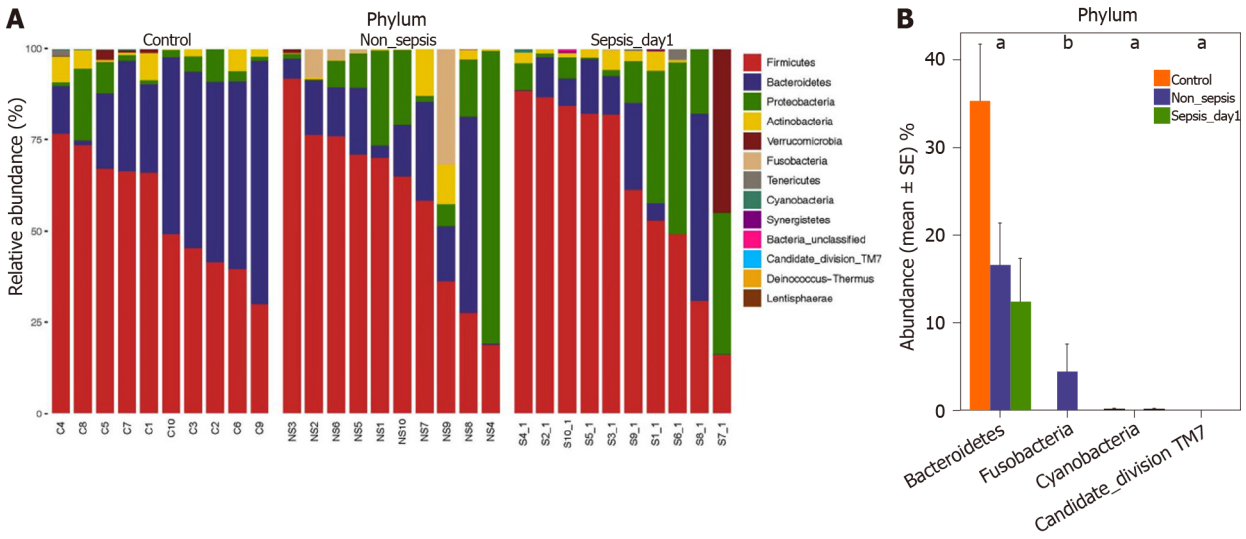



**Figure 3 Composition and comparison of the fecal microbiota among normal individuals, sepsis patients, and non-sepsis patients at the phylum level.** A: Composition of the fecal microbiota at the phylum level among three groups; B: Comparison of the fecal microbiota at the phylum level among three groups. The average abundance of each phylum is depicted as the mean ± SE. *P* values were calculated using the Kruskal–Wallis rank-sum test: $^{a}P < 0.05$, $^{b}P < 0.01$.

### *Correlation analysis between clinical indicators and abundance of intestinal bacterial genera in patients with sepsis*

There were ten patients in the sepsis group (S1–S10). Venous blood samples were collected on days 1, 3, and 7 after admission to the ICU to determine the levels of permeability-related D-Lac, endotoxin, and DAO and infection-related PCT. In the blood of sepsis patients, the levels of D-Lac, endotoxin, DAO, and PCT were 20.96 ± 11.90 mg/L, 10.65 ± 7.92 U/L, 19.58 ± 17.61 U/L, and 7.23 ± 13.92 ng/mL, respectively.

Based on the Spearman correlation analysis, significant correlations were established between clinical indicators and abundance of intestinal bacterial genera in sepsis patients (Figure 8). The number of days of admission in the ICU negatively correlated with the abundance of *Streptococcus* and *Ruminococcus* ($P < 0.05$). The abundance of *Roseburia* and *Parasutterella* was positively correlated ($P < 0.05$), while that of *Prevotella*, *Lactobacillus*, and *Finegoldia* was negatively correlated with the levels of D-Lac ($P < 0.05$). Additionally, the abundance of *Ruminococcus*, *Roseburia*, *Sutterella*, *Peptostreptococcus*, *Escherichia-Shigella*, *Dorea*, *Bilophila*, *Coprobacter*, *Clostridium_sensu_stricto_1*, *Parabacteroides*, *Bifidobacterium*, *Alistipes*, and *Parasutterella*, as well as some genera belonging to Lachnospiraceae, Christensenellaceae, Ruminococcaceae, and Erysipelotrichaceae, was positively correlated with the levels of PCT ($P < 0.05$). Conversely, the abundance of *Stenotrophomonas* and *Enterococcus* was negatively correlated with the levels of PCT ($P < 0.05$). The abundance of *Peptostreptococcus*, *Roseburia*, *Collinsella*, *Dorea*, and VadinBB60 group norank was also positively correlated with the endotoxin levels ($P < 0.05$), while that of *Dialister*, *Pepto streptococcus*, with the endotoxin levPeptostreptococcus, *Dorea*, *Hemophilus*, *Bifidobacterium*, *Collinsella*, and *Blautia* was positively correlated with the levels of DAO ($P < 0.05$).

## DISCUSSION

### *Characteristics of intestinal flora structure in sepsis patients*

Sepsis is associated with high morbidity and mortality worldwide, and its timely identification and treatment are difficult. Therefore, improving the diagnosis and treatment of sepsis is essential from a global health perspective[8]. In February 2016, the SCCM and the European Society of Intensive Care Medicine jointly released the definition of sepsis in "Sepsis 3.0" as follows: "Life-threatening organ dysfunction due to a dysregulated host response to infection"[7]. This definition emphasizes the close relationship between sepsis and organ dysfunction; of note, the intestinal tract is recognized as the "engine" of organ dysfunction. The intestinal flora, as an important component of the intestinal tract, plays major physiological roles in the context of

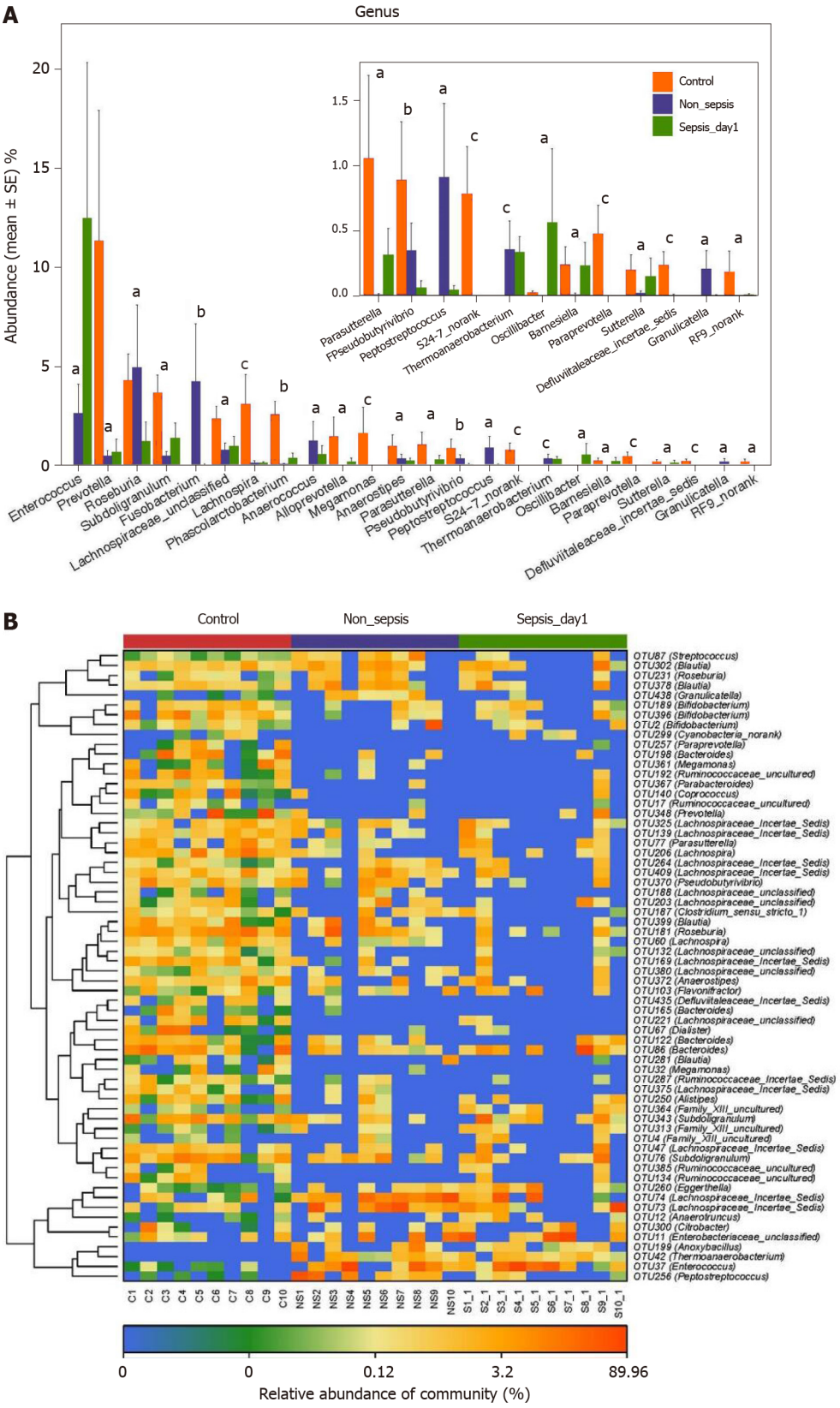



**Figure 4 Key phylotypes showing significant differences among normal individuals, sepsis patients, and non-sepsis patients.** A: Comparison of the fecal microbiota at the genus level among the three groups. The average abundance of each genus is depicted as the mean ± SE. *P* values were calculated using the Kruskal–Wallis rank-sum test: $^{a}P < 0.05$; $^{b}P < 0.01$, $^{c}P < 0.001$; B: Heatmap representing the relative abundance of differential operational taxonomic units (OTUs) among the three groups. The key OTUs whose relative abundance was above 1%, as per random forest-based models are displayed in the "figure".

biosynthesis and metabolism; however, critical diseases lead to many changes in the diversity of the intestinal flora, causing excessive growth of pathogenic bacteria[9,10].

By comparing the intestinal flora of sepsis patients and healthy controls, we confirmed that there are marked differences in the abundance, distribution, and structure of the intestinal flora in the context of sepsis. In sepsis patients, normal bacteria decreased or disappeared, while the abundance of abnormal bacteria increased sharply. At the phylum level, the abundance of Firmicutes increased significantly, while that of Bacteroidetes decreased significantly in the intestinal flora of sepsis patients *vs* non-septic critically ill patients; of note, the number of bacteria in the phylum Fusobacterium also decreased significantly. At the genus level, in addition to the decrease in the abundance of beneficial symbiotic bacteria such as *Prevotella* and *Lachnospira*, as well as of other genera, including *Fusobacterium* and *Peptostreptococcus*, we found that the abundance of *Enterococcus* increased significantly in the intestinal flora of sepsis patients *vs* non-septic critically ill patients. Therefore, our results suggest that dysbiosis in sepsis shows three major features. First, as the abundance and diversity of the intestinal flora decrease, the structure of the intestinal flora changes, and the differences among individuals are greater[11]. Second, the abundance of dominant obligate anaerobes decreases and that of facultative anaerobes increases[11,12]. Third, the abundance of beneficial symbiotic bacteria decreases, while that of pathogenic bacteria increases, possibly becoming predominant[11,13].

The occurrence of intestinal microecological disorders in sepsis patients is not surprising. First, the physiological state of sepsis patients is completely different from that at homeostasis; specifically, intestinal hypoperfusion and reperfusion injury lead to changes in the intestinal environment and blood supply to the intestinal mucosa, resulting in intestinal mucosal inflammation and, consequently, a series of changes in the intestinal environment, such as increased nitrate concentrations[11] and altered mucosal oxygen gradient[12]. These changes are favorable to the growth of Proteobacteria, leading to the expansion of many clinically familiar pathogenic Gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Escherichia coli*, as well as *Staphylococcus aureus* and *Enterococcus*[14,15]. Second, ICU patients are exposed to various endogenous regulators (such as increased catecholamine production and changes in glucose metabolism) and clinical interventions (such as proton pump inhibitors, opioids, nutritional support, and antibiotics), which affect the living environment of the intestinal flora to varying degrees and, thus, affect the flora structure[16]. Finally, the intestinal mucosa will be damaged and thinned in critically ill patients[17,18]; such mucosal damage leads to the loss of the normal habitat of symbiotic microorganisms, subsequently leading to intestinal microecological disorders.

### *Dynamic changes in the structure of the intestinal flora of sepsis patients within 1 wk after treatment initiation*

Our study suggests that the abundance, distribution, and diversity of the intestinal flora in sepsis patients do not change significantly within 1 wk of ICU admittance. However, in relation to the gut microbiome of healthy subjects, the numbers of most beneficial bacteria, such as *Prevotella* and *Bifidobacterium*, were below the detection limit in sepsis patients, whereas the abundance of infection-related bacteria, such as *Enterococcus* and *Hemophilus*, increased despite the action of antibiotics (with the exception of *Coprococcus*, which decreased in abundance to below the detection limit). These results indicate that patients with intestinal flora disorders associated with sepsis could not recover in a short time, and while the drugs were effective against the bacteria causing infection, they also affected bacteria in the intestines. In fact, the effects of drug treatment, especially antibiotics, on bacteria were not limited to harmful bacteria; the proportion of beneficial intestinal bacteria also decreased. Other studies have also shown that the long-term use of antibiotics changes the normally healthy intestinal flora and leads to the emergence of drug resistance; worrisome enough, the long-term use of antibiotics has the potential to generate organism reservoirs with a multi-drug resistance gene pool[19]. Ma *et al*[20] used rat models with third-degree burns on 30% of the total surface area of the back and quantified/identified the intestinal bacteria after treatment. The results showed that the number of cocci in the gastrointestinal contents of rats increased significantly, and the coccus/bacillus ratio was seriously inverted after treatment with Rocephin. It was considered that broad-spectrum antibiotics destroyed the intestinal microecological balance; of note, the conditional pathogenic intestinal flora showed potential to affect health, disease, and drug action. Our study also revealed that the abundance of *Enterococcus* increased significantly in sepsis patients, which might be related to the use of broad-spectrum antibiotics.

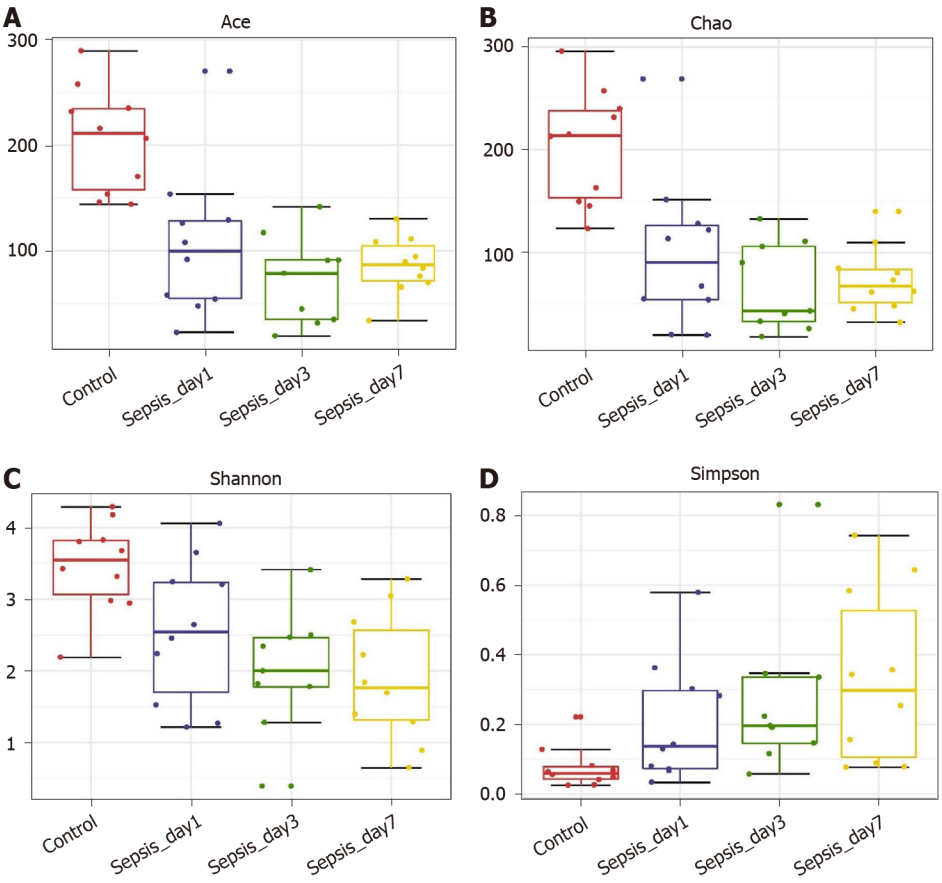



**Figure 5 α-diversity indexes of the fecal microbiota in sepsis patients on days 1, 3, and 7 after admission to the intensive care unit *vs* healthy individuals.** A: ACE estimator; B: Chao 1 estimator; C: Shannon index; D: Simpson index.

### *Correlation between the abundance of intestinal bacterial genera and clinical indicators in patients with sepsis*

Our study showed a clear correlation between the abundance of intestinal flora components and clinical indicators in sepsis. The clinical indicators used in this study were D-Lac, endotoxin, DAO, and PCT. Under normal circumstances, D-Lac is produced *via* the methylglyoxal metabolism, and its content in the blood is very small. However, when glycolysis results in increases in a large number of gastrointestinal bacteria and the intestinal barrier function is impaired, the content of D-Lac increases sharply. Endotoxins are cell wall components of Gram-negative bacteria that are released only when bacteria are lysed and die. DAO is a highly active intracellular enzyme in the upper villi of the intestinal mucosa of mammals, including humans. Its activity is closely associated with nucleic acid and protein synthesis in mucosal cells. Therefore, the above three indicators can reflect the integrity and damage degree of the intestinal mechanical barrier. Additionally, PCT reflects the active level of the systemic inflammatory response, and the factors affecting its levels include the size and type of the infected organ, the type of pathogenic bacteria, the degree of inflammation, and the state of immune responses. Our study showed that serum PCT, endotoxin, DAO, and D-Lac levels in patients with sepsis were correlated with the abundance of various intestinal bacterial genera. Of note, some of these genera were correlated with multiple clinical indicators at the same time. For example, the abundance of *Peptostreptococcus* and *Dorea* was positively correlated with the serum levels of PCT, endotoxin, and DAO; moreover, the abundance of *Roseburia* was positively correlated with the serum levels of PCT, endotoxin, and D-Lac.

Among these genera, the abundance of *Ruminococcus* had the highest positive correlation with PCT. *Ruminococcus* is an important constituent of the normal intestinal microbiota. A study published in 2017[21] using samples from young Han Chinese individuals revealed changes in the intestinal flora of the Chinese population and showed that the genera *Ruminococcus* and *Fusobacterium*, among others, were relatively highly abundant in obese Chinese individuals, whereas the abundance of *Bacteroides* was greatly reduced. Of note, two *Ruminococcus* species, *Ruminococcus torques* and

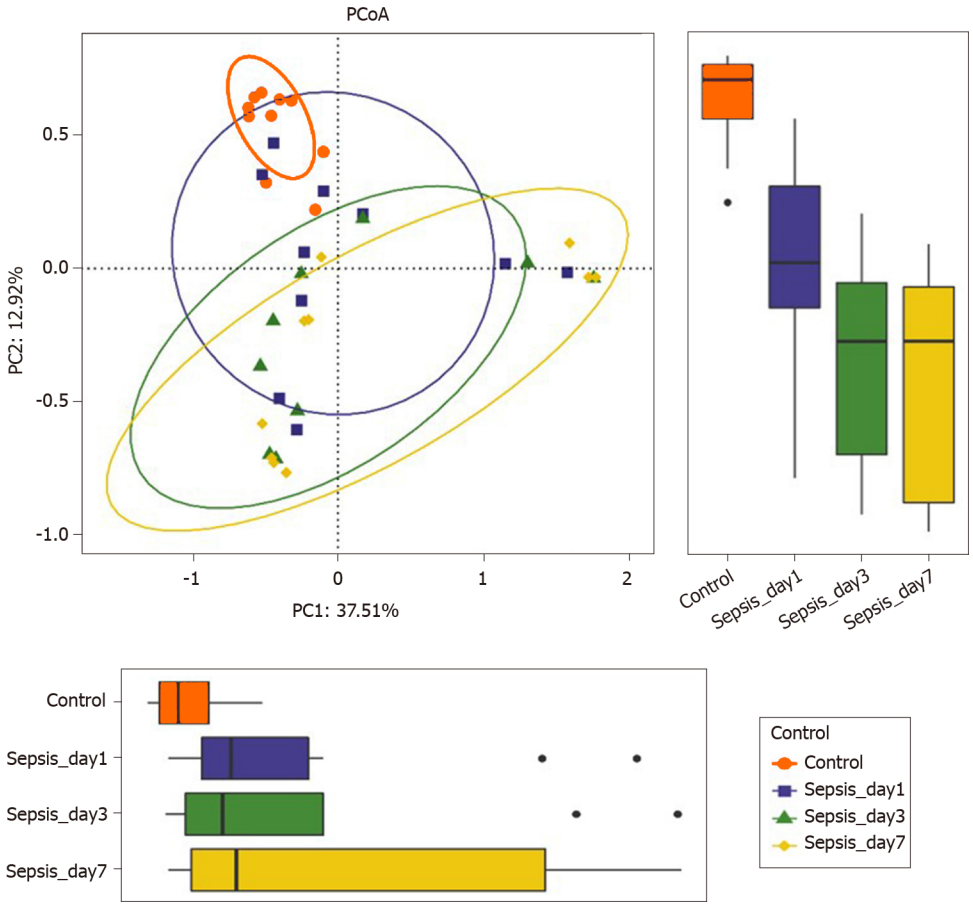



**Figure 6 Principal coordinate analysis of the intestinal flora in sepsis patients on days 1, 3, and 7 after admission to the intensive care unit versus healthy individuals.** Principal coordinate analysis was plotted based on the Bray–Curtis dissimilarity between samples. The ellipses highlight the clustering of the fecal microbiomes according to the groups (red: Healthy control group; blue: Sepsis patients on day 1; green: Sepsis patients on day 3; yellow: Sepsis patients on day 7).

*Ruminococcus gnavus*, should be mentioned because they are related to inflammatory bowel disease[22] and metabolic disorder[23]. Importantly, from the significant decrease in the number and abundance of intestinal bacteria, including the decrease in abundance of the phylum Bacteroidetes, the increase in abundance of the phylum Verrucomicrobia, and the increase in abundance of the genus *Ruminococcus* in sepsis patients, we can establish a parallel with the results of the study of obese Chinese individuals. In clinical practice, it is known that obese individuals have more significant characteristics of insulin resistance and dyslipidemia, with a more significant inflammatory phenotype, which are risk factors for cardiovascular diseases, diabetes, osteoporosis, and some cancers among other diseases[24]. Interestingly, patients with sepsis also have clinical manifestations such as insulin resistance, dyslipidemia, and inflammatory responses, which have adverse effects on the severity of illness and prognosis[25]. Therefore, we boldly speculate that the intestinal phenotype of obese patients makes them more susceptible to the complications of sepsis, which might be of great significance for the treatment and prevention of sepsis. Of course, the sample size in this study was small, and thus, it is necessary to conduct larger, multicenter studies to support our findings; moreover, the proposed mechanisms require further validation in animal studies for clarification.

There is a correlation between intestinal flora disorders and intestinal barrier dysfunction in sepsis patients. Our research showed that the abundance of *Roseburia* in the intestinal tract of sepsis patients was definitely related to the levels of serum markers of intestinal barrier function. *Roseburia* is one of the main bacterial genera producing butyrate in the human intestinal flora[26,27]. Butyrate is the main energy source of colonic epithelial cells. Research suggests that the abundance of bacteria producing butyrate in the intestinal tract of critically ill patients decreases or disappears, which leads to a decrease in butyrate production and the apoptosis of intestinal epithelial cells due to "starvation"[26]. Importantly, one study showed that the genus *Roseburia* can improve the intestinal ecosystem, prevent intestinal leakage,

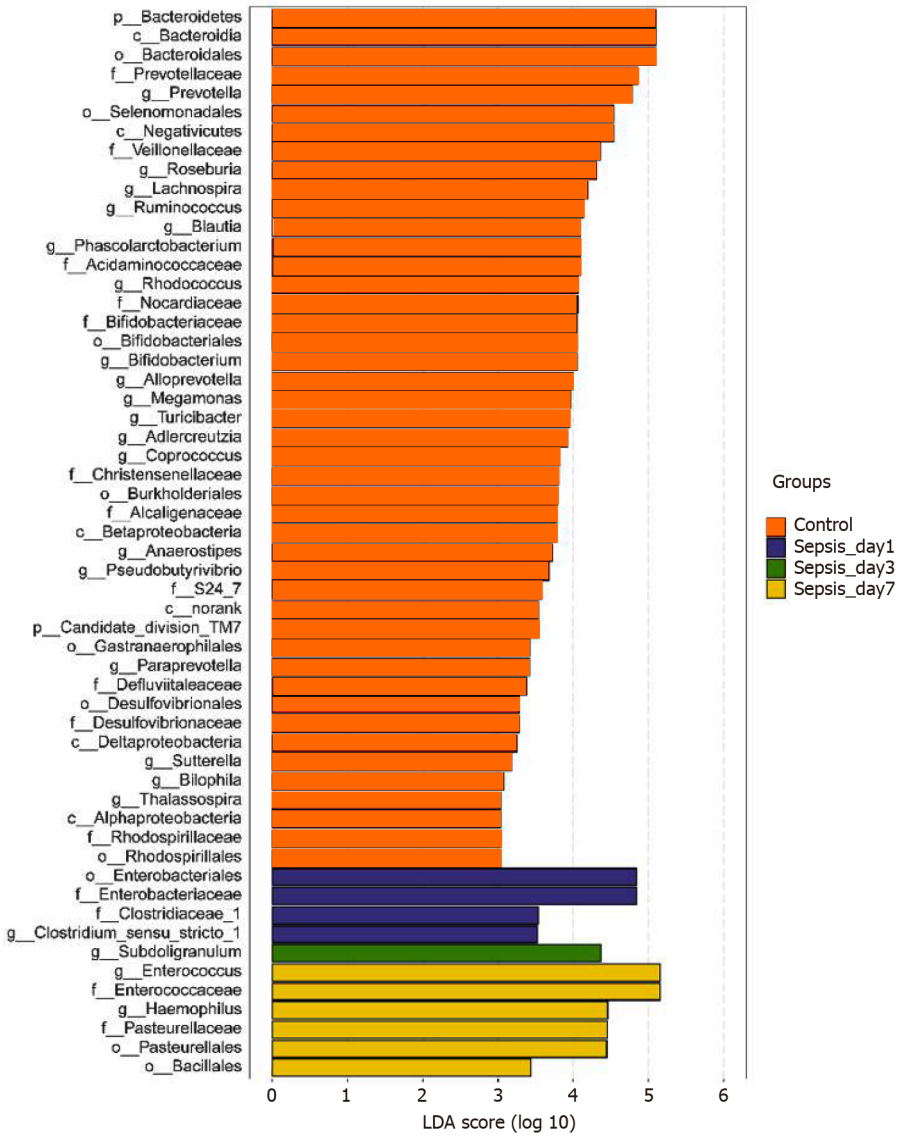



**Figure 7 Linear discriminant analysis scores indicating significant differences in the fecal microbiota between the sepsis patients within 1 wk after admission to the intensive care unit and the normal control group.** Red: Enriched taxa in the healthy control group; blue: Enriched taxa in sepsis patients on day 1; green: Enriched taxa in sepsis patients on day 3; yellow: Enriched taxa in sepsis patients on day 7.

and reduce the incidence of diabetes[27]. Conversely, other studies have shown that the abundance of *Roseburia* is significantly reduced in patients with Crohn's disease[28] and inflammatory bowel disease[29]. Our study showed that the abundance of intestinal *Roseburia* was positively correlated with serum PCT, endotoxin, and D-Lac levels in sepsis patients, indicating that the intestinal barrier function is more severely damaged and the systemic inflammatory response is more serious with a higher content of *Roseburia*, which seems to contradict the results of existing research. We speculate that systemic inflammatory responses in sepsis patients cause intestinal damage and that the body has a self-regulatory effect on this damage. In addition, a recently published study[30] showed that immune cells and autoantibodies of patients with antiphospholipid syndrome (an autoimmune disease) could cross-react with the mimic epitopes of *Roseburia*; in line with this, *Roseburia* could also cause autoimmune diseases in susceptible mice. Once again, these results suggest that the positive and negative effects of intestinal bacteria are not invariable.

Bacteria can perceive the changes in the host internal environment and then change their own toxic factors to become pathogenic. Under various stimuli such as sepsis, trauma, and burns, a healthy bacterial strain might be transformed into a pathogenic bacterium within a few hours[31]. For example, *Pseudomonas aeruginosa* was injected into the ceca of mice in a sham operation group for culture, and then, the bacteria were collected and implanted into the undamaged abdominal cavity of mice; it was found that no death occurred. However, the same strain was injected into the ceca of mice

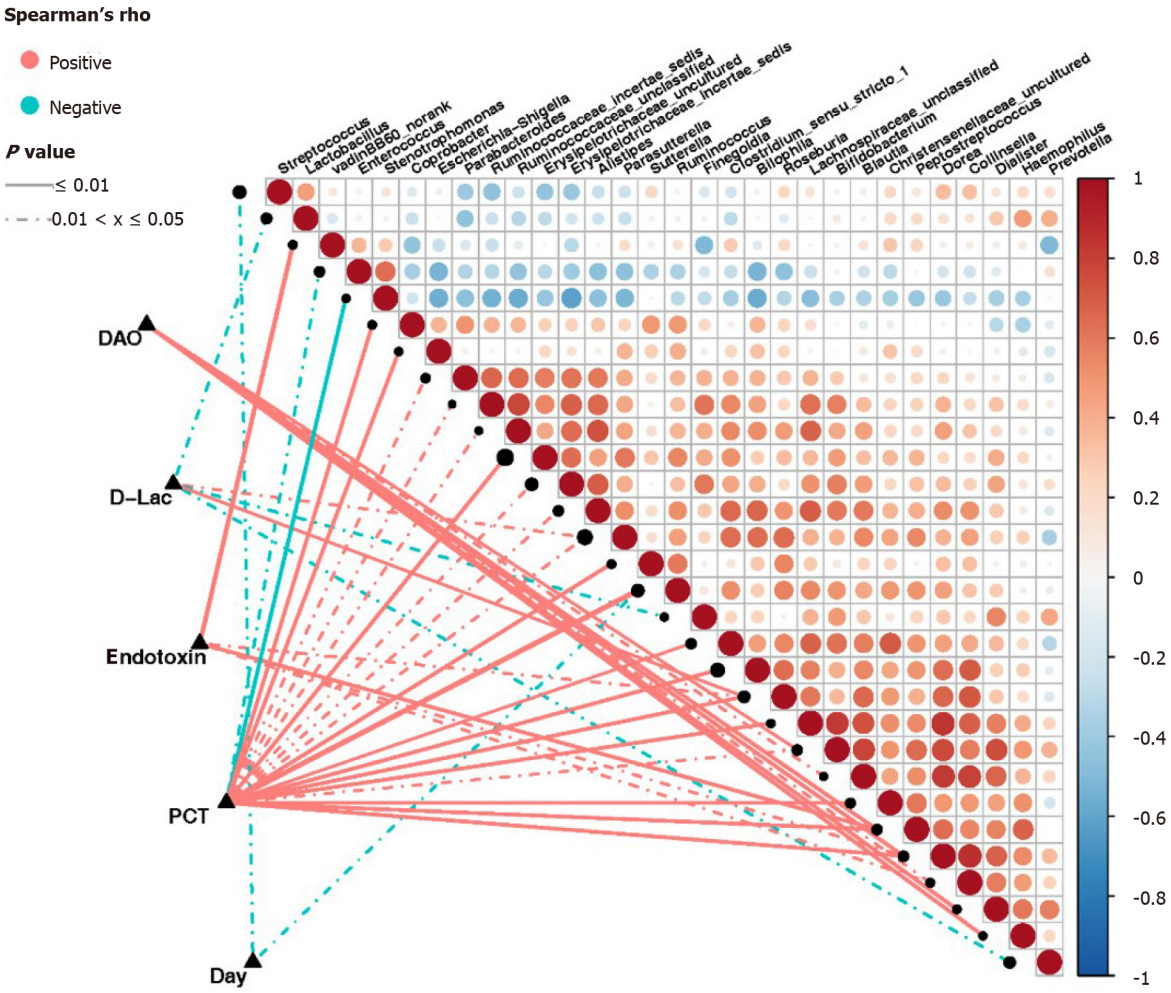



**Figure 8 Correlation between clinical indicators and abundance of fecal microbiota in sepsis patients.** Heatmap showing partial Spearman's correlation coefficients among 30 genera and clinical indexes. Connecting lines represent the correlation coefficient values above 0.4 (red, positive correlation) or below -0.4 (blue, negative correlation). Solid lines represent $P \leq 0.01$. Dotted lines represent $0.01 < P \leq 0.05$. The intensity of shading in the circles is proportional to the magnitude of the association. In the figure, "day" indicates the collection time of stool samples from sepsis patients after admission to the intensive care unit (ICU) (days 1, 3, and 7 after admission to the ICU). D-Lac: d-lactic acid; PCT: Procalcitonin; DAO: Diamine oxidase.

after 30% hepatectomy for culture, and then were collected and implanted into the undamaged abdominal cavities of mice, which led to their deaths[32]. Although the mechanisms underlying such transformations remain inconclusive, these results suggest that the host environment can not only change the diversity of bacterial species but also change their virulence. Therefore, the beneficial or harmful effects of a specific bacterium are not absolute; they can be influenced by many factors.

## CONCLUSION

In this study, we report that sepsis patients in the ICU showed intestinal microecological disorders, lasting for at least 1 wk. Furthermore, intestinal microecological disorder was correlated with inflammation-related and intestinal barrier-related indexes in sepsis. However, this study is not without limitations. The sample size was small, and thus, it is necessary to carry out larger and multicenter studies to support these findings. Additional animal studies might be needed to clarify the related molecular mechanisms.

## ARTICLE HIGHLIGHTS

### Research background

Sepsis is a common disease in intensive care units, with high morbidity and mortality. Intestinal microecology plays a vital part in the development and progression of this disease, possibly because sepsis and its treatment cause specific changes to the intestinal flora. However, there are few studies on the sepsis-related intestinal flora and its dynamic changes. An improved understanding of the status and degree of intestinal flora disturbances in sepsis patients is of great significance, to allow for the accurate evaluation of the disease condition and prognosis and to optimize the treatment measures.

### Research motivation

Studies have shown a dose–response relationship between the degree of intestinal microecological disturbance and the incidence of subsequent severe sepsis. Critical illness leading to abnormal changes in the types, quantities, proportions, and locations of microorganisms in the intestinal flora may, thus, increase susceptibility to sepsis. Therefore, an improved understanding of the status and degree of intestinal flora disturbances in sepsis patients is of great clinical significance.

### Research objectives

The main objective of this study was to investigate the characteristics of intestinal flora disturbance in sepsis patients treated with antibiotics.

### Research methods

We enrolled ten patients with sepsis admitted to the intensive care unit (ICU), ten patients without sepsis admitted to the ICU in the same period, and ten healthy individuals (sepsis group, non-sepsis group, and control group, respectively). Using *16S rRNA* gene sequencing technology, the fecal samples of the three groups were analyzed, and the intestinal flora diversity, structure, and composition were compared. The fecal samples of sepsis patients on days 1, 3, and 7 after ICU admittance were also analyzed, and the dynamics of the diversity, structure, and composition of the intestinal flora of sepsis patients were compared. Lastly, the serum levels of procalcitonin, endotoxin, diamine oxidase, and D-lactic acid were determined in sepsis patients on days 1, 3, and 7 after ICU admittance and correlated with the abundance of intestinal bacteria.

### Research results

Sepsis patients showed a reduced intestinal flora α-diversity and a different flora structure, with Firmicutes as the dominant bacteria, and significantly decreased proportions of Bacteroidetes, as well as *Prevotella*, *Lachnospira*, and other genera. *Enterococcus* was significantly increased in the intestinal tract of sepsis patients. Additionally, from days 1 to 7 of treatment, the α-diversity of the intestinal flora in the sepsis group decreased gradually, although without statistical significance. Of note, some harmful bacteria such as *Coprococcus* disappeared, the abundance of beneficial bacteria such as *Prevotella* and *Bifidobacterium* decreased, while that of *Enterococcus* and other genera increased. Interestingly, the serum levels of procalcitonin, endotoxin, diamine oxidase, and D-lactic acid in sepsis patients correlated with the abundance of various intestinal bacterial genera.

### Research conclusions

In this study, we report the characteristics of sepsis intestinal flora disturbance and reveal, for the first time, the dynamic characteristics of the intestinal flora in sepsis patients under antibiotic treatment. Altogether, our results suggest that sepsis patients in the ICU show intestinal microecological disorders, lasting for at least 1 wk. Importantly, we also show that the intestinal microecological disorder in sepsis patients is correlated with inflammation-related and intestinal barrier-related indexes. Of note, the sample size of this study was small, and thus, it is necessary to conduct larger and multicenter studies to support these findings.

### Research perspectives

We plan to carry out animal studies to clarify the molecular mechanisms of intestinal flora disturbance in sepsis.

## ACKNOWLEDGEMENTS

We thank all the patients who participated in this study and the physicians in the Department of Critical Care Medicine, General Hospital, Ningxia Medical University. We also thank Shanghai Mobio Biomedical Technology Co. for technical support and to Ren HY for her help in writing the manuscript.

## REFERENCES

1 **Vincent JL**, Opal SM, Marshall JC, Tracey KJ. Sepsis definitions: time for change. *Lancet* 2013; **381**: 774-775 [PMID: 23472921 DOI: 10.1016/S0140-6736(12)61815-7]
2 **O'Hara AM**, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006; **7**: 688-693 [PMID: 16819463 DOI: 10.1038/sj.embor.7400731]
3 **Clarke G**, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: Gut microbiota: the neglected endocrine organ. *Mol Endocrinol* 2014; **28**: 1221-1238 [PMID: 24892638 DOI: 10.1210/me.2014-1108]
4 **McDonald D**, Ackermann G, Khailova L, Baird C, Heyland D, Kozar R, Lemieux M, Derenski K, King J, Vis-Kampen C, Knight R, Wischmeyer PE. Extreme Dysbiosis of the Microbiome in Critical Illness. *mSphere* 2016; **1** [PMID: 27602409 DOI: 10.1128/mSphere.00199-16]
5 **Prescott HC**, Dickson RP, Rogers MA, Langa KM, Iwashyna TJ. Hospitalization Type and Subsequent Severe Sepsis. *Am J Respir Crit Care Med* 2015; **192**: 581-588 [PMID: 26016947 DOI: 10.1164/rccm.201503-0483OC]
6 **Suau A**, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, Doré J. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. *Appl Environ Microbiol* 1999; **65**: 4799-4807 [PMID: 10543789 DOI: 10.1128/AEM.65.11.4799-4807.1999]
7 **Bassetti M**, Vena A, Meroi M, Cardozo C, Cuervo G, Giacobbe DR, Salavert M, Merino P, Gioia F, Fernández-Ruiz M, López-Cortés LE, Almirante B, Escolà-Vergé L, Montejo M, Aguilar-Guisado M, Puerta-Alcalde P, Tasias M, Ruiz-Gaitán A, González F, Puig-Asensio M, Marco F, Pemán J, Fortún J, Aguado JM, Soriano A, Carratalá J, Garcia-Vidal C, Valerio M, Sartor A, Bouza E, Muñoz P. Factors associated with the development of septic shock in patients with candidemia: a post hoc analysis from two prospective cohorts. *Crit Care* 2020; **24**: 117 [PMID: 32216822 DOI: 10.1186/s13054-020-2793-y]
8 **Klingensmith NJ**, Coopersmith CM. The Gut as the Motor of Multiple Organ Dysfunction in Critical Illness. *Crit Care Clin* 2016; **32**: 203-212 [PMID: 27016162 DOI: 10.1016/j.ccc.2015.11.004]
9 **Shimizu K**, Ogura H, Goto M, Asahara T, Nomoto K, Morotomi M, Yoshiya K, Matsushima A, Sumi Y, Kuwagata Y, Tanaka H, Shimazu T, Sugimoto H. Altered gut flora and environment in patients with severe SIRS. *J Trauma* 2006; **60**: 126-133 [PMID: 16456446 DOI: 10.1097/01.ta.0000197374.99755.fe]
10 **Lankelma JM**, van Vught LA, Belzer C, Schultz MJ, van der Poll T, de Vos WM, Wiersinga WJ. Critically ill patients demonstrate large interpersonal variation in intestinal microbiota dysregulation: a pilot study. *Intensive Care Med* 2017; **43**: 59-68 [PMID: 27837233 DOI: 10.1007/s00134-016-4613-z]
11 **Winter SE**, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tsolis RM, Stewart VJ, Bäumler AJ. Host-derived nitrate boosts growth of E. coli in the inflamed gut. *Science* 2013; **339**: 708-711 [PMID: 23393266 DOI: 10.1126/science.1232467]
12 **Albenberg L**, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, Grunberg S, Baldassano RN, Lewis JD, Li H, Thom SR, Bushman FD, Vinogradov SA, Wu GD. Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* 2014; **147**: 1055-63. e8 [PMID: 25046162 DOI: 10.1053/j.gastro.2014.07.020]
13 **Lupp C**, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe* 2007; **2**: 119-129 [PMID: 18005726 DOI: 10.1016/j.chom.2007.06.010]
14 **Honda K**, Littman DR. The microbiome in infectious disease and inflammation. *Annu Rev Immunol* 2012; **30**: 759-795 [PMID: 22224764 DOI: 10.1146/annurev-immunol-020711-074937]
15 **Grootjans J**, Lenaerts K, Derikx JP, Matthijsen RA, de Bruïne AP, van Bijnen AA, van Dam RM, Dejong CH, Buurman WA. Human intestinal ischemia-reperfusion-induced inflammation characterized: experiences from a new translational model. *Am J Pathol* 2010; **176**: 2283-2291 [PMID: 20348235 DOI: 10.2353/ajpath.2010.091069]
16 **Haak BW**, Levi M, Wiersinga WJ. Microbiota-targeted therapies on the intensive care unit. *Curr Opin Crit Care* 2017; **23**: 167-174 [PMID: 28092309 DOI: 10.1097/MCC.0000000000000389]
17 **Lu Q**, Xu DZ, Sharpe S, Doucet D, Pisarenko V, Lee M, Deitch EA. The anatomic sites of disruption of the mucus layer directly correlate with areas of trauma/hemorrhagic shock-induced gut injury. *J Trauma* 2011; **70**: 630-635 [PMID: 20664373 DOI: 10.1097/TA.0b013e3181e1221b]
18 **Rupani B**, Caputo FJ, Watkins AC, Vega D, Magnotti LJ, Lu Q, Xu DZ, Deitch EA. Relationship

between disruption of the unstirred mucus layer and intestinal restitution in loss of gut barrier function after trauma hemorrhagic shock. *Surgery* 2007; **141**: 481-489 [PMID: 17383525 DOI: 10.1016/j.surg.2006.10.008]

19 **Jandhyala SM**, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* 2015; **21**: 8787-8803 [PMID: 26269668 DOI: 10.3748/wjg.v21.i29.8787]

20 **Ma LQ**, Chen DC, Liu S. Selective action of broad-spectrum antibiotics on intestinal flora in sepsis in rats. *Zhongguo Weizhongbing Jijiuyixue* 2007; **19**: 456-459 [DOI: 10.3760/j.issn:1003-0603.2007.08.003]

21 **Liu R**, Hong J, Xu X, Feng Q, Zhang D, Gu Y, Shi J, Zhao S, Liu W, Wang X, Xia H, Liu Z, Cui B, Liang P, Xi L, Jin J, Ying X, Zhao X, Li W, Jia H, Lan Z, Li F, Wang R, Sun Y, Yang M, Shen Y, Jie Z, Li J, Chen X, Zhong H, Xie H, Zhang Y, Gu W, Deng X, Shen B, Yang H, Xu G, Bi Y, Lai S, Wang J, Qi L, Madsen L, Ning G, Kristiansen K, Wang W. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat Med* 2017; **23**: 859-868 [PMID: 28628112 DOI: 10.1038/nm.4358]

22 **Peterson CT**, Sharma V, Elmén L, Peterson SN. Immune homeostasis, dysbiosis and therapeutic modulation of the gut microbiota. *Clin Exp Immunol* 2015; **179**: 363-377 [PMID: 25345825 DOI: 10.1111/cei.12474]

23 **Le Chatelier E**, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, Leonard P, Li J, Burgdorf K, Grarup N, Jørgensen T, Brandslund I, Nielsen HB, Juncker AS, Bertalan M, Levenez F, Pons N, Rasmussen S, Sunagawa S, Tap J, Tims S, Zoetendal EG, Brunak S, Clément K, Doré J, Kleerebezem M, Kristiansen K, Renault P, Sicheritz-Ponten T, de Vos WM, Zucker JD, Raes J, Hansen T; MetaHIT consortium, Bork P, Wang J, Ehrlich SD, Pedersen O. Richness of human gut microbiome correlates with metabolic markers. *Nature* 2013; **500**: 541-546 [PMID: 23985870 DOI: 10.1038/nature12506]

24 **Yu E**, Malik VS, Hu FB. Cardiovascular Disease Prevention by Diet Modification. *J Am Coll Cardiol* 2018; **72**: 914-926 [PMID: 30115231 DOI: 10.1016/j.jacc.2018.02.085]

25 **Golucci APBS**, Marson F, Ribeiro AF, Nogueira RJN. Lipid profile associated with the systemic inflammatory response syndrome and sepsis in critically ill patients. *Nutrition* 2018; **55-56**: 7-14 [PMID: 29960160 DOI: 10.1016/j.nut.2018.04.007]

26 **Donohoe DR**, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, Bultman SJ. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 2011; **13**: 517-526 [PMID: 21531334 DOI: 10.1016/j.cmet.2011.02.018]

27 **Seo B**, Jeon K, Moon S, Lee K, Kim WK, Jeong H, Cha KH, Lim MY, Kang W, Kweon MN, Sung J, Kim W, Park JH, Ko G. Roseburia spp. Abundance Associates with Alcohol Consumption in Humans and Its Administration Ameliorates Alcoholic Fatty Liver in Mice. *Cell Host Microbe* 2020; **27**: 25-40. e6 [PMID: 31866426 DOI: 10.1016/j.chom.2019.11.001]

28 **Quan Y**, Song K, Zhang Y, Zhu C, Shen Z, Wu S, Luo W, Tan B, Yang Z, Wang X. Roseburia intestinalis-derived flagellin is a negative regulator of intestinal inflammation. *Biochem Biophys Res Commun* 2018; **501**: 791-799 [PMID: 29772233 DOI: 10.1016/j.bbrc.2018.05.075]

29 **Ananthakrishnan AN**, Luo C, Yajnik V, Khalili H, Garber JJ, Stevens BW, Cleland T, Xavier RJ. Gut Microbiome Function Predicts Response to Anti-integrin Biologic Therapy in Inflammatory Bowel Diseases. *Cell Host Microbe* 2017; **21**: 603-610. e3 [PMID: 28494241 DOI: 10.1016/j.chom.2017.04.010]

30 **Ruff WE**, Dehner C, Kim WJ, Pagovich O, Aguiar CL, Yu AT, Roth AS, Vieira SM, Kriegel C, Adeniyi O, Mulla MJ, Abrahams VM, Kwok WW, Nussinov R, Erkan D, Goodman AL, Kriegel MA. Pathogenic Autoreactive T and B Cells Cross-React with Mimotopes Expressed by a Common Human Gut Commensal to Trigger Autoimmunity. *Cell Host Microbe* 2019; **26**: 100-113. e8 [PMID: 31227334 DOI: 10.1016/j.chom.2019.05.003]

31 **Hayakawa M**, Asahara T, Henzan N, Murakami H, Yamamoto H, Mukai N, Minami Y, Sugano M, Kubota N, Uegaki S, Kamoshida H, Sawamura A, Nomoto K, Gando S. Dramatic changes of the gut flora immediately after severe and sudden insults. *Dig Dis Sci* 2011; **56**: 2361-2365 [PMID: 21384123 DOI: 10.1007/s10620-011-1649-3]

32 **Babrowski T**, Romanowski K, Fink D, Kim M, Gopalakrishnan V, Zaborina O, Alverdy JC. The intestinal environment of surgical injury transforms Pseudomonas aeruginosa into a discrete hypervirulent morphotype capable of causing lethal peritonitis. *Surgery* 2013; **153**: 36-43 [PMID: 22862900 DOI: 10.1016/j.surg.2012.06.022]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2394-2414

DOI: 10.3748/wjg.v27.i19.2394

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

ORIGINAL ARTICLE

Observational Study

# Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study

Xia Liu, Yi-Wen Cheng, Li Shao, Shu-Hong Sun, Jian Wu, Qing-Hai Song, Hong-Sheng Zou, Zong-Xin Ling

**ORCID number:** Xia Liu 0000-0002-9987-3332; Yi-Wen Cheng 0000-0003-3095-9311; Li Shao 0000-0003-4006-0641; Shu-Hong Sun 0000-0002-8694-308X; Jian Wu 0000-0003-0087-3744; Qing-Hai Song 0000-0002-8749-313X; Hong-Sheng Zou 0000-0002-1854-5741; Zong-Xin Ling 0000-0001-9662-099X.

**Author contributions:** Ling ZX was the guarantor and designed the study; Liu X, Cheng YW, Shao L, Sun SH, Wu J, Song QH, and Zou HS participated in the acquisition, analysis, and interpretation of the data, and drafted the initial manuscript; Liu X, Cheng YW, Shao L, and Ling ZX revised the article critically for important intellectual content.

**Supported by** National Natural Science Foundation of China, No. 31700800, No. 81771724, and No. 81790631; and National S&T Major Project of China, No. 2018YFC2000500.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University (Zhejiang, China).

**Informed consent statement:** All study participants, or their legal

**Xia Liu,** Department of Intensive Care Unit, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

**Yi-Wen Cheng, Jian Wu, Zong-Xin Ling,** Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

**Li Shao,** Institute of Hepatology and Metabolic Diseases, Hangzhou Normal University, Hangzhou 310000, Zhejiang Province, China

**Li Shao,** Institute of Translational Medicine, The Affiliated Hospital of Hangzhou Normal University, Hangzhou 310000, Zhejiang Province, China

**Shu-Hong Sun,** Department of Laboratory Medicine, Linyi People's Hospital, Linyi 276000, Shandong Province, China

**Qing-Hai Song,** Department of Geriatrics, Lishui Second People's Hospital, Lishui 323000, Zhejiang Province, China

**Hong-Sheng Zou,** Department of Intensive Care Unit, People's Hospital of Rongcheng, Rongcheng 264300, Shandong Province, China

**Corresponding author:** Zong-Xin Ling, PhD, Professor, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, the First Affiliated Hospital, School of Medicine, Zhejiang University, No. 79 Qingchun Road, Hangzhou 310003, Zhejiang Province, China. lingzongxin_lzx@163.com

## Abstract

### *BACKGROUND*

Gut microbiota dysbiosis is reportedly actively involved in autoimmune diseases such as type 1 diabetes mellitus (T1DM). However, the alterations in the gut microbiota and their correlation with fasting blood glucose (FBG) in Chinese children with T1DM remain unclear.

### *AIM*

To investigate alterations in the gut microbiota in Chinese children with T1DM

and their associations with clinical indicators.

*METHODS*
Samples from 51 children with T1DM and 47 age-matched and gender-matched healthy controls were obtained, to explore the structural and functional alterations in the fecal microbiota. The V3-V4 regions of the 16S rRNA gene were sequenced on a MiSeq instrument, and the association with FBG were analyzed.

*RESULTS*
We found that the bacterial diversity was significantly increased in the T1DM-associated fecal microbiota, and changes in the microbial composition were observed at different taxonomic levels. The T1DM-reduced differential taxa, such as *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, and *Flavonifractor plautii*, were negatively correlated with FBG, while the T1DM-enriched taxa, such as *Blautia*, *Eubacterium hallii* group, *Anaerostipes hadrus*, and *Dorea longicatena*, were positively correlated with FBG. *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, the *Eubacterium hallii* group, and *Anaerostipes hadrus*, either alone or in combination, could be used as noninvasive diagnostic biomarkers to discriminate children with T1DM from healthy controls. In addition, the functional changes in the T1DM-associated fecal microbiota also suggest that these fecal microbes were associated with altered functions and metabolic activities, such as glycan biosynthesis and metabolism and lipid metabolism, which might play vital roles in the pathogenesis and development of T1DM.

*CONCLUSION*
Our present comprehensive investigation of the T1DM-associated fecal microbiota provides novel insights into the pathogenesis of the disease and sheds light on the diagnosis and treatment of T1DM.

**Key Words:** Dysbiosis; Fasting blood glucose; Sequencing; Metabolism; Type 1 diabetes mellitus

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** Alterations in the gut microbiota play vital roles in the development of autoimmune diseases such as type 1 diabetes mellitus (T1DM). Our present study explores the overall structure and composition of the fecal microbiota in Chinese children with T1DM and its association with fasting blood glucose (FBG). We found that the bacterial diversity increased significantly in children with T1DM and that several key functional taxa were correlated with FBG. These key functional bacteria could be used as noninvasive diagnostic biomarkers to discriminate T1DM patients from healthy controls. This comprehensive investigation of the T1DM-associated fecal microbiota provides novel insights into the pathogenesis of T1DM.

**Citation:** Liu X, Cheng YW, Shao L, Sun SH, Wu J, Song QH, Zou HS, Ling ZX. Gut microbiota dysbiosis in Chinese children with type 1 diabetes mellitus: An observational study. *World J Gastroenterol* 2021; 27(19): 2394-2414
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2394.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2394

## INTRODUCTION

Type 1 diabetes mellitus (T1DM), a chronic autoimmune disease that usually begins in childhood, results from the destruction (or loss) of pancreatic β-cells, which leads to an inability to produce insulin and a need for the administration of exogenous insulin. T1DM ranks as the second most common autoimmune disease among children. The peak incidence of T1DM is clearly within the age group of 10–14 years and declines thereafter. One nationwide, population based study demonstrated that the incidence

guardian, provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** There are no conflicts of interest to report.

**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. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/Licenses/by-nc/4.0/

**Manuscript source:** Invited manuscript

**Specialty type:** Gastroenterology and hepatology

**Country/Territory of origin:** China

**Peer-review report's scientific quality classification**
Grade A (Excellent): A
Grade B (Very good): B, B, B, B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

**Received:** January 23, 2021
**Peer-review started:** January 23, 2021
**First decision:** February 10, 2021
**Revised:** February 17, 2021
**Accepted:** April 14, 2021
**Article in press:** April 14, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Athyros VG, de Souza HSP, Roysommuti S, Yang YJ

**S-Editor:** Fan JR
**L-Editor:** Wang TQ
**P-Editor:** Ma YJ

of T1DM in children is 1.93 (ranging from 0.83 to 3.03) per 100000 person-years in China, with an annual increase of approximately 6.5%[1]. T1DM can cause life-changing and life-threatening health complications in many organs and tissues rich in capillary vessels, such as the kidney, retina, and nerves, resulting in premature death. Children with T1DM often have to be treated for hypertension, dyslipidemia, microalbuminuria, and nephropathy, among other conditions[2]. Its early onset and chronicity make T1DM a disease of considerable importance. Currently, T1DM and its related comorbidities are considered major public health concerns.

Over the last few decades, considerable progress has been made in research on the pathogenesis and treatment of T1DM. However, its precise etiology and pathological mechanisms remain largely unclear. Both genetic susceptibility and environmental factors contribute to the development of T1DM[3]. The genetic susceptibility associated with T1DM is fairly well known, whereas the environmental factors remain poorly defined despite intensive research. Among the environmental risk factors associated with T1DM are industrial and economic advances (such as high levels of hygiene), changes in diet, and the emergence of more sedentary lifestyles[4], which can affect the environmental exposure of children. This altered environmental exposures can directly and indirectly influence the early-life gut microbiota.

Numerous clinical and experimental reports provide growing evidence of a close link between an altered gut microbiota (also defined as dysbiosis) and T1DM[5-12]. A previous study found that T1DM-related dysbiosis is associated with reduced integrity and increased permeability of the gut mucosa, which leads to bacterial penetration, and can stimulate the immune system to produce antibodies. Cross-reaction of these antibodies and surface antigens of pancreatic beta cells, as well as T cell cross-reactivity, results in the destruction of pancreatic β-cells and the development of T1DM[13]. Specifically, the Firmicutes/Bacteroidetes ratio is significantly reduced in Finnish children with T1DM[14]. Leiva-Gea *et al*[6] demonstrated that *Bacteroides* and *Veillonella* are markedly enriched in patients with TIDM, whereas *Faecalibacterium* and *Roseburia* are significantly reduced[6]. Livanos *et al*[11] showed that early-life antibiotic treatments alter the gut microbiota and its metabolic capacities, intestinal gene expression, and T-cell populations, thereby accelerating T1DM onset in non-obese diabetic mice[11]. Taken together, these findings show that early dysbiosis of the gut microbiota can be used as a promising biomarker for T1DM.

Most of the previous studies on the T1DM-associated microbiota were conducted in the United States and Europe. However, differences in lifestyle, dietary constitution, environmental exposures, and host genetic background between Chinese and Western populations may contribute to disparities in the baseline microbiota composition, which may influence the roles of specific bacteria in the etiopathology of T1DM. The present study aimed to explore the T1DM-associated gut microbiota in Chinese children by using the 16S rRNA gene high-throughput sequencing platform. We also evaluated the correlation between the T1DM-associated gut microbiota and fasting blood glucose (FBG). Our findings suggest novel targets for noninvasive early diagnosis and personalized treatment of T1DM.

## MATERIALS AND METHODS

### *Participant selection*

A total of 51 children with newly confirmed T1DM (aged 6-14 years) and 47 age-, gender-, and education-matched healthy controls were enrolled from Linyi People's Hospital (Linyi, China) and the First Affiliated Hospital, School of Medicine, Zhejiang University (Hangzhou, China) from June 2019 to November 2019. The diagnosis of T1DM was based on the criteria of the American Diabetes Association: T1DM-associated autoimmunity (*i.e.*, formation of islet autoantibodies); the classic trio of symptoms associated with disease onset, *i.e.*, polydipsia, polyphagia, and polyuria along with hyperglycemia; an immediate need for exogenous insulin replacement and lifetime treatment. All the T1DM children were treated with only insulin. The FBG level of these participants was determined in the morning (Table 1). The following exclusion criteria were established: Body mass index (BMI) ≥ 30 kg/m$^2$; use of antibiotics, probiotics, prebiotics, or synbiotics in the previous month; known active infections such as bacterial, fungal, chlamydial, or viral infections; and other diseases such as irritable bowel syndrome (IBS), inflammatory bowel disease or other autoimmune diseases. The protocols for the present study were approved by the Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University. Informed written consent was obtained from the subjects' guardians before

**Table 1 Fundamental information of subjects**

| Parameter | T1DM children (*n* = 51) | Healthy controls (*n* = 47) | *P* value |
|---|---|---|---|
| Age (yr) | 10.38 ± 3.59 | 9.58 ± 4.35 | 0.284 |
| Gender (male/female) | 24/27 | 21/26 | 0.816 |
| BMI (mean ± SD) | 18.54 ± 4.21 | 18.98 ± 3.26 | 0.732 |
| Age of onset (years, mean ± SD) | 5.58 ± 3.45 | - | - |
| FBG (mmol/L, mean ± SD) | 11.87 ± 2.75 | 4.83 ± 0.41 | 0.000 |
| Triglycerides (mmol/L, mean ± SD) | 0.95 ± 0.38 | 0.88 ± 0.29 | 0.257 |
| Antibiotics use, *n* | 0 | 0 | - |
| Insulin use, *n* | 51 | 0 | - |
| Other autoimmune diseases | 0 | 0 | - |

T1DM: Type 1 diabetes mellitus; FBG: Fasting blood glucose; BMI: Body mass index; SD: Standard deviation.

enrollment.

### *Fecal sample collection and microbial DNA extraction*

According to our previous studies, approximately 2 g of fresh fecal sample was collected in a sterile plastic cup, and stored at -80 °C within 15 min after preparation until use. Bacterial genomic DNA was extracted from 300 mg of homogenized feces using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with additional glass-bead beating steps on a Mini-Beadbeater (FastPrep; Thermo Electron Corporation, Boston, MA, United States). The amount of DNA was determined using a NanoDrop ND-1000 spectrophotometer (Thermo Electron Corporation); the integrity and size were checked by 1.0% agarose gel electrophoresis on a gel containing 0.5 mg/mL ethidium bromide. All DNA was stored at -20 °C before further analysis.

### *Amplicon library construction and sequencing*

Amplicon libraries were constructed with the Illumina sequencing-compatible and barcode-indexed bacterial polymerase chain reaction primers 338F (5'-ACTCCTRCGG-GAGGCAGCAG-3') and 806R (5'-GGACTACCVGGGTATCTAAT-3'), which targeted the V3-V4 regions of 16S rRNA gene. All PCRs were performed with KAPA HiFi HotStart ReadyMix using the manufacturer's protocol (KAPA Biosystems, Wilmington, MA) and approximately 50 ng of extracted DNA was used per reaction. Thermocycling conditions were as follows: 30 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. All PCRs were performed in triplicate in a volume of 50 mL, and the samples were combined after PCR. The amplicon library was prepared using a TruSeq™ DNA Sample Preparation Kit (Illumina Inc, San Diego, CA, United States). Prior to sequencing, the PCR products were extracted with the MiniElute Gel Extraction Kit (Qiagen) and quantified on a NanoDrop ND-1000 spectrophotometer (Thermo Electron Corporation) and Qubit 2.0 fluorometer (Invitrogen). The purified amplicons were then pooled in equimolar concentrations and the final concentration of the library was determined by Qubit (Invitrogen). Negative DNA extraction controls (lysis buffer and kit reagents only) were amplified and sequenced as contamination controls. Sequencing was performed on a MiSeq instrument (Illumina) using a 300 × 2 V3 kit together with PhiX Control V3 (Illumina)[15,16].

### *Bioinformatic analysis*

The 16S rRNA gene sequence data set generated from the Illumina MiSeq platform was inputted to QIIME2 (version 2020.11), and all steps of sequence processing and quality control were performed in QIIME2 with default parameters[15,16]. Before the following data analysis, these reads of each sample were normalized to even sampling depths and annotated using the Greengenes reference database (version 13.8) with both the Ribosomal Database Project Classifier and UCLUST version 1.2.22 methods implemented in QIIME. Alpha diversity, including the observed species, abundance-based coverage estimator (ACE), Chao1 estimator, Shannon, Simpson, Evenness, and

PD whole tree indices, was calculated at a 97% similarity level. Beta diversity was measured by the unweighted UniFrac, weighted UniFrac, Jaccard, and Bray-Curtis distances calculated by QIIME2, which were visualized by principal coordinate analysis (PCoA). The differences in the composition of the fecal microbiota at different taxonomic levels were analyzed with Statistical Analysis of Metagenomic Profiles (STAMP) software package v2.1.3 and by the linear discriminant analysis effect size (LEfSe) method. PiCRUSt v1.0.0 was used to identify predicted gene families and associated pathways from inferred metagenomes of taxa of interest identified from the compositional analyses. The sparse compositional correlation (SparCC) algorithm was used for correlation analysis, and the results were visualized using Cytoscape v3.4.1.

### Statistical analysis

For continuous variables, independent *t*-tests, White's nonparametric *t*-tests, and Mann-Whitney *U*-tests were applied. For categorical variables between groups, Pearson's chi-square test or Fisher's exact test was used, depending on assumption validity. For correlation analyses, Spearman's rank correlation test was used. Statistical analyses were performed using SPSS V19.0 (SPSS Inc., Chicago, IL, United States) and STAMP V2.1.3. GraphPad Prism version 6.0 (San Diego, CA, United States) was used to prepare graphs. All tests of significance were two sided, and $P < 0.05$ or corrected $P < 0.05$ was considered statistically significant.

### Accession number

The sequence data from this study are deposited in the GenBank Sequence Read Archive with the accession number SRP287193.

## RESULTS

### Altered bacterial diversity in children with T1DM

No significant differences were noted in age, gender, race, BMI, or lipid levels between Chinese children with T1DM and healthy controls ($P > 0.05$). The FBG level was significantly higher in children with T1DM than in healthy controls (Figure 1; $P < 0.05$). A total of 3961145 high-quality reads (1857135 reads in healthy controls and 2104010 reads in children with T1DM) with an average of 40420 reads per sample were obtained for subsequent microbiota analysis. Deeper sequencing identified the majority of the bacterial phylotypes [640 operational taxonomic units (OTUs)] present in the fecal microbiota. The Good's estimator of coverage was 99.93%.

For bacterial diversity analyses, the Shannon and Simpson indices differed significantly between children with T1DM and healthy controls ($P < 0.05$; Figure 2A and B), with an increased diversity in the T1DM-associated fecal microbiota. However, richness indices, such as the ACE and Chao1 indices, showed no significant changes between the two groups ($P > 0.05$; Figure 2C and D). Owing to the significant interindividual variations, PCoA based on unweighted UniFrac, weighted UniFrac, and Bray–Curtis algorithms could not separate the two groups into different clusters (Figure 2E-G). Based on the Venn diagram in Figure 2H and Shannon rarefaction curves (Figure 2I), we observed a slightly higher number of OTUs in children with T1DM. Taken together, the results of our deeper sequencing analysis indicated increased fecal microbial diversity in children with T1DM.

### Altered fecal microbiota composition in children with T1DM

The overall microbial compositions in children with T1DM and healthy controls were examined at different taxonomic levels from phylum to species. Using the RDP Classifier, sequences were annotated as follows: 11 phyla, 29 orders, 53 families, 184 genera, and 271 species. LEfSe identified many key bacterial phylotypes, mainly at the genus and species levels, that could potentially distinguish children with T1DM from healthy controls (Figure 3A and B). A representative cladogram demonstrated dysbiosis of the T1DM-associated fecal microbiota among children with T1DM.

Specifically, no one phylum was observed to differ significantly between children with T1DM and healthy controls. However, the dysbiotic indicator, the Firmicutes/Bacteroidetes (F/B) ratio, was markedly increased in children with T1DM (Supplementary Figure 1), which suggested that fecal microbial dysbiosis occurred in patients with T1DM. At the order level, we found that Erysipelotrichales, Enterobacteriales, and Coriobacteriales were enriched in children with T1DM, while Selenomonadales was markedly reduced ($P < 0.05$; Figure 3C). At the family level, Lachnospiraceae,

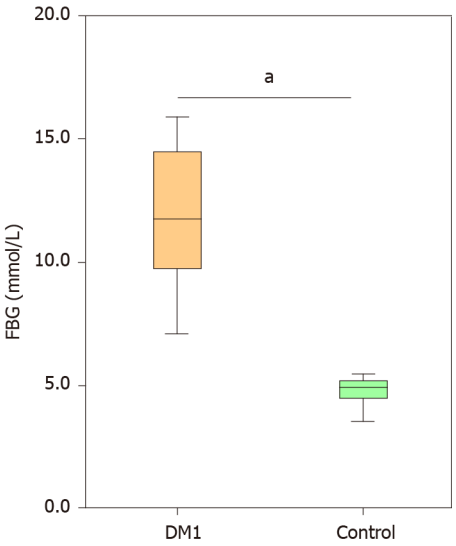



**Figure 1 Comparison of the levels of fasting blood glucose between Chinese children with type 1 diabetes mellitus and healthy controls.**
Data are presented as the mean ± SD. $^{a}P < 0.05$, compared with control group. FBG: Fasting blood glucose; DM1: Type 1 diabetes mellitus.

Erysipelotrichaceae, Enterobacteriaceae, and Coriobacteriaceae were significantly enriched in children with T1DM ($P < 0.05$; Figure 3C).

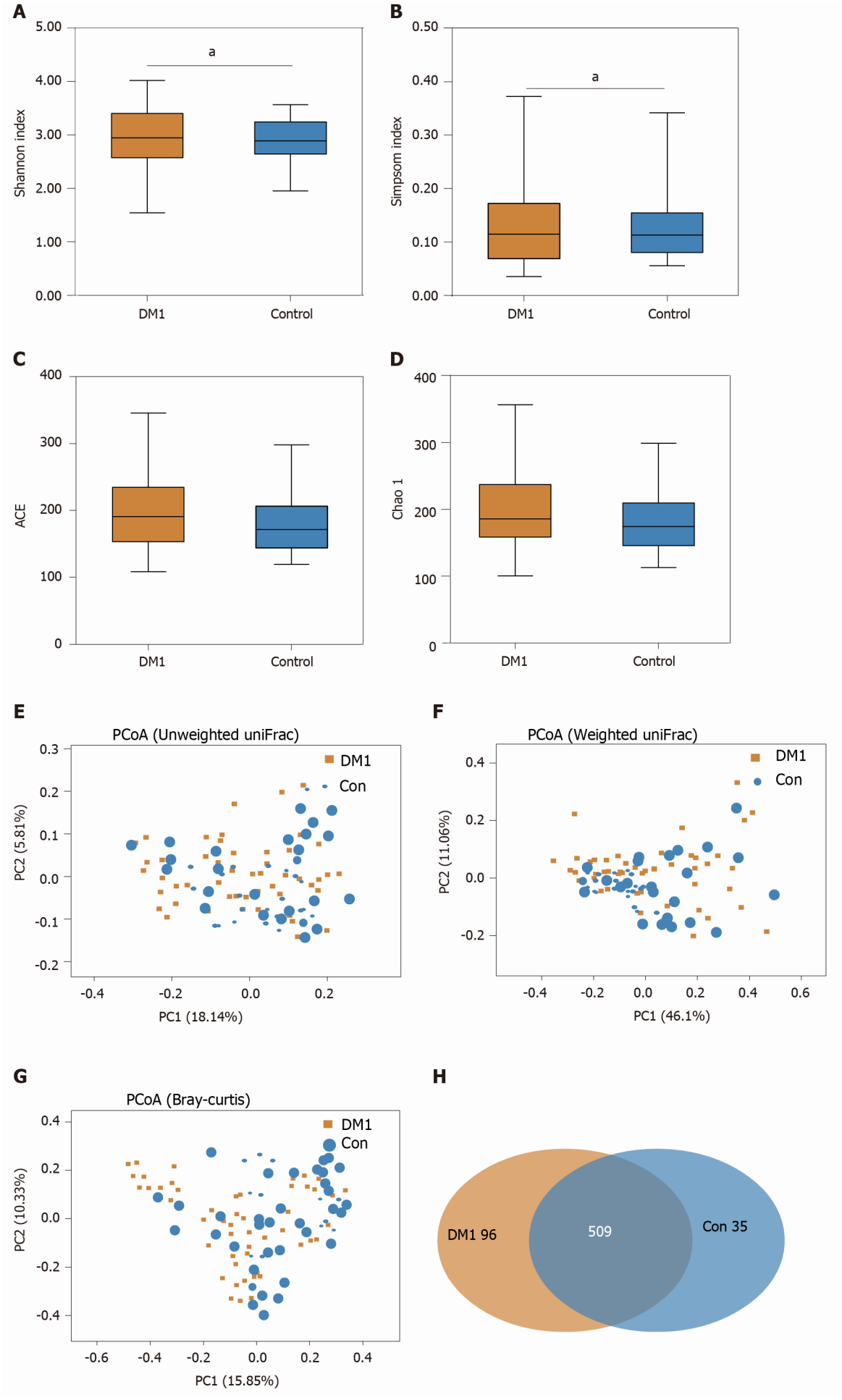
At the genus level, *Blautia*, *Anaerostipes*, unclassified Lachnospiraceae, the *Eubacterium hallii* group, unclassified Peptostreptococcaceae, *Dorea*, *Collinsella*, and *Klebsiella* were significantly enriched in children with T1DM, whereas *Parabacteroides*, *Flavonifractor*, and uncultured Ruminococcaceae were markedly reduced ($P < 0.05$; Figure 3C). At the species level, *Anaerostipes hadrus*, *Ruminococcus* sp._5_1_39BFAA, *Dorea longicatena*, and *Collinsella aerofaciens* were enriched in children with T1DM, while seven species, namely, *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, *Bacteroides dorei*, *Flavonifractor plautii*, *Parabacteroides merdae*, and *Parabacteroides distasonis* ATCC8503 were markedly reduced ($P < 0.05$; Figure 3C). Figure 4 shows a heatmap of bacterial genera in children with T1DM and healthy controls, presenting the relative percentages of most genera identified in each sample.

The overall structure of the T1DM-associated fecal microbiota was the result of dynamic interactions between community members. The SparCC algorithm with false discovery rate adjustment was employed to generate correlation-based microbial interaction networks based on the relative abundance of OTUs between the two groups (Figure 5). We found a more complex network of interactions in healthy controls than in children with T1DM. More positive and negative correlations among bacteria were found in the healthy controls. Based on our present findings, dysbiosis of the T1DM-associated fecal microbiota was observed in children with T1DM.

### *A fecal microbiota-based signature discriminates patients with T1DM from healthy controls*

As mentioned above, several taxa were identified as key functional differentially abundant bacteria. These differentially abundant taxa, including *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, and *Flavonifractor plautii*, were negatively correlated with FBG, while *Blautia*, *Eubacterium hallii* group, *Anaerostipes hadrus*, and *Dorea longicatena* were positively correlated with FBG ($P < 0.05$; Figure 6). These correlation analyses indicated that key T1DM-associated functional bacteria actively participated in the regulation of glycemic levels in children.

We evaluated the potential value of the key functional differentially abundant taxa as biomarkers, including *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, the *Eubacterium hallii* group, and *Anaerostipes hadrus*. First, using only one of the differential bacteria as a predictor, we generated the receiver operating characteristic curves, with the area under the curve (AUC) ranging from 0.294 to 0.690 (Figure 7A). Multivariable stepwise logistic regression analysis was then performed to evaluate the list of T1DM-associated taxa, to distinguish T1DM patients from healthy controls (Figure 7B). We found that a combination of four taxa, including *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, the *Eubacterium hallii* group, and *Anaerostipes hadrus*, could significantly improve the predictive performance (AUC = 0.830). Based on our present findings, these key differentially abundant taxa could be used as potential


A
Shannon index
5.00
4.00
3.00
2.00
1.00
0.00
a
DM1
Control
B
Simpsom index
0.50
0.40
0.30
0.20
0.10
0.00
a
DM1
Control
C
ACE
400
300
200
100
0
DM1
Control
D
Chao 1
400
300
200
100
0
DM1
Control
E
PCoA (Unweighted uniFrac)
DM1
Con
PC2 (5.81%)
PC1 (18.14%)
F
PCoA (Weighted uniFrac)
DM1
Con
PC2 (11.06%)
PC1 (46.1%)
G
PCoA (Bray-curtis)
DM1
Con
PC2 (10.33%)
PC1 (15.85%)
H
DM1 96
509
Con 35

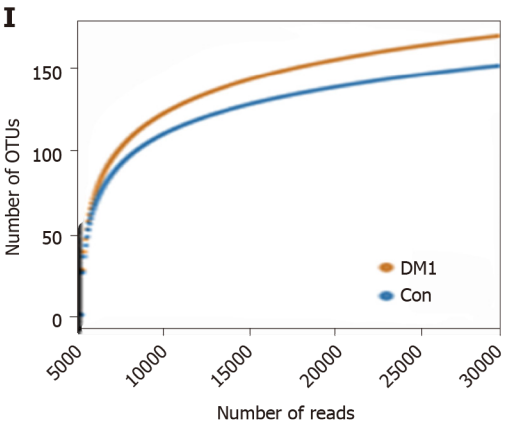



**Figure 2 Altered bacterial diversity and richness of the fecal microbiota in Chinese children with type 1 diabetes mellitus.** A-D: The diversity indices, such as Shannon (A), and Simpson (B), and the richness indices, such as the abundance-based coverage estimator (C), and Chao1 (D), were used to evaluate the overall structure of the fecal microbiota in patients with type 1 diabetes mellitus (T1DM) and healthy controls. Data are presented as the mean ± SD. Unpaired *t*-tests (two-tailed) were used to analyze variation between the two groups; E-G: Principal coordinate analysis plots of individual fecal microbiota based on unweighted (E) and weighted (F) UniFrac distance, and Bray–Curtis dissimilarity (G) in patients with T1DM and healthy controls. Each symbol represents a single sample; H: Venn diagram illustrating the overlap of operational taxonomic units (OTUs) in T1DM-associated fecal microbiota between the two groups; I: Rarefaction curves used to estimate the richness (at a 97% level of similarity) of T1DM-associated fecal microbiota between the two groups. The vertical axis shows the expected number of OTUs after sampling the number of tags or sequences shown on the horizontal axis. $^{a}P < 0.05$. OTUs: Operational taxonomic units; ACE: Abundance-based coverage estimator; PCoA: Principal coordinate analysis; Con: Control; DM1: Type 1 diabetes mellitus.

biomarkers for discriminating between T1DM patients and healthy controls.

### *T1DM-associated microbial functional alterations*

To study the functional and metabolic changes in microbial communities between patients with T1DM and controls, we inferred the metagenomes from the 16S rRNA data and analyzed the functional potential of the fecal microbiota using PiCRUSt software, based on closed-reference OTU picking. We compared 64 Kyoto Encyclopedia of Genes and Genome (KEGG) pathways at level 2, and identified five KEGG categories with significantly differential abundances between children with T1DM and healthy controls. We found that glycan biosynthesis and metabolism and lipid metabolism were significantly reduced in children with T1DM ($P < 0.05$; Figure 8), which suggests that they might play crucial roles in the development of T1DM.

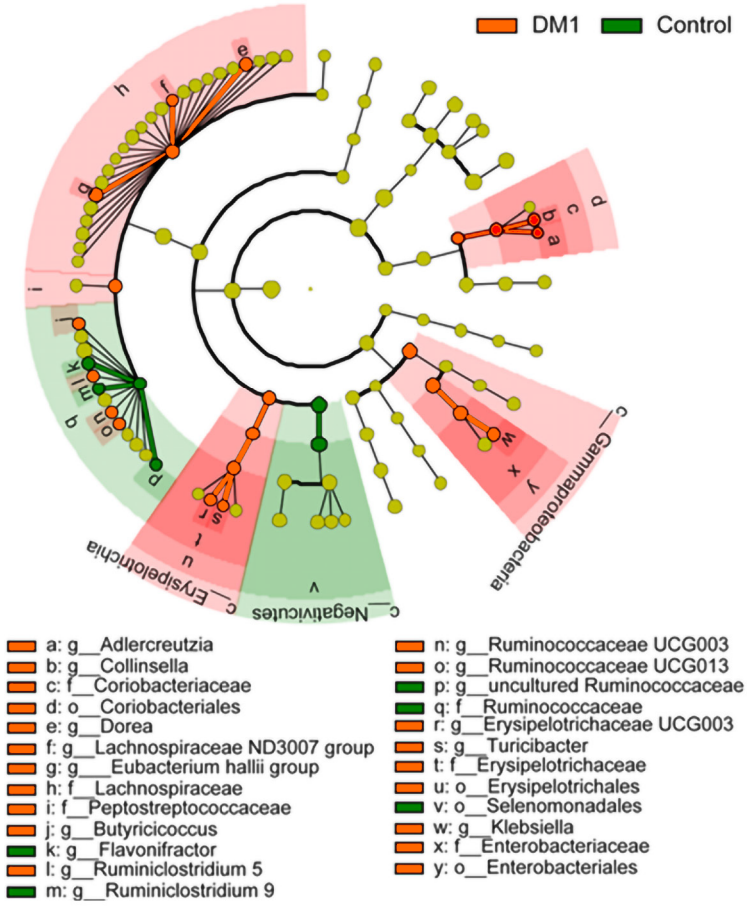
Specifically, nine pathways at level 3, including transcription factors, the phosphotransferase system, methane metabolism, and peptidoglycan biosynthesis, were significantly increased in the T1DM-associated fecal microbiota. Furthermore, 18 other pathways, including glycan degradation, glycosaminoglycan degradation, and insulin signaling pathway, were markedly reduced in the T1DM-associated microbiota. Together, these functional alterations in the fecal microbiota, especially that in glycan metabolism, were likely associated with the pathogenesis and development of T1DM.

## DISCUSSION

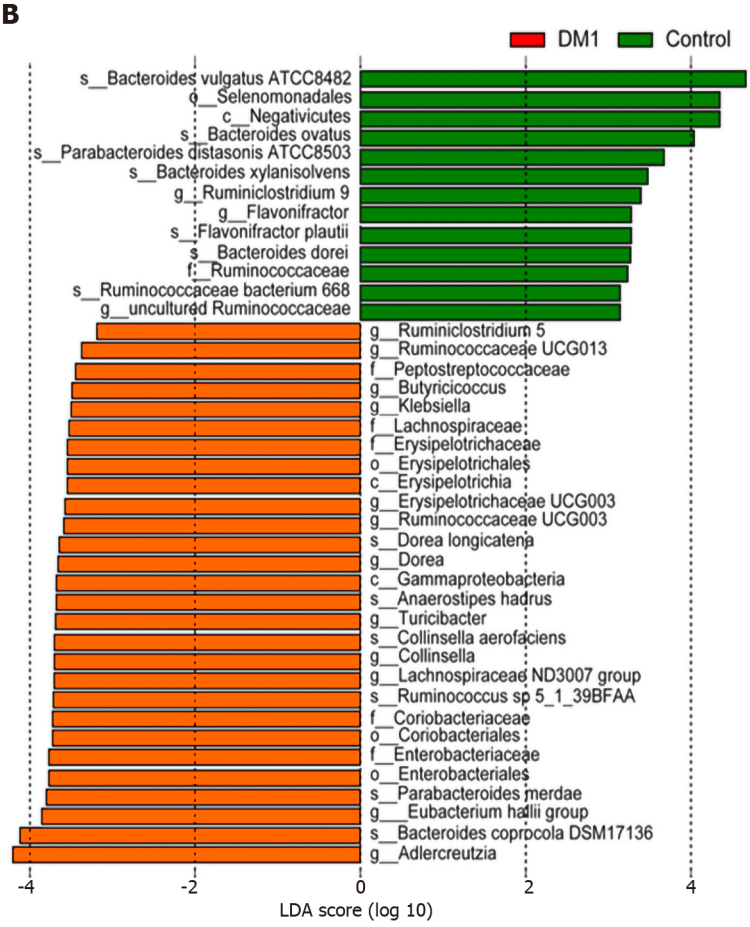
As a chronic autoimmune disease, T1DM is affected by genetic and non-genetic factors. With the advent of high-throughput sequencing technology, numerous studies have found that non-genetic factors, such as an optimal balance of the gut microbiota, play vital roles in regulating the host immune system and preventing the development of T1DM. A recent study demonstrated that a dysbiotic gut microbiota limits the effects of therapy in T1DM, while depletion of gut microbiota resistance enhances stem cell therapy in T1DM[17]. Our present T1DM-associated gut microbiota analysis excluded the influence of physiological factors such as the age, gender, and race of the enrolled participants. Most of the children with T1DM were newly diagnosed cases and were treated with only insulin. Without any additional treatment options, our present microbiota analysis could determine the actual correlations and roles of gut bacteria in the development of T1DM.

In the present study, the bacterial diversity of the T1DM-associated fecal microbiota was significantly increased[6,8,10,18], which is inconsistent with the findings of previous studies. Several case-control studies have reported that the bacterial diversity

**A**


DM1
Control
c__Negativicutes
c__Erysipelotrichia
c__Gammaproteobacteria
a: g__Adlercreutzia
b: g__Collinsella
c: f__Coriobacteriaceae
d: o__Coriobacteriales
e: g__Dorea
f: g__Lachnospiraceae ND3007 group
g: g__Eubacterium hallii group
h: f__Lachnospiraceae
i: f__Peptostreptococcaceae
j: g__Butyricicoccus
k: g__Flavonifractor
l: g__Ruminiclostridium 5
m: g__Ruminiclostridium 9
n: g__Ruminococcaceae UCG003
o: g__Ruminococcaceae UCG013
p: g__uncultured Ruminococcaceae
q: f__Ruminococcaceae
r: g__Erysipelotrichaceae UCG003
s: g__Turicibacter
t: f__Erysipelotrichaceae
u: o__Erysipelotrichales
v: o__Selenomonadales
w: g__Klebsiella
x: f__Enterobacteriaceae
y: o__Enterobacteriales

**B**


DM1
Control
s__Bacteroides vulgatus ATCC8482
o__Selenomonadales
c__Negativicutes
s__Bacteroides ovatus
s__Parabacteroides distasonis ATCC8503
s__Bacteroides xylanisolvens
g__Ruminiclostridium 9
g__Flavonifractor
s__Flavonifractor plautii
s__Bacteroides dorei
f__Ruminococcaceae
s__Ruminococcaceae bacterium 668
g__uncultured Ruminococcaceae
g__Ruminiclostridium 5
g__Ruminococcaceae UCG013
f__Peptostreptococcaceae
g__Butyricicoccus
g__Klebsiella
f__Lachnospiraceae
f__Erysipelotrichaceae
o__Erysipelotrichales
c__Erysipelotrichia
g__Erysipelotrichaceae UCG003
g__Ruminococcaceae UCG003
s__Dorea longicatena
g__Dorea
c__Gammaproteobacteria
s__Anaerostipes hadrus
g__Turicibacter
s__Collinsella aerofaciens
g__Collinsella
g__Lachnospiraceae ND3007 group
s__Ruminococcus sp 5_1_39BFAA
f__Coriobacteriaceae
o__Coriobacteriales
f__Enterobacteriaceae
o__Enterobacteriales
s__Parabacteroides merdae
g__Eubacterium hallii group
s__Bacteroides coprocola DSM17136
g__Adlercreutzia
-4
-2
0
2
4
LDA score (log 10)

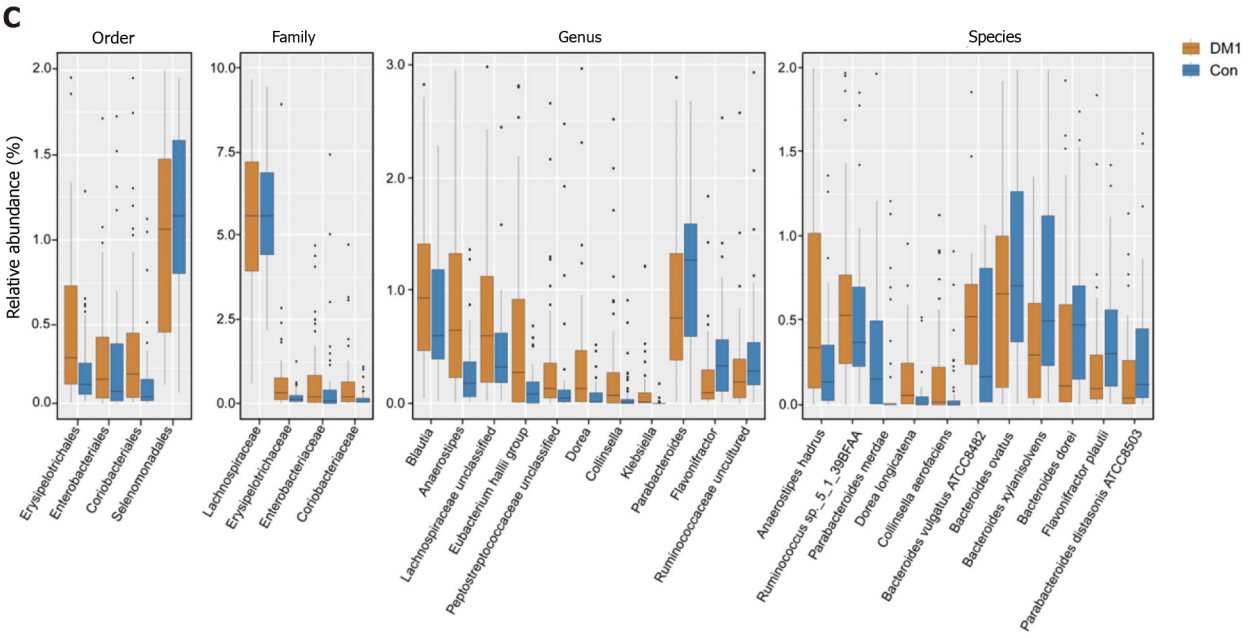



**Figure 3 Differential bacterial taxa between Chinese children with type 1 diabetes mellitus and healthy controls.** The linear discriminant analysis effect size identifies the taxa with the greatest differences in abundance between children with type 1 diabetes mellitus (T1DM) and healthy controls. A and B: Only the taxa meeting a significant linear discriminant analysis threshold value of > 2 are shown; C: Comparisons of the relative abundance of abundant bacterial taxa at the levels of the order, family, genus, and species. Data are presented as the mean ± SD. Mann–Whitney *U*-tests were used to analyze variation between children with T1DM and healthy controls. Con: Control; DM1: Type 1 diabetes mellitus.

of the gut microbiota is not significantly different in children with T1DM[8,10,18]. However, Leiva-Gea *et al*[6] found that T1DM is associated with a significantly lower microbiota diversity[6]. Furthermore, de Goffau *et al*[5] indicated that the age of children may influence bacterial diversity, and older children with T1DM tend to have a higher bacterial diversity[5]. In addition, differences in geographic location could influence the gut microbiota in early childhood, which might explain the disparity in bacterial diversity among different T1DM-associated microbiota studies. As significant inter-personal variations were observed, our PCoA could not separate the children with T1DM from healthy controls in these case-control studies. This indicates that β-diversity was similar between children with T1DM and healthy controls. In contrast to microbiota shifts in other childhood diseases, such as antibiotic-associated diarrhea, our present α- and β-diversity analyses showed increased bacterial diversity in children with T1DM, which could be used as a potential target dietary intervention in T1DM.

Inconsistent with previous findings regarding bacterial diversity, our LEfSe analysis showed that several taxa could be used as biomarkers to discriminate T1DM children from healthy controls. Consistent with previous human and animal studies, Bacteroidetes and Firmicutes were the two most predominant phyla, accounting for more than 93% of the gut microbiota in children. Most of the differentially abundant taxa at the genus and species levels belonged to these two phyla. However, the dominant phyla showed no significant differences between the two groups. The composition of the gut microbiota at the phylum level varied with age, and a greater relative abundance of Bacteroidetes was observed among older individuals. Despite the absence of differentially abundant phyla, the F/B ratio was significantly higher in children with T1DM. The F/B ratio is an indicator of gut dysbiosis, which is positively correlated with BMI[19]. Alterations in the F/B ratio may be important, as this ratio can influence efficiency in the processing of indigestible complex polysaccharides in the diet[20,21]. A previous study reported that the F/B ratio showed a significant decline in adults with type 2 diabetes mellitus (T2DM)[22]. Although the change patterns are not always consistent in patients with diabetes, an altered F/B ratio demonstrates a dysbiotic gut microbiota compared with healthy controls.

Specifically, we found that several genera and species of the phyla Firmicutes and Bacteroidetes were significantly altered in the T1DM-associated fecal microbiota. Interestingly, the co-network analysis indicated that interactions among altered bacterial species and abundant species play an important role in shaping the overall structure and composition of the T1DM-associated fecal microbiota. We found a more

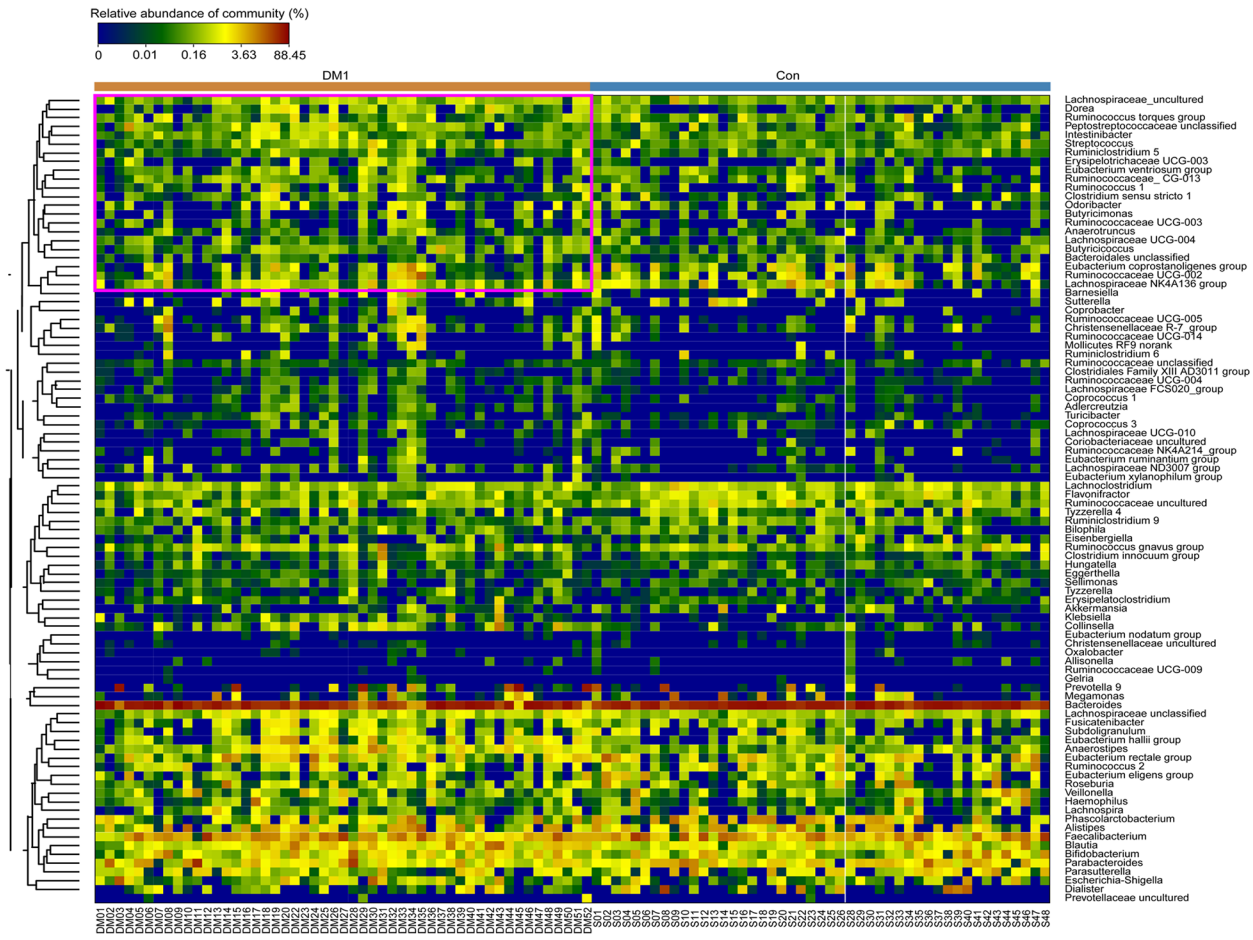



**Figure 4 Heatmap of the type 1 diabetes mellitus-associated fecal microbiota at the genus level.** The color of the spots in the panel represents the relative abundance (normalized and log10-transformed) of the genus in each sample. Relative abundance of the bacteria in each genus is indicated by a gradient of color from blue (low abundance) to red (high abundance). Genera were organized according to Spearman's correlation analysis, based on their relative abundances. Taxonomic classifications of the genus are shown on the right.

complex network of interactions in healthy controls than in children with T1DM, with more positive and negative correlations in healthy controls. These differentially abundant bacterial species played vital roles in regulating blood glucose in children. *Bacteroides vulgatus* ATCC8482, a highly abundant gram-negative obligate anaerobe, constitutes part of the core gut microbiota in healthy humans and is generally considered beneficial[23]. We found that the level of *Bacteroides vulgatus* ATCC8482 was significantly reduced in the T1DM-associated fecal microbiota and negatively correlated with FBG. Leiva-Gea *et al*[6] also found that the prevalence of *Bacteroides vulgatus* was significantly reduced in patients with T2DM, which can be considered a gut microbiota signature associated with the development of T2DM. Pedersen *et al*[24] identified *Bacteroides vulgatus* as the main species driving the association between the biosynthesis of branched-chain amino acids (BCAAs) and insulin resistance, suggesting that it may directly impact host metabolism[24]. Similar to our present findings, Yoshida *et al*[25] also revealed a significantly lower abundance of *Bacteroides vulgatus* in patients with coronary artery disease. Gavage with live *Bacteroides vulgatus* can attenuate atherosclerotic lesion formation in atherosclerosis-prone mice. Such action can thereby markedly ameliorate endotoxemia, directly reduce gut microbial lipopolysaccharide (LPS) production, and effectively suppress proinflammatory immune responses. These studies suggest that *Bacteroides vulgatus* plays a beneficial role in regulating blood glucose and preventing the development of T1DM.

Another dominant species of the genus *Bacteroides* in the human gut microbiota, *Bacteroides dorei*, shares similar 16S rRNA sequencing patterns with *Bacteroides vulgatus*. Leonard *et al*[26] found that cesarean section delivery is associated with a reduced abundance of the beneficial species, *Bacteroides vulgatus* and *Bacteroides dorei* [27]. Altered relative abundance of *Bacteroides dorei* can significantly influence the composition of the gut microbiota, and this species can be considered a keystone species[27]. A previous study showed that increased abundance of *Bacteroides dorei*

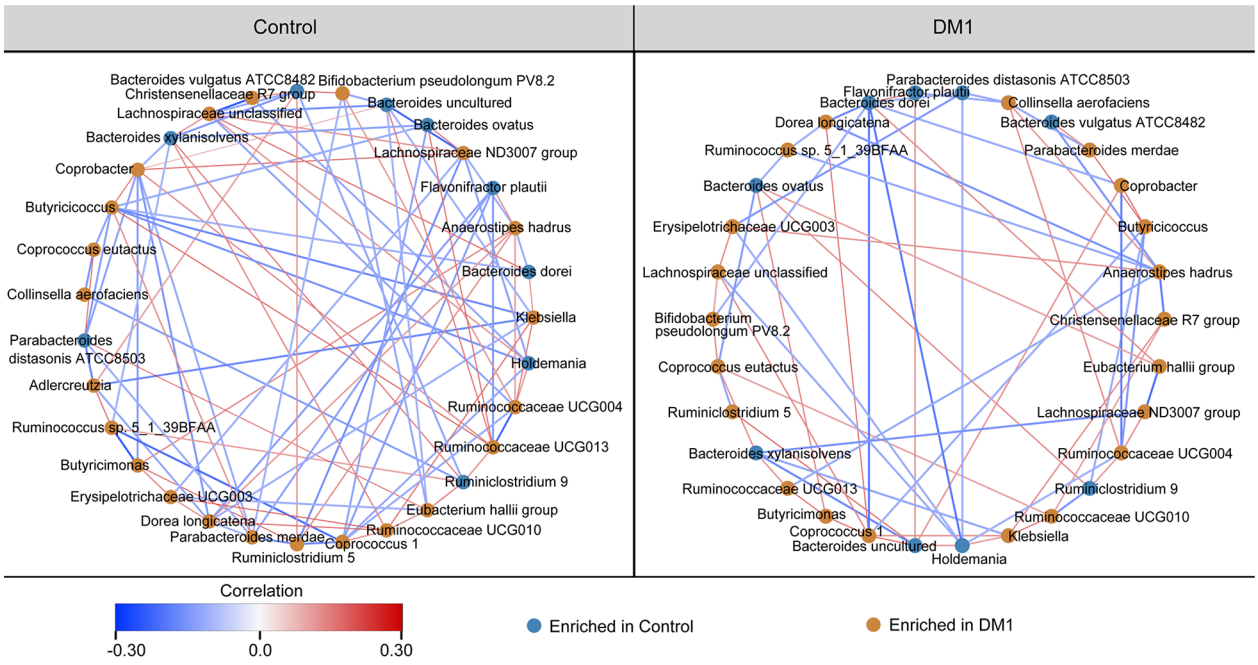



**Figure 5 Correlation strengths of the abundant fecal microbiota in Chinese children with type 1 diabetes mellitus and healthy controls.** Correlation network of the abundant fecal microbiota in healthy controls and children with type 1 diabetes mellitus (T1DM) is shown. Correlation coefficients were calculated with the sparse correlation for compositional data algorithm. The Cytoscape version 3.4.0 software was used for network construction. Red and blue lines represent positive and negative correlations, respectively. The correlation network became simpler in T1DM. DM1: Type 1 diabetes mellitus.

leads to reduced gut microbial production of LPS, which improves immune function through mechanisms such as major histocompatibility complex production and T cell activation[25]. Anonye *et al*[28] also found that *Clostridium difficile* growth is significantly reduced in the presence of *Bacteroides dorei*[28]. However, a recent study on gestational diabetes identified *Bacteroides dorei* as a putative biomarker of impaired carbohydrate tolerance, and suggested that it played a role in the regulation of glucose tolerance in pregnant women[29]. However, our present correlation analysis found no significant association between *Bacteroides dorei* and FBG. Inconsistent with our present alteration patterns, previous studies have found that higher levels of *Bacteroides dorei* may contribute to the onset of T1DM, and may be potential monitoring and therapeutic microbial markers for T1DM[30]. *Bacteroides ovatus* is a common member of the human gut microbiota, with a broad capability to degrade complex glycans[31]. It makes considerable contributions to the overall differences between T1DM cases and controls. Consistent with our results, Giongo *et al*[14] found that *Bacteroides ovatus* accounted for nearly 24% of the total increase in the phylum Bacteroidetes among children with T1DM[14] and for the first time established a causal relationship between *Bacteroides ovatus* and metabolic homeostasis. Their findings demonstrated that *Bacteroides ovatus* may be a potentially beneficial intestinal bacterial species. Similar to *Bacteroides vulgatus*, *Bacteroides ovatus* can also regulate BCAA metabolism, and alleviate metabolic syndrome. A recent study performed by Yang *et al*[32] demonstrated that specific strains of *Bacteroides ovatus* are capable of inducing high levels of mucosal immunoglobulin A (IgA) production in the large intestine, which can be used to modulate the host immune response[32]. In addition, as one of the active immunomodulators, oral gavage of *Bacteroides ovatus* could significantly increase the efficacy of erlotinib and induce the expression of CXCL9 and interferon-gamma in a murine lung cancer model, which was positively correlated with treatment outcomes[33]. Another *Bacteroides* species with reduced levels in T1DM, *Bacteroides xylanisolvens*, is a xylan-degrading bacterium isolated from human feces. Following a safety evaluation of a *Bacteroides xylanisolvens* strain (DSM 23964), a previous study reported its potential probiotic properties[34]. Consistent with a previous study on patients with atherosclerosis[35], *Bacteroides xylanisolvens* is reportedly an important contributor to folate transformations II and glycolysis III, and it is significantly more abundant in healthy controls than in patients with T1DM. Qiao *et al*[36] also found that *Bacteroides xylanisolvens* can alleviate nonalcoholic hepatic steatosis and provided evidence of the benefits of the gut *Bacteroides*-folate-liver pathway[36]. *Bacteroides xylanisolvens* is considered a probiotic bacterium that is positively correlated with anti-

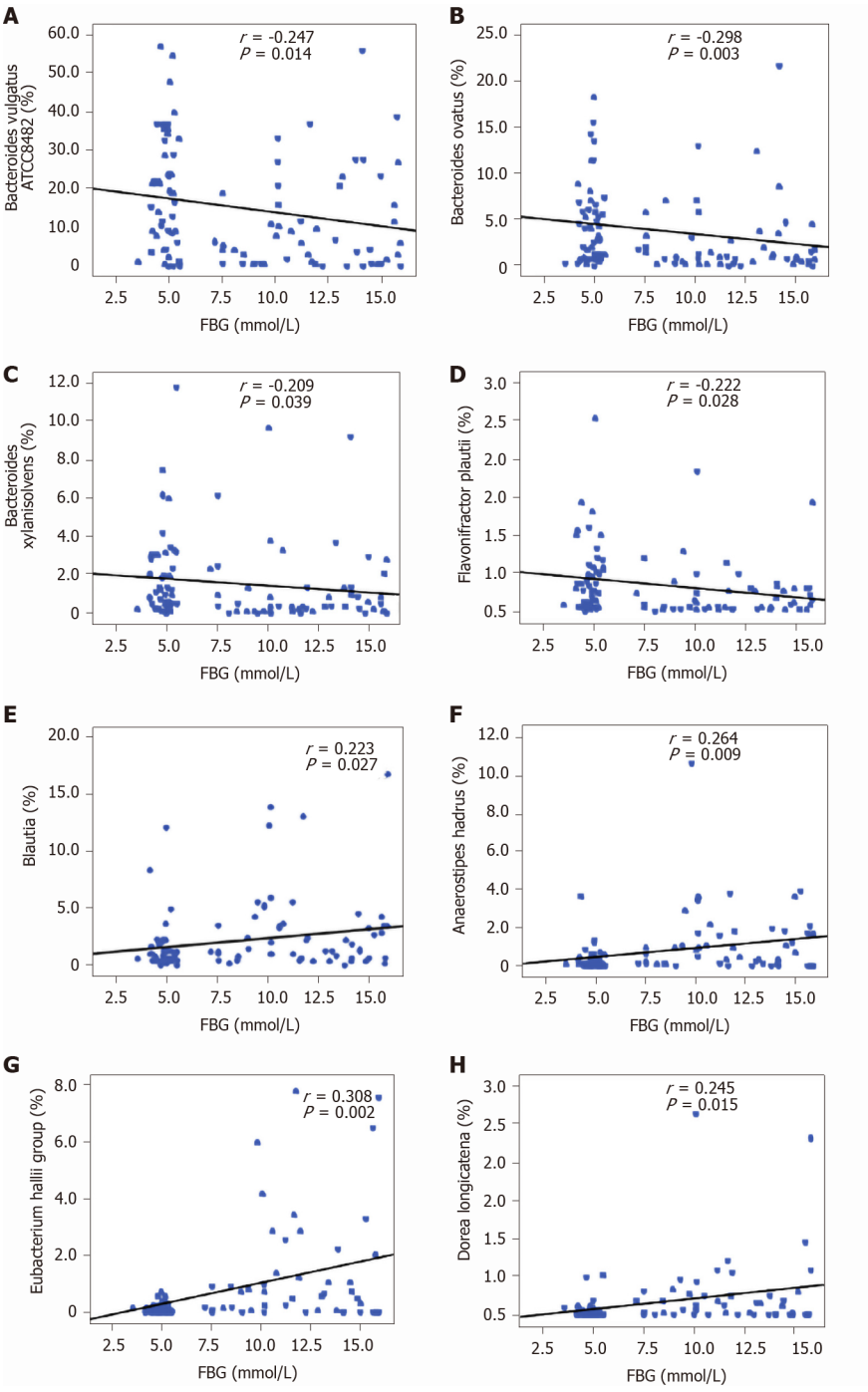



**Figure 6 Correlation between key bacteria in type 1 diabetes mellitus-associated fecal microbiota and fasting blood glucose.** The different taxa, including *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, and *Flavonifractor plautii*, were negatively correlated with fasting blood glucose (FBG), while *Blautia*, the *Eubacterium hallii* group, *Anaerostipes hadrus*, and *Dorea longicatena* were positively correlated with FBG. Spearman's rank correlation and probability were used to evaluate statistical significance. A: *Bacteroides vulgatus* ATCC8482; B: *Bacteroides ovatus*; C: *Bacteroides xylanisolvens*; D: *Flavonifractor plautii*; E: *Blautia*; F: *Anaerostipes hadrus*; G: *Eubacterium hallii*; H: *Dorea longicatena*. FBG: Fasting blood glucose.

inflammatory/tumor markers and negatively correlated with proinflammatory/tumor markers[37]. Sufficient evidence has supported and facilitated authorization of the use of heat-inactivated *Bacteroides xylanisolvens* in the European Union[38]. *Flavonifractor plautii*, a Gram-positive anaerobic bacterium, is a member of *Clostridium* cluster IV in the Ruminococcaceae family, and has been isolated worldwide from human feces. Our data showed a lower level of *Flavonifractor plautii* in children with T1DM than in healthy controls. This finding suggests that *Flavonifractor plautii* plays a beneficial role in regulating the metabolism of blood glucose. Similar to our present findings, Borgo *et al*[39] found that *Flavonifractor plautii* is negatively correlated with BMI[39]. Kasai *et al*[40] found that the fraction of *Flavonifractor plautii* is significantly lower in feces from obese subjects than in feces from non-obese[40]. Recently, Mikami *et al*[41] suggested

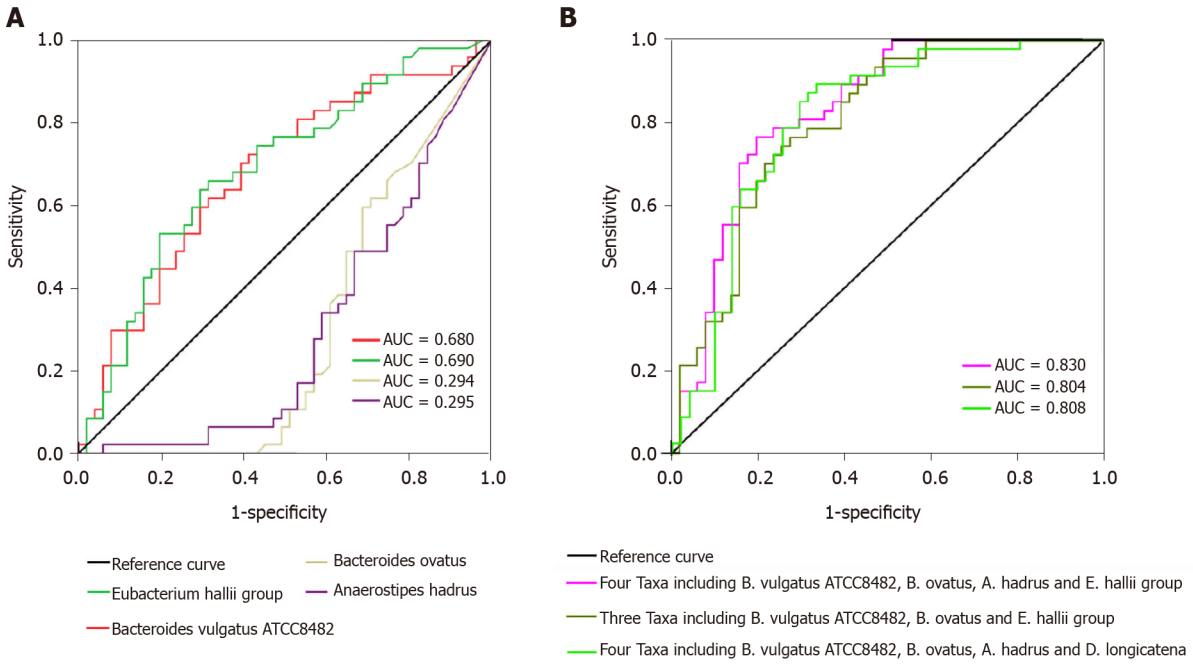



**Figure 7 Receiver operating characteristic curves for different taxa, including *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Anaerostipes hadrus*, and the *Eubacterium hallii* group, which were used either alone or in combination to discriminate between patients with type 1 diabetes mellitus and healthy controls.** A: Alone; B: Combination. AUC: Area under the curve.

that oral administration of *Flavonifractor plautii* prevents the accumulation of tumor necrosis factor-α-encoding transcripts in the adipose tissue of obese mice, thereby suppressing adipose tissue-associated chronic inflammation[41]. In addition, their group also found that *Flavonifractor plautii* alleviates antigen-induced Th2 immune responses, and can be used as a potential anti-allergic probiotic[42]. *Flavonifractor plautii* abundance in fecal samples has now been proposed as a biomarker of health status[39]. *Parabacteroides distasonis*, a core member of the gut microbiota in humans, is also reportedly a beneficial commensal gut microorganism in different pathophysiological models due to its anti-inflammatory and barrier restorative abilities. The abundance of *Parabacteroides distasonis* is relatively low in patients affected by obesity, nonalcoholic fatty liver disease (NAFLD), and multiple sclerosis[43-45]. A recent study indicated that *Parabacteroides distasonis* modulates host metabolism and alleviates obesity and metabolic dysfunctions *via* the production of succinate and secondary bile acids[46]. Colonization of antibiotic-treated or germ-free mice with a single *Parabacteroides distasonis* strain induced Treg differentiation[43]. The abundance of another *Parabacteroides* species, *Parabacteroides merdae*, was also reduced in children with T1DM, indicating its beneficial role during T1DM development. Wang *et al*[47] recently reported that enrichment of *Parabacteroides merdae* is positively correlated with longevity[47]. These bacteria exhibit promising potential beneficial effects on human health in a strain-dependent manner. Thus, several strains could contribute to the development of chronic diseases.

The proportional abundances of four species, namely, *Anaerostipes hadrus*, *Ruminococcus* sp. 5_1_39BFAA, *Dorea longicatena*, and *Collinsella aerofaciens*, were significantly increased in children with T1DM. *Anaerostipes hadrus*, one of the core species isolated from human feces, is able to produce large amounts of butyrate from both L-sorbose and xylitol and can consume acetate[48]. Zhang *et al*[49] found that *Anaerostipes hadrus* can significantly aggravate colitis in dextran sulfate sodium-treated mice, but exerts no detrimental effects in healthy mice[49]. Recently, Zeevi *et al*[50] observed that several genomic structural variants of *Anaerostipes hadrus* are negatively correlated with weight, waist circumference, median blood glucose levels, and BMI and positively correlated with HDL cholesterol levels[50]. The functions of *Anaerostipes hadrus* structural variants are not consistent with those of the wild type, which is strongly associated with lower metabolic risk. *Ruminococcus* sp. 5_1_39BFAA, a member of the genus *Blautia*, is positively associated with FBG. A previous study reported that *Ruminococcus* sp. 5_1_39BFAA is enriched among elderly patients with hypertension and reduced exercise capacity[51]. To date, no studies have extensively investigated the roles and mechanisms of *Ruminococcus* sp. 5_1_39BFAA. *Blautia* is associated with

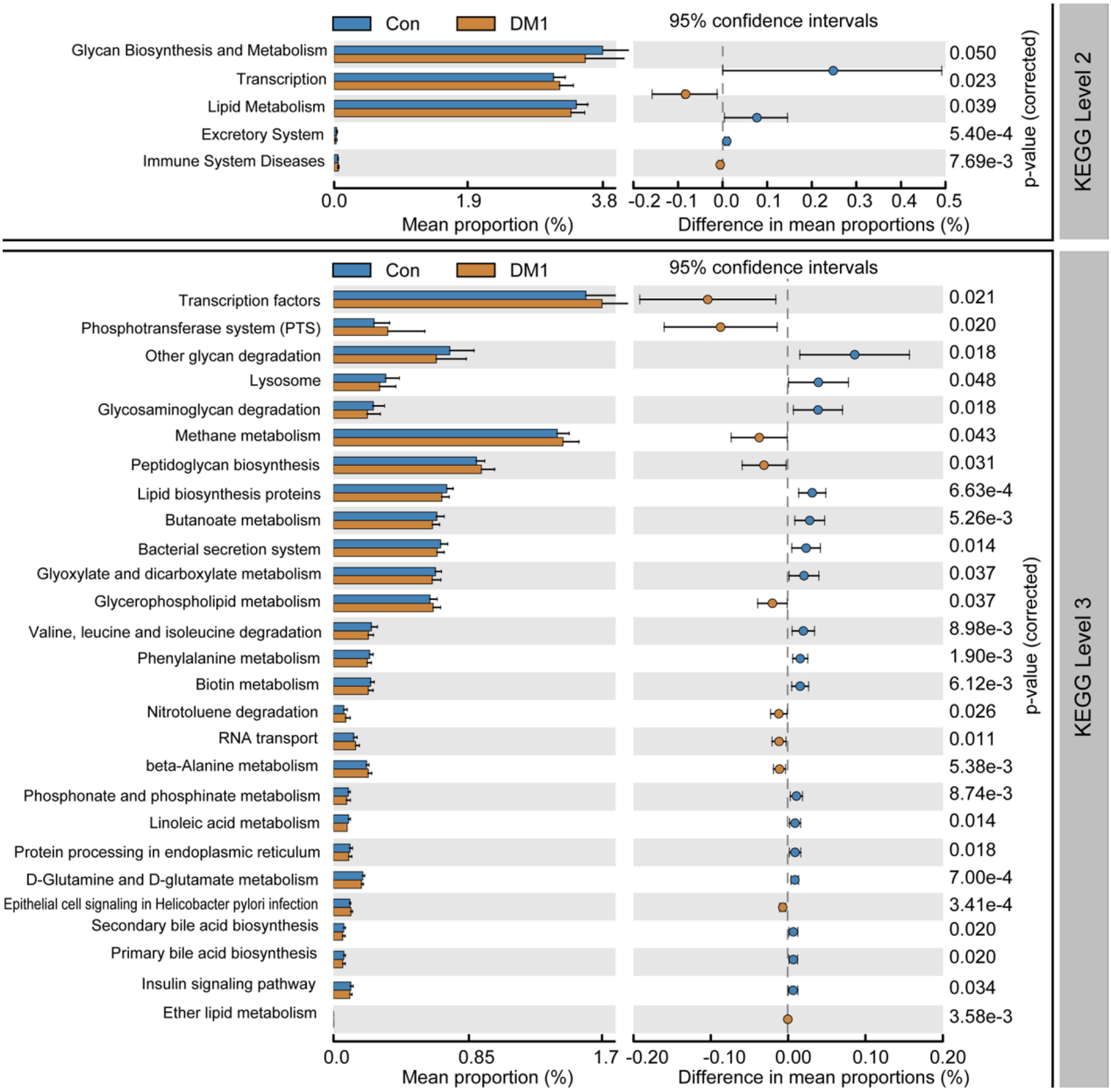



**Figure 8 PiCRUSt-based fecal microbiome study among Chinese children with type 1 diabetes mellitus and healthy controls.** Different bacterial functions were evaluated based on the two-sided Welch's *t*-test. Comparisons between the two groups for each Kyoto Encyclopedia of Genes and Genome functional category (level 2 and level 3) are shown by percentage. The Benjamini–Hochberg method was used for multiple testing correction, based on the false discovery rate using the Statistical Analysis of Metagenomic Profiles software. KEGG: Kyoto Encyclopedia of Genes and Genome; Con: Control; DM1: Type 1 diabetes mellitus.

blood glucose regulation, lipid metabolism, and regulation of T cell differentiation [6,52]. However, increased proportions of *Blautia* have been reported in various diseases, such as IBS, NAFLD, and Crohn's disease. A previous case-control study also found that the abundance of *Blautia* is increased in children with T1DM, and is positively correlated with HbA1c, the number of T1DM autoantibodies, and the titers of tyrosine phosphatase autoantibodies[52]. Consistent with the findings of Kostic *et al*[53], the present study demonstrated that *Blautia* is positively correlated with the levels of FBG in children with T1DM. *Dorea longicatena*, a new member of *Clostridium* cluster XIVa in the Lachnospiraceae family, can produce acetate as a fermentation product. Mortaş *et al*[54] found that *Dorea longicatena* is significantly more abundant in individuals working the night shift[54]. Yang *et al*[55] found that *Dorea* is a biomarker of the risk for colorectal cancer[55]. Higher abundance of the *Dorea* genus is associated with increased intestinal permeability. In contrast with our present findings, Brahe *et al*[56] reported that *Dorea longicatena* is negatively correlated with markers for insulin resistance, such as glucose and insulin, in obese female participants [56]. Nevertheless, the increased abundance of *Dorea longicatena* in T1DM could be actively

involved in regulating host metabolism. *Collinsella aerofaciens*, one of the most abundant Actinobacteria in the gastrointestinal tract of healthy humans, shows increased abundance in the feces of patients with T1DM; one of its subspecies is capable of butyrate production[57]. Increased *Collinsella* abundance has been associated with both positive and negative health conditions, but there is no consensus on its health effects. Cohort studies have identified increases in *Collinsella* abundance in the fecal microbiota of patients with T2DM, atherosclerosis, and IBS[58-60]. Turnbaugh *et al*[61] reported that the enrichment of *Collinsella aerofaciens* is linked to BMI, with an increased prominence of *Collinsella aerofaciens* in obese individuals than in lean twins and their mothers[61]. Another abundant bacterium in T1DM, the *Eubacterium hallii* group (an anaerobic, Gram-positive, catalase-negative bacterium of the Lachnospiraceae family), is a metabolically versatile species that can contribute to intestinal butyrate and propionate formation[62,63]. We observed a positive correlation between the *Eubacterium hallii* group and FBG. Consistent with the present data, Ye *et al*[64] found that the abundance of the *Eubacterium hallii* group was significantly higher in patients with gestational diabetes mellitus (GDM) who failed to make lifestyle modifications for glycemic control, which is also positively correlated with FBG[64]. They also showed that the *Eubacterium hallii* group can be used to distinguish GDM patients and patients who failed glycemic control from healthy controls. As mentioned by Schwab *et al*[63], the *Eubacterium hallii* group may actively contribute to metabolic interactions[63]. However, Udayappan *et al*[65] observed that oral treatment with the *Eubacterium hallii* group can improve insulin sensitivity in db/db mice[65], which is inconsistent with the present findings. Our ROC analysis found that the bacteria mentioned above, such as *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, the *Eubacterium hallii* group, and *Anaerostipes hadrus*, can be used as potential biomarkers to discriminate children with T1DM from healthy controls. These bacteria in patients with T1DM might contribute to alterations in microbial functions and actively participate in the development of T1DM. Therefore, they could be used as novel targets for non-invasive diagnostic biomarkers and personalized treatment of T1DM in the future.

There are several limitations of the present study. First, our case-control study only explored the characteristics of the T1DM-associated fecal microbiota, whereas alterations in the fecal microbiota after successful treatment were not investigated. Second, the fecal microbial signature and corresponding metabolites, as well as the diagnostic model associated with T1DM, still require further clinical studies with a larger sample size to validate the results. Third, the relatively weak correlations between key differential functional bacteria and FBG could not indicate an obvious primary or secondary relationship. More clinical indicators should be added into these correlation analyses in future studies. Fourth, culturomics should be used to identify T1DM-associated bacteria. Further animal experiments could help to determine the cause-effect relationship between these bacteria and the pathogenesis of T1DM.

## CONCLUSION

In summary, the present study investigated the altered profiles of fecal microbiota in children with T1DM. High-throughput sequencing identified the detailed composition and diversity of the T1DM-associated fecal microbiota at a much deeper level. We found that bacterial diversity was significantly increased in the T1DM-associated fecal microbiota. We also observed microbial compositional changes at different taxonomic levels. The proportions of *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, *Flavonifractor plautii*, *Anaerostipes hadrus*, and *Dorea longicatena* at the species level and *Blautia* and the *Eubacterium hallii* group at the genus level showed significant differences between children with T1DM and healthy controls, and were markedly correlated with FBG. Furthermore, *Bacteroides vulgatus* ATCC8482 and *Bacteroides ovatus*, either alone or in combination, can be used as non-invasive diagnostic biomarkers to distinguish between patients with T1DM and healthy controls. In addition, functional changes in the T1DM-associated fecal microbiota suggest that the alterations are associated with the functions and metabolic activities of the microbiota, which might play vital roles in the pathogenesis and development of T1DM. Thus, our comprehensive investigation of the T1DM-associated fecal microbiota provides novel insights into the pathogenesis of T1DM.

## ARTICLE HIGHLIGHTS

### *Research background*

Gut microbiota dysbiosis is reportedly actively involved in autoimmune diseases such as type 1 diabetes mellitus (T1DM). However, the alterations in the gut microbiota and their correlation with fasting blood glucose (FBG) in Chinese children with T1DM remain unclear.

### *Research motivation*

Most of the previous studies on the T1DM-associated microbiota were conducted in the United States and Europe. However, differences in lifestyle, dietary constitution, environmental exposure, and host genetic background between Chinese and Western populations may contribute to disparities in the baseline microbiota composition, which may influence the roles of specific bacteria in the etiopathology of T1DM.

### *Research objectives*

Our present study aimed to investigate alterations in the gut microbiota in Chinese children with T1DM and their associations with clinical indicators.

### *Research methods*

Samples from 51 children with T1DM and 47 age- and gender-matched healthy controls were obtained to explore the structural and functional alterations in the fecal microbiota. The V3-V4 regions of the 16S rRNA gene were sequenced on a MiSeq instrument and the association with FBG was analyzed.

### *Research results*

We found that the bacterial diversity was significantly increased in the T1DM-associated fecal microbiota, and changes in the microbial composition were observed at different taxonomic levels. The T1DM-reduced differentially abundant taxa, such as *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, *Bacteroides xylanisolvens*, and *Flavonifractor plautii*, were negatively correlated with FBG, while the T1DM-enriched taxa, such as *Blautia*, *Eubacterium hallii* group, *Anaerostipes hadrus*, and *Dorea longicatena*, were positively correlated with FBG. *Bacteroides vulgatus* ATCC8482, *Bacteroides ovatus*, the *Eubacterium hallii* group, and *Anaerostipes hadrus*, either alone or in combination, could be used as non-invasive diagnostic biomarkers to discriminate children with T1DM from healthy controls. In addition, the functional changes in the T1DM-associated fecal microbiota also suggest that these fecal microbes were associated with altered functions and metabolic activities, such as glycan biosynthesis and metabolism and lipid metabolism, which might play vital roles in the pathogenesis and development of T1DM.

### *Research conclusions*

Our present comprehensive investigation of the T1DM-associated fecal microbiota provides novel insights into the pathogenesis of the disease and sheds light on the diagnosis and treatment of T1DM.

### *Research perspectives*

Further causal-development studies based on the present results will expand the knowledge on the pathogenesis of T1DM, which will help to provide microbiota-targeted T1DM diagnosis and treatment.

## ACKNOWLEDGEMENTS

The authors thank all of the participants who recruited patients in this study.

## REFERENCES

1 **Weng J**, Zhou Z, Guo L, Zhu D, Ji L, Luo X, Mu Y, Jia W; T1D China Study Group. Incidence of type 1 diabetes in China, 2010-13: population based study. *BMJ* 2018; **360**: j5295 [PMID: 29298776 DOI: 10.1136/bmj.j5295]

2 **Rak K**, Bronkowska M. Immunomodulatory Effect of Vitamin D and Its Potential Role in the

Prevention and Treatment of Type 1 Diabetes Mellitus-A Narrative Review. *Molecules* 2018; **24** [PMID: 30586887 DOI: 10.3390/molecules24010053]

3 **Ilonen J**, Lempainen J, Veijola R. The heterogeneous pathogenesis of type 1 diabetes mellitus. *Nat Rev Endocrinol* 2019; **15**: 635-650 [PMID: 31534209 DOI: 10.1038/s41574-019-0254-y]

4 **Gowd V**, Xie L, Zheng X, Chen W. Dietary fibers as emerging nutritional factors against diabetes: focus on the involvement of gut microbiota. *Crit Rev Biotechnol* 2019; **39**: 524-540 [PMID: 30810398 DOI: 10.1080/07388551.2019.1576025]

5 **de Goffau MC**, Fuentes S, van den Bogert B, Honkanen H, de Vos WM, Welling GW, Hyöty H, Harmsen HJ. Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia* 2014; **57**: 1569-1577 [PMID: 24930037 DOI: 10.1007/s00125-014-3274-0]

6 **Leiva-Gea I**, Sánchez-Alcoholado L, Martín-Tejedor B, Castellano-Castillo D, Moreno-Indias I, Urda-Cardona A, Tinahones FJ, Fernández-García JC, Queipo-Ortuño MI. Gut Microbiota Differs in Composition and Functionality Between Children With Type 1 Diabetes and MODY2 and Healthy Control Subjects: A Case-Control Study. *Diabetes Care* 2018; **41**: 2385-2395 [PMID: 30224347 DOI: 10.2337/dc18-0253]

7 **Demirci M**, Bahar Tokman H, Taner Z, Keskin FE, Çağatay P, Ozturk Bakar Y, Özyazar M, Kiraz N, Kocazeybek BS. Bacteroidetes and Firmicutes levels in gut microbiota and effects of hosts TLR2/TLR4 gene expression levels in adult type 1 diabetes patients in Istanbul, Turkey. *J Diabetes Complications* 2020; **34**: 107449 [PMID: 31677982 DOI: 10.1016/j.jdiacomp.2019.107449]

8 **Murri M**, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, Queipo-Ortuño MI. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med* 2013; **11**: 46 [PMID: 23433344 DOI: 10.1186/1741-7015-11-46]

9 **Wen L**, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 2008; **455**: 1109-1113 [PMID: 18806780 DOI: 10.1038/nature07336]

10 **Huang Y**, Li SC, Hu J, Ruan HB, Guo HM, Zhang HH, Wang X, Pei YF, Pan Y, Fang C. Gut microbiota profiling in Han Chinese with type 1 diabetes. *Diabetes Res Clin Pract* 2018; **141**: 256-263 [PMID: 29733871 DOI: 10.1016/j.diabres.2018.04.032]

11 **Livanos AE**, Greiner TU, Vangay P, Pathmasiri W, Stewart D, McRitchie S, Li H, Chung J, Sohn J, Kim S, Gao Z, Barber C, Kim J, Ng S, Rogers AB, Sumner S, Zhang XS, Cadwell K, Knights D, Alekseyenko A, Bäckhed F, Blaser MJ. Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *Nat Microbiol* 2016; **1**: 16140 [PMID: 27782139 DOI: 10.1038/nmicrobiol.2016.140]

12 **Vatanen T**, Franzosa EA, Schwager R, Tripathi S, Arthur TD, Vehik K, Lernmark Å, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B, Krischer JP, Stewart CJ, Ajami NJ, Petrosino JF, Gevers D, Lähdesmäki H, Vlamakis H, Huttenhower C, Xavier RJ. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature* 2018; **562**: 589-594 [PMID: 30356183 DOI: 10.1038/s41586-018-0620-2]

13 **Cole DK**, Bulek AM, Dolton G, Schauenberg AJ, Szomolay B, Rittase W, Trimby A, Jothikumar P, Fuller A, Skowera A, Rossjohn J, Zhu C, Miles JJ, Peakman M, Wooldridge L, Rizkallah PJ, Sewell AK. Hotspot autoimmune T cell receptor binding underlies pathogen and insulin peptide cross-reactivity. *J Clin Invest* 2016; **126**: 2191-2204 [PMID: 27183389 DOI: 10.1172/JCI85679]

14 **Giongo A**, Gano KA, Crabb DB, Mukherjee N, Novelo LL, Casella G, Drew JC, Ilonen J, Knip M, Hyöty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW. Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011; **5**: 82-91 [PMID: 20613793 DOI: 10.1038/ismej.2010.92]

15 **Ling Z**, Shao L, Liu X, Cheng Y, Yan C, Mei Y, Ji F. Regulatory T Cells and Plasmacytoid Dendritic Cells Within the Tumor Microenvironment in Gastric Cancer Are Correlated With Gastric Microbiota Dysbiosis: A Preliminary Study. *Front Immunol* 2019; **10**: 533 [PMID: 30936882 DOI: 10.3389/fimmu.2019.00533]

16 **Liu X**, Shao L, Liu X, Ji F, Mei Y, Cheng Y, Liu F, Yan C, Li L, Ling Z. Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. *EBioMedicine* 2019; **40**: 336-348 [PMID: 30584008 DOI: 10.1016/j.ebiom.2018.12.034]

17 **Lv W**, Graves DT, He L, Shi Y, Deng X, Zhao Y, Dong X, Ren Y, Liu X, Xiao E, Zhang Y. Depletion of the diabetic gut microbiota resistance enhances stem cells therapy in type 1 diabetes mellitus. *Theranostics* 2020; **10**: 6500-6516 [PMID: 32483466 DOI: 10.7150/thno.44113]

18 **Mejía-León ME**, Petrosino JF, Ajami NJ, Domínguez-Bello MG, de la Barca AM. Fecal microbiota imbalance in Mexican children with type 1 diabetes. *Sci Rep* 2014; **4**: 3814 [PMID: 24448554 DOI: 10.1038/srep03814]

19 **Koliada A**, Syzenko G, Moseiko V, Budovska L, Puchkov K, Perederiy V, Gavalko Y, Dorofeyev A, Romanenko M, Tkach S, Sineok L, Lushchak O, Vaiserman A. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. *BMC Microbiol* 2017; **17**: 120 [PMID: 28532414 DOI: 10.1186/s12866-017-1027-1]

20 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]

21 **Turnbaugh PJ**, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008; **3**: 213-223

[PMID: 18407065 DOI: 10.1016/j.chom.2008.02.015]

22 **Larsen N**, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; **5**: e9085 [PMID: 20140211 DOI: 10.1371/journal.pone.0009085]

23 **Bittinger K**, Zhao C, Li Y, Ford E, Friedman ES, Ni J, Kulkarni CV, Cai J, Tian Y, Liu Q, Patterson AD, Sarkar D, Chan SHJ, Maranas C, Saha-Shah A, Lund P, Garcia BA, Mattei LM, Gerber JS, Elovitz MA, Kelly A, DeRusso P, Kim D, Hofstaedter CE, Goulian M, Li H, Bushman FD, Zemel BS, Wu GD. Bacterial colonization reprograms the neonatal gut metabolome. *Nat Microbiol* 2020; **5**: 838-847 [PMID: 32284564 DOI: 10.1038/s41564-020-0694-0]

24 **Pedersen HK**, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, Forslund K, Hildebrand F, Prifti E, Falony G, Le Chatelier E, Levenez F, Doré J, Mattila I, Plichta DR, Pöhö P, Hellgren LI, Arumugam M, Sunagawa S, Vieira-Silva S, Jørgensen T, Holm JB, Trošt K; MetaHIT Consortium, Kristiansen K, Brix S, Raes J, Wang J, Hansen T, Bork P, Brunak S, Oresic M, Ehrlich SD, Pedersen O. Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* 2016; **535**: 376-381 [PMID: 27409811 DOI: 10.1038/nature18646]

25 **Yoshida N**, Emoto T, Yamashita T, Watanabe H, Hayashi T, Tabata T, Hoshi N, Hatano N, Ozawa G, Sasaki N, Mizoguchi T, Amin HZ, Hirota Y, Ogawa W, Yamada T, Hirata KI. Bacteroides vulgatus and Bacteroides dorei Reduce Gut Microbial Lipopolysaccharide Production and Inhibit Atherosclerosis. *Circulation* 2018; **138**: 2486-2498 [PMID: 30571343 DOI: 10.1161/CIRCULATIONAHA.118.033714]

26 **Leonard MM**, Karathia H, Pujolassos M, Troisi J, Valitutti F, Subramanian P, Camhi S, Kenyon V, Colucci A, Serena G, Cucchiara S, Montuori M, Malamisura B, Francavilla R, Elli L, Fanelli B, Colwell R, Hasan N, Zomorrodi AR, Fasano A; CD-GEMM Team. Multi-omics analysis reveals the influence of genetic and environmental risk factors on developing gut microbiota in infants at risk of celiac disease. *Microbiome* 2020; **8**: 130 [PMID: 32917289 DOI: 10.1186/s40168-020-00906-w]

27 **Gutiérrez N**, Garrido D. Species Deletions from Microbiome Consortia Reveal Key Metabolic Interactions between Gut Microbes. *mSystems* 2019; **4** [PMID: 31311843 DOI: 10.1128/mSystems.00185-19]

28 **Anonye BO**, Hassall J, Patient J, Detamornrat U, Aladdad AM, Schüller S, Rose FRAJ, Unnikrishnan M. Probing *Clostridium difficile* Infection in Complex Human Gut Cellular Models. *Front Microbiol* 2019; **10**: 879 [PMID: 31114553 DOI: 10.3389/fmicb.2019.00879]

29 **Wu Y**, Bible PW, Long S, Ming WK, Ding W, Long Y, Wen X, Li X, Deng X, Deng Y, Guo S, Doçi CL, Wei L, Chen H, Wang Z. Metagenomic analysis reveals gestational diabetes mellitus-related microbial regulators of glucose tolerance. *Acta Diabetol* 2020; **57**: 569-581 [PMID: 31820107 DOI: 10.1007/s00592-019-01434-2]

30 T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M, Kolde R, Vlamakis H, Arthur TD, Hämäläinen AM, Peet A, Tillmann V, Uibo R, Mokurov S, Dorshakova N, Ilonen J, Virtanen SM, Szabo SJ, Porter JA, Lähdesmäki H, Huttenhower C, Gevers D, Cullen TW, Knip M; DIABIMMUNE Study Group, Xavier RJ. Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell* 2016; **165**: 842-853 [PMID: 27133167 DOI: 10.1016/j.cell.2016.04.007]

31 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Jian M, Zhou Y, Li Y, Zhang X, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65 [PMID: 20203603 DOI: 10.1038/nature08821]

32 **Yang C**, Mogno I, Contijoch EJ, Borgerding JN, Aggarwala V, Li Z, Siu S, Grasset EK, Helmus DS, Dubinsky MC, Mehandru S, Cerutti A, Faith JJ. Fecal IgA Levels Are Determined by Strain-Level Differences in Bacteroides ovatus and Are Modifiable by Gut Microbiota Manipulation. *Cell Host Microbe* 2020; **27**: 467-475. e6 [PMID: 32075742 DOI: 10.1016/j.chom.2020.01.016]

33 **Heshiki Y**, Vazquez-Uribe R, Li J, Ni Y, Quainoo S, Imamovic L, Sørensen M, Chow BKC, Weiss GJ, Xu A, Sommer MOA, Panagiotou G. Predictable modulation of cancer treatment outcomes by the gut microbiota. *Microbiome* 2020; **8**: 28 [PMID: 32138779 DOI: 10.1186/s40168-020-00811-2]

34 **Ulsemer P**, Toutounian K, Schmidt J, Karsten U, Goletz S. Preliminary safety evaluation of a new Bacteroides xylanisolvens isolate. *Appl Environ Microbiol* 2012; **78**: 528-535 [PMID: 22101046 DOI: 10.1128/AEM.06641-11]

35 **Liu S**, Zhao W, Liu X, Cheng L. Metagenomic analysis of the gut microbiome in atherosclerosis patients identify cross-cohort microbial signatures and potential therapeutic target. *FASEB J* 2020; **34**: 14166-14181 [PMID: 32939880 DOI: 10.1096/fj.202000622R]

36 **Qiao S**, Bao L, Wang K, Sun S, Liao M, Liu C, Zhou N, Ma K, Zhang Y, Chen Y, Liu SJ, Liu H. Activation of a Specific Gut Bacteroides-Folate-Liver Axis Benefits for the Alleviation of Nonalcoholic Hepatic Steatosis. *Cell Rep* 2020; **32**: 108005 [PMID: 32783933 DOI: 10.1016/j.celrep.2020.108005]

37 **Huang G**, Khan I, Li X, Chen L, Leong W, Ho LT, Hsiao WLW. Ginsenosides Rb3 and Rd reduce polyps formation while reinstate the dysbiotic gut microbiota and the intestinal microenvironment in $Apc^{Min/+}$ mice. *Sci Rep* 2017; **7**: 12552 [PMID: 28970547 DOI: 10.1038/s41598-017-12644-5]

38 **Brodmann T**, Endo A, Gueimonde M, Vinderola G, Kneifel W, de Vos WM, Salminen S, Gómez-Gallego C. Safety of Novel Microbes for Human Consumption: Practical Examples of Assessment in the European Union. *Front Microbiol* 2017; **8**: 1725 [PMID: 28955311 DOI: 10.3389/fmicb.2017.01725]

39 **Borgo F**, Garbossa S, Riva A, Severgnini M, Luigiano C, Benetti A, Pontiroli AE, Morace G, Borghi E. Body Mass Index and Sex Affect Diverse Microbial Niches within the Gut. *Front Microbiol* 2018; **9**: 213 [PMID: 29491857 DOI: 10.3389/fmicb.2018.00213]

40 **Kasai C**, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, Tameda M, Shiraki K, Ito M, Takei Y, Takase K. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol* 2015; **15**: 100 [PMID: 26261039 DOI: 10.1186/s12876-015-0330-2]

41 **Mikami A**, Ogita T, Namai F, Shigemori S, Sato T, Shimosato T. Oral administration of Flavonifractor plautii attenuates inflammatory responses in obese adipose tissue. *Mol Biol Rep* 2020; **47**: 6717-6725 [PMID: 32808115 DOI: 10.1007/s11033-020-05727-6]

42 **Ogita T**, Yamamoto Y, Mikami A, Shigemori S, Sato T, Shimosato T. Oral Administration of *Flavonifractor plautii* Strongly Suppresses Th2 Immune Responses in Mice. *Front Immunol* 2020; **11**: 379 [PMID: 32184789 DOI: 10.3389/fimmu.2020.00379]

43 **Cekanaviciute E**, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL, Crabtree-Hartman E, Sand IK, Gacias M, Zhu Y, Casaccia P, Cree BAC, Knight R, Mazmanian SK, Baranzini SE. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci USA* 2017; **114**: 10713-10718 [PMID: 28893978 DOI: 10.1073/pnas.1711235114]

44 **Del Chierico F**, Nobili V, Vernocchi P, Russo A, De Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* 2017; **65**: 451-464 [PMID: 27028797 DOI: 10.1002/hep.28572]

45 **Verdam FJ**, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, Buurman WA, de Vos WM, Rensen SS. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. *Obesity (Silver Spring)* 2013; **21**: E607-E615 [PMID: 23526699 DOI: 10.1002/oby.20466]

46 **Wang K**, Liao M, Zhou N, Bao L, Ma K, Zheng Z, Wang Y, Liu C, Wang W, Wang J, Liu SJ, Liu H. Parabacteroides distasonis Alleviates Obesity and Metabolic Dysfunctions *via* Production of Succinate and Secondary Bile Acids. *Cell Rep* 2019; **26**: 222-235. e5 [PMID: 30605678 DOI: 10.1016/j.celrep.2018.12.028]

47 **Wang N**, Li R, Lin H, Fu C, Wang X, Zhang Y, Su M, Huang P, Qian J, Jiang F, Wang H, Jiang L, Yu X, Liu J, Chen Y, Jiang Q. Enriched taxa were found among the gut microbiota of centenarians in East China. *PLoS One* 2019; **14**: e0222763 [PMID: 31639130 DOI: 10.1371/journal.pone.0222763]

48 **Sato T**, Kusuhara S, Yokoi W, Ito M, Miyazaki K. Prebiotic potential of L-sorbose and xylitol in promoting the growth and metabolic activity of specific butyrate-producing bacteria in human fecal culture. *FEMS Microbiol Ecol* 2017; **93** [PMID: 27810878 DOI: 10.1093/femsec/fiw227]

49 **Zhang Q**, Wu Y, Wang J, Wu G, Long W, Xue Z, Wang L, Zhang X, Pang X, Zhao Y, Zhao L, Zhang C. Accelerated dysbiosis of gut microbiota during aggravation of DSS-induced colitis by a butyrate-producing bacterium. *Sci Rep* 2016; **6**: 27572 [PMID: 27264309 DOI: 10.1038/srep27572]

50 **Zeevi D**, Korem T, Godneva A, Bar N, Kurilshikov A, Lotan-Pompan M, Weinberger A, Fu J, Wijmenga C, Zhernakova A, Segal E. Structural variation in the gut microbiome associates with host health. *Nature* 2019; **568**: 43-48 [PMID: 30918406 DOI: 10.1038/s41586-019-1065-y]

51 **Yu Y**, Mao G, Wang J, Zhu L, Lv X, Tong Q, Fang Y, Lv Y, Wang G. Gut dysbiosis is associated with the reduced exercise capacity of elderly patients with hypertension. *Hypertens Res* 2018; **41**: 1036-1044 [PMID: 30291307 DOI: 10.1038/s41440-018-0110-9]

52 **Qi CJ**, Zhang Q, Yu M, Xu JP, Zheng J, Wang T, Xiao XH. Imbalance of Fecal Microbiota at Newly Diagnosed Type 1 Diabetes in Chinese Children. *Chin Med J (Engl)* 2016; **129**: 1298-1304 [PMID: 27231166 DOI: 10.4103/0366-6999.182841]

53 **Kostic AD**, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen AM, Peet A, Tillmann V, Pöhö P, Mattila I, Lähdesmäki H, Franzosa EA, Vaarala O, de Goffau M, Harmsen H, Ilonen J, Virtanen SM, Clish CB, Orešič M, Huttenhower C, Knip M; DIABIMMUNE Study Group, Xavier RJ. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 2015; **17**: 260-273 [PMID: 25662751 DOI: 10.1016/j.chom.2015.01.001]

54 **Mortaş H**, Bilici S, Karakan T. The circadian disruption of night work alters gut microbiota consistent with elevated risk for future metabolic and gastrointestinal pathology. *Chronobiol Int* 2020; **37**: 1067-1081 [PMID: 32602753 DOI: 10.1080/07420528.2020.1778717]

55 **Yang J**, McDowell A, Kim EK, Seo H, Lee WH, Moon CM, Kym SM, Lee DH, Park YS, Jee YK, Kim YK. Development of a colorectal cancer diagnostic model and dietary risk assessment through gut microbiome analysis. *Exp Mol Med* 2019; **51**: 1-15 [PMID: 31582724 DOI: 10.1038/s12276-019-0313-4]

56 **Brahe LK**, Le Chatelier E, Prifti E, Pons N, Kennedy S, Hansen T, Pedersen O, Astrup A, Ehrlich SD, Larsen LH. Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. *Nutr Diabetes* 2015; **5**: e159 [PMID: 26075636 DOI: 10.1038/nutd.2015.9]

57 **Qin P**, Zou Y, Dai Y, Luo G, Zhang X, Xiao L. Characterization a Novel Butyric Acid-Producing Bacterium *Collinsella aerofaciens* Subsp. *Shenzhenensis* Subsp. Nov. *Microorganisms* 2019; **7** [PMID: 30871249 DOI: 10.3390/microorganisms7030078]

58 **Candela M**, Biagi E, Soverini M, Consolandi C, Quercia S, Severgnini M, Peano C, Turroni S, Rampelli S, Pozzilli P, Pianesi M, Fallucca F, Brigidi P. Modulation of gut microbiota dysbioses in type 2 diabetic patients by macrobiotic Ma-Pi 2 diet. *Br J Nutr* 2016; **116**: 80-93 [PMID: 27151248 DOI: 10.1017/S0007114516001045]

59 **Karlsson FH**, Fåk F, Nookaew I, Tremaroli V, Fagerberg B, Petranovic D, Bäckhed F, Nielsen J. Symptomatic atherosclerosis is associated with an altered gut metagenome. *Nat Commun* 2012; **3**: 1245 [PMID: 23212374 DOI: 10.1038/ncomms2266]

60 **Masoodi I**, Alshanqeeti AS, Alyamani EJ, AlLehibi AA, Alqutub AN, Alsayari KN, Alomair AO. Microbial dysbiosis in irritable bowel syndrome: A single-center metagenomic study in Saudi Arabia. *JGH Open* 2020; **4**: 649-655 [PMID: 32782952 DOI: 10.1002/jgh3.12313]

61 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]

62 **Shetty SA**, Ritari J, Paulin L, Smidt H, De Vos WM. Complete Genome Sequence of *Eubacterium hallii* Strain L2-7. *Genome Announc* 2017; **5** [PMID: 29074659 DOI: 10.1128/genomeA.01167-17]

63 **Schwab C**, Ruscheweyh HJ, Bunesova V, Pham VT, Beerenwinkel N, Lacroix C. Trophic Interactions of Infant Bifidobacteria and *Eubacterium hallii* during L-Fucose and Fucosyllactose Degradation. *Front Microbiol* 2017; **8**: 95 [PMID: 28194144 DOI: 10.3389/fmicb.2017.00095]

64 **Ye G**, Zhang L, Wang M, Chen Y, Gu S, Wang K, Leng J, Gu Y, Xie X. The Gut Microbiota in Women Suffering from Gestational Diabetes Mellitus with the Failure of Glycemic Control by Lifestyle Modification. *J Diabetes Res* 2019; **2019**: 6081248 [PMID: 31772944 DOI: 10.1155/2019/6081248]

65 **Udayappan S**, Manneras-Holm L, Chaplin-Scott A, Belzer C, Herrema H, Dallinga-Thie GM, Duncan SH, Stroes ESG, Groen AK, Flint HJ, Backhed F, de Vos WM, Nieuwdorp M. Oral treatment with *Eubacterium hallii* improves insulin sensitivity in *db/db* mice. *NPJ Biofilms Microbiomes* 2016; **2**: 16009 [PMID: 28721246 DOI: 10.1038/npjbiofilms.2016.9]

World Journal of Gastroenterology

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

*World J Gastroenterol* 2021 May 21; 27(19): 2415-2433

DOI: 10.3748/wjg.v27.i19.2415

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

META-ANALYSIS

# Selection of first-line systemic therapies for advanced hepatocellular carcinoma: A network meta-analysis of randomized controlled trials

Yue Han, Wei-Hua Zhi, Fei Xu, Chen-Bo Zhang, Xiao-Qian Huang, Jian-Feng Luo

**ORCID number:** Yue Han 0000-0001-7274-009X; Wei-Hua Zhi 0000-0001-5920-3548; Fei Xu 0000-0002-6529-3508; Chen-Bo Zhang 0000-0003-3320-7828; Xiao-Qian Huang 0000-0003-3320-7829; Jian-Feng Luo 0000-0002-9833-0416.

**Author contributions:** Han Y, Zhi WH, and Xu F made contributions to the literature search; Zhi WH and Xu F were involved in figure preparation; Han Y and Luo JF made contributions to the study design; Luo JF and Huang XQ were involved in collecting the data; Luo JF, Huang XQ, and Zhang CB were involved in analyzing the data; Han Y, Luo JF, and Zhi WH made contributions to the data interpretation; Han Y and Zhi WH were involved in manuscript writing; Zhang CB and Han Y made contributions to the verification of the data.

**Conflict-of-interest statement:** The authors have no declarations of interest to declare.

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

**Open-Access:** This article is an open-access article that was selected by an in-house editor and

**Yue Han, Wei-Hua Zhi, Fei Xu,** Department of Interventional Therapy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

**Chen-Bo Zhang, Xiao-Qian Huang, Jian-Feng Luo,** Department of Biostatistics, School of Public Health, Fudan University, Shanghai 200034, China

**Corresponding author:** Yue Han, PhD, Doctor, Department of Interventional Therapy, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 9 Dongdan 3rd Alley, Dongcheng District, Beijing 100021, China. doctorhan@163.com

## Abstract

### *BACKGROUND*

The majority of clinical trials of first-line systemic treatments for hepatocellular carcinoma (HCC) used placebo or sorafenib as comparators, and there are limited data providing a cross comparison of treatments in this setting, especially for newly-approved immune checkpoint inhibitor and vascular endothelial growth factor inhibitor combination treatments.

### *AIM*

To systematically review and compare response rates, survival outcomes, and safety of first-line systemic therapies for advanced hepatocellular carcinoma.

### *METHODS*

We searched PubMed, Science Direct, the Cochrane Database, Excerpta Medica Database, and abstracts from the American Society of Clinical Oncology 2020 annual congress. Eligible studies were randomized controlled trials of systemic therapy enrolling adults with advanced/unresectable HCC. Risk of bias was assessed with the Cochrane risk of bias tool for randomized controlled trials. A network meta-analysis was used to synthesize data and perform direct and indirect comparisons between treatments. *P* value, a frequentist analog to the surface under the cumulative ranking curve, was used to rank treatments.

### *RESULTS*

In total, 1398 articles were screened and 27 included. Treatments compared were atezolizumab plus bevacizumab, brivanib, donafenib, dovitinib, FOLFOX4, lenvatinib, linifanib, nintedanib, nivolumab, sorafenib, sunitinib, vandetanib, 11 sorafenib combination therapies, and three other combination therapies. For

fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**Manuscript source:** Unsolicited manuscript

**Specialty type:** Oncology

**Country/Territory of origin:** China

**Peer-review report's scientific quality classification**

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

**Received:** January 26, 2021
**Peer-review started:** January 26, 2021
**First decision:** February 27, 2021
**Revised:** March 10, 2021
**Accepted:** April 21, 2021
**Article in press:** April 21, 2021
**Published online:** May 21, 2021

**P-Reviewer:** Kamimura K
**S-Editor:** Zhang L
**L-Editor:** Wang TQ
**P-Editor:** Wang LL

overall response rate, lenvatinib ranked 1/19, followed by atezolizumab plus bevacizumab and nivolumab. For progression-free survival (PFS), atezolizumab + bevacizumab was ranked 1/15, followed by lenvatinib. With the exception of atezolizumab + bevacizumab [hazard ratios $(HR)_{PFS}$ = 0.90; 95% confidence interval (CI): 0.64-1.25], the estimated HRs for PFS for all included treatments *vs* lenvatinib were > 1; however, the associated 95%CI passed through unity for bevacizumab plus erlotinib, linifanib, and FOLFOX4. For overall survival, atezolizumab plus bevacizumab was ranked 1/25, followed by vandetanib 100 mg/d and donafinib, with lenvatinib ranked 6/25. Atezolizumab + bevacizumab was associated with a lower risk of death *vs* lenvatinib ($HR_{os}$ = 0.63; 95%CI: 0.44-0.89), while the HR for overall survival for most other treatments *vs* lenvatinib had associated 95%CIs that passed through unity. Vandetanib 300 mg/d and 100 mg/d were ranked 1/13 and 2/13, respectively, for the lowest incidence of treatment terminations due to adverse events, followed by sorafenib (5/13), lenvatinib (10/13), and atezolizumab + bevacizumab (13/13).

*CONCLUSION*

There is not one single first-line treatment for advanced HCC associated with superior outcomes across all outcome measurements. Therefore, first-line systemic treatment should be selected based on individualized treatment goals.

**Key Words:** Hepatocellular carcinoma; Systemic therapy; Meta-analysis; Lenvatinib; First-line; Immune therapy

**©The Author(s) 2021.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core Tip:** The present network meta-analysis is the first to compare data from randomized trials of all first-line systemic therapies for hepatocellular carcinoma including chemotherapy, targeted drugs, immunotherapy, and combination therapies. Furthermore, the analysis represents a comprehensive cross comparison of outcomes, including tumor response rates, survival, and safety and included a sub-analysis in patients with hepatitis B virus infection. Our results showed that atezolizumab plus bevacizumab was ranked first for progression-free survival and overall survival but also had the highest rate of discontinuations due to adverse events. Lenvatinib ranked first for overall response rate and second for progression-free survival.

**Citation:** Han Y, Zhi WH, Xu F, Zhang CB, Huang XQ, Luo JF. Selection of first-line systemic therapies for advanced hepatocellular carcinoma: A network meta-analysis of randomized controlled trials. *World J Gastroenterol* 2021; 27(19): 2415-2433
**URL:** https://www.wjgnet.com/1007-9327/full/v27/i19/2415.htm
**DOI:** https://dx.doi.org/10.3748/wjg.v27.i19.2415

## INTRODUCTION

Liver cancer is the sixth most common cancer globally, accounting for 4.7% of all new cancer cases in 2018, and represents the third most common cause of cancer-related death worldwide behind lung and colorectal cancer[1]. Of the primary liver cancers, hepatocellular carcinoma (HCC) is the most prevalent histological subtype and accounts for 80%-85% of cases[2]. Surgical resection and liver transplant are associated with the best survival outcomes for patients with HCC, and are potentially curative treatments[3]. Locoregional therapies including arterially directed therapies, ablation, and radiotherapy are also associated with good survival outcomes in patients with unresectable disease confined to the liver[4]. However, over 50% of patients with HCC are diagnosed at an advanced stage or with other characteristics that preclude surgical or locoregional treatment[5]. For these patients, systemic therapy is usually the recommended treatment option[4,6].

Over the past 3 years, the number of approved first-line systemic therapies for patients with HCC has expanded greatly, and numerous drugs and drug combinations have been evaluated in this setting[7]. Between 2007 and 2018, sorafenib was the only

approved systemic treatment for HCC based on the results of the Phase III SHARP trial, which showed a survival benefit for sorafenib *vs* placebo[8]. In the decade following the approval of sorafenib, numerous unsuccessful trials of systemic therapies in advanced HCC were conducted until the approval of lenvatinib in 2018[9]. Lenvatinib was approved for first-line use in advanced HCC following the successful outcome of the Phase III REFLECT trial. In this trial, lenvatinib showed a non-inferior overall survival (OS) *vs* sorafenib for the treatment of advanced HCC[10]. Since the approval of lenvatinib, the immunotherapy drugs nivolumab and pembrolizumab, as well as other tyrosine kinase inhibitors (TKI), have been approved for the second-line treatment of HCC. Most recently, combination therapy with the anti-PD-L1 agent atezolizumab plus bevacizumab demonstrated better OS and progression-free survival (PFS) than sorafenib in the Phase III IMbrave 150 trial[11].

The expansion of first-line treatment options for advanced HCC represents a significant advance in the treatment of this disease. However, further data would be useful to inform treatment selection. Most clinical trials of first-line therapies for HCC used placebo or sorafenib as comparators and there are limited data providing a cross comparison of the efficacy and safety of drugs in this setting. Furthermore, although lenvatinib is widely seen as a standard of care in real clinical practice and is a recommended first-line therapy in most international treatment guidelines[4,12,13], there are limited head-to-head data comparing lenvatinib with other systemic therapies. Finally, although historically systemic treatments for HCC were associated with low tumor response rates, recently approved therapies have been associated with response rates > 30%[14]. This has led to renewed interest in tumor response rates in HCC, and investigation of downstaging and conversion therapy strategies. A comparison of response rates for all currently available therapies would therefore be of clinical value.

This network meta-analysis was conducted to systematically review and compare the response rates, survival outcomes, and safety reported by randomized trials of first-line systemic therapies in patients with advanced unresectable HCC, and to provide a comparison between lenvatinib and other systemic therapies in this setting. Two recent meta-analyses have investigated a similar topic to the present study; however, one did not include data on atezolizumab plus bevacizumab and excluded non-targeted therapies[15], and a more recent analysis focused on treatment sequencing by investigating survival outcomes only[16]. Therefore, although there is some overlap with the present analysis, these studies are complementary to each other. In particular, the present analysis is the first to include data on donafenib, a Chinese drug that has shown a superior OS to sorafenib in a Phase III trial[17]. Furthermore, our analysis includes data on survival, response rate, and safety, which in combination are important for treatment decision-making, particularly for patients who may be candidates for downstaging. Finally, the present meta-analysis included a sub-group analysis of patients with HBV infection, which is an important population in the Asia-Pacific region and has not been covered by other current meta-analyses.

## MATERIALS AND METHODS

The analysis methods and inclusion criteria for this study were specified in advance and the protocol was prospectively submitted for registration in the PROSPERO database on May 26, 2020. This report has been written in line with the PRISMA guidelines for network meta-analyses.

### *Eligibility criteria*

This analysis included randomized controlled trials conducted in adult patients (age ≥ 18 years) with advanced or unresectable HCC not eligible for, or with disease progression after, surgical or locoregional therapies. Eligible studies included patients with Child-Pugh Class A or B liver function, ≥ 1 measurable lesion, and no evidence of untreated brain or meningeal metastases. Eligible studies were also required to report at least an assessment of tumor response, survival [OS, PFS, or time to progression (TTP)], and safety. The analysis excluded studies including patients with Child-Pugh Class C liver function, patients receiving anticoagulation therapy or antiretroviral therapy for HIV, and patients who had received previous systemic treatment. These broad eligibility criteria covered a number of trials reporting negative results *vs* sorafenib. Although the analysis therefore includes multiple therapies that failed clinical trials in HCC, this allowed the collection of data for sorafenib from studies conducted over a wide time range, which improved the precision of the analysis.

### Information sources, search strategy, and study selection

Studies were identified by searching the following electronic databases: PubMed, Science Direct, and the Cochrane Database, and Excerpta Medica Database Abstracts from the American Society of Clinical Oncology (ASCO) 2020 annual congress were also searched. The search was completed on May 21, 2020 using the search terms shown in Figure 1.

The Cochrane risk of bias tool for randomized controlled trials was used to assess the quality and risk of bias of studies included in the analysis[18].

### Data extraction

Data were independently extracted by two evaluators (Luo JF and Huang XQ) and cross-checked. In the case of disagreement, the original documents were checked and the correct data confirmed. General information extracted included journal name, document title, publication time, author, country, region where the lead author was located, and the country and region where the research was conducted. Demographics and baseline characteristics extracted were patient age, gender, Barcelona clinic liver cancer classification, Eastern Co-operative Oncology Group performance status, prevalence of HBV infection, and presence of extrahepatic vascular infiltration and extrahepatic metastasis. Details of interventions extracted included dosage and dose schedule. Efficacy and safety endpoints extracted (where available) were overall response rate [ORR; assessed by response evaluation criteria in solid tumours (RECIST) v1/1.1 for all included studies], OS, PFS, TTP, incidence of Grade ≥ 3 adverse events (AE), incidence of treatment interruption due to adverse events (AEs), and incidence of dose reductions due to AEs.

### Statistical analysis

For OS, PFS, TTP, and other survival endpoints, hazard ratios (HR) were estimated to compare treatments. For discrete variables such as ORR, and incidence of AEs, estimated risk ratios were calculated to compare treatments. Selection of a fixed effect or random effect model was based on the level of heterogeneity in the data, assessed using the Higgins $I^2$ statistic and defined as $I^2 \leq 50\%$ and $P > 0.1$. If no obvious data heterogeneity was found, a fixed effect model was adopted, otherwise a random effect model was utilized. For endpoints reported in a relatively small number of studies (< 6), a fixed effects model was adopted.

A network meta-analysis was used to synthesize information from the included studies, and perform direct and indirect comparisons using a method based on the frequency school of Rücker *et al*[19,20]. The Q statistic was used to assess the consistency of direct and indirect evidence in the treatment network(s) studied. If no obvious inconsistency ($P > 0.1$) was found, a fixed effect model was adopted, otherwise a random effect model was utilized. *P* value, a frequentist analog to the surface under the cumulative ranking curve, was used to rank treatments[21]. A funnel chart was used to evaluate publication bias; a symmetrical graph indicates a low influence of publication bias and an asymmetric graph indicates possible publication bias. A post-hoc analysis of all studies reporting data from patients with HBV-related HCC was also included to assess OS, PFS, and safety in these patients.

All statistical analyses were performed using Rv3.6. The Robias toolkit was used for evaluation of literature quality and Netmeta was used for the network meta-analysis.

## RESULTS

### Studies included in the analysis

In total, 1398 articles were screened: PubMed/MEDLINE, *n* = 114; Science Direct, *n* = 312; Cochrane Database, *n* = 355; Excerpta Medica Database, *n* = 561; and the ASCO 2020 abstract book, *n* = 12 (Figure 1). After removing duplicates and top-line screening of abstracts for suitability, a total of 86 articles were reviewed in detail, of which 27 met the full inclusion criteria (Table 1). These 27 articles corresponded to 27 different studies (Supplementary Figure 1).

### Study characteristics

Of the 27 studies included, 25 investigated targeted treatment regimens (nintedanib, mapatumumab + sorafenib, atezolizumab + bevacizumab, doxorubicin + sorafenib, dovitinib, tigatuzumab + sorafenib, vandetanib, brivanib, linifanib, lenvatinib, nivolumab, sunitinib, sorafenib + erlotinib, sorafenib (two studies), nintedanib,

**Table 1 Details of included studies**

| Ref. | Year | Experimental arm(s) | Comparator arm | Primary endpoint | Analysis timing | Survival outcomes, mo |
|---|---|---|---|---|---|---|
| Yen *et al*[47] | 2018 | Nintedanib | Sorafenib | TTP | PFS: Final; OS: Final | PFS: 2.7 *vs* 3.7; OS: 10.2 *vs* 1.1 |
| Ciuleanu *et al*[48] | 2016 | Mapatumumab + sorafenib | Placebo + sorafenib | TTP | PFS: Final; OS: Final | PFS: 3.2 *vs* 4.3; OS: 10.0 *vs* 10.1 |
| Finn *et al*[11] | 2020 | Atezolizumab + bevacizumab | Sorafenib | OS and PFS | PFS: Final; OS: Final | PFS: 6.8 *vs* 4.3; OS: NE *vs* 13.2 |
| Abou-Alfa *et al*[49] | 2010 | Doxorubicin + sorafenib | Doxorubicin + placebo | TTP | PFS: Final; OS: Final | PFS: 6.0 *vs* 2.7; OS: 13.7 *vs* 6.5 |
| Cheng *et al*[50] | 2016 | Dovitinib | Sorafenib | OS and TTP | TTP: Final; OS: Final | TTP: 4.1 *vs* 4.1; OS: 8.0 *vs* 8.4 |
| Cheng *et al*[28] | 2015 | Tigatuzumab (6 + 2) + sorafenib; Tigatuzumab (6 + 6) + sorafenib | Sorafenib | TTP | TTP: Final; OS: Final | TTP: 3.0 *vs* 3.9 *vs* 2.8; OS: 8.2 *vs* 12.2 *vs* 8.2 |
| Hsu *et al*[51] | 2012 | Vandetanib 300 mg/d; Vandetanib 100 mg/d | Placebo | Tumor stabilization rate | PFS: Final; OS: Final | PFS: 1.1 *vs* 0.7 *vs* 1.0; OS: 6.0 *vs* 5.8 *vs* 4.3 |
| Johnson *et al*[22] | 2013 | Sorafenib | Brivanib | OS | PFS: No; OS: Final | PFS: 4.1 *vs* 4.2; OS: 9.9 *vs* 9.5 |
| Cainap *et al*[24] | 2015 | Linifanib | Sorafenib | OS | PFS: Final; OS: Final | PFS: 4.2 *vs* 2.9; OS: 9.1 *vs* 9.8 |
| Kudo *et al*[10] | 2018 | Lenvatinib | Sorafenib | OS | PFS: No; OS: Final | PFS: 7.4 *vs* 3.7; OS: 13.6 *vs* 12.3 |
| Yau *et al*[23] | 2019 | Nivolumab | Sorafenib | OS | PFS: Final; OS: Final | PFS: 3.7 *vs* 3.8; OS: 16.4 *vs* 14.7 |
| Cheng *et al*[29] | 2013 | Sunitinib | Sorafenib | OS | PFS: Final;OS: Final | PFS: 3.6 *vs* 3.0; OS: 7.9 *vs* 10.2 |
| Zhu *et al*[26] | 2015 | Sorafenib + erlotinib | Sorafenib + placebo | OS | TTP: Final; OS: Final | TTP: 3.2 *vs* 4.0; OS: 9.5 *vs* 8.5 |
| Llovet *et al*[52] | 2008 | Sorafenib | Placebo | OS and TTP | TTP: Final; OS: Final | TSP: 5.5 *vs* 2.8; OS: 10.7 *vs* 7.9 |
| Cheng *et al*[25] | 2009 | Sorafenib | Placebo | - | TTP: Final; OS: Final | TTP: 2.8 *vs* 1.4; OS: 6.5 *vs* 4.2 |
| Palmer *et al*[53] | 2018 | Nintedanib | Sorafenib | TTP | PFS: Final; OS: Final | PFS: 5.3 *vs* 3.9; OS: 11.9 *vs* 11.4 |
| Thomas *et al*[54] | 2018 | Bevacizumab + erlotinib | Sorafenib | OS | PFS: No; OS: Final | PFS: 4.4 *vs* 2.8; OS: 8.6 *vs* 8.6 |
| Abou-Alfa *et al*[55] | 2019 | Sorafenib + doxorubicin | Sorafenib | OS | PFS: Final; OS: Final | PFS: 4.0 *vs* 3.7; OS: 9.3 vs. 9.4 |
| Tak *et al*[27] | 2018 | Sorafenib | Sorafenib + resminostat | TTP | TTP: Final; OS: Final | TTP: 2.8 *vs* 2.8; OS: 14.1 *vs* 11.8 |
| Jouve *et al*[56] | 2019 | Sorafenib + pravastatin | Sorafenib | OS | PFS: Final; OS: Final | PFS: 5.0 *vs* 5.4; OS: 10.7 *vs* 10.5 |
| Lee *et al*[57] | 2016 | AEG35156 + sorafenib | Sorafenib | PFS | PFS: Final; OS: Final | PFS: 4.0 *vs* 2.6; OS: 6.5 *vs* 5.4 |
| Assenat *et al*[58] | 2019 | Sorafenib + GEMOX | Sorafenib | PFS | PFS: Final; OS: Final | PFS: 6.2 *vs* 4.6; OS:13.5 *vs* 14.8 |
| Azim *et al*[59] | 2018 | Sorafenib + tegafur–uracil | Sorafenib | TTP | PFS: Final; OS: Final | PFS: 6.0 *vs* 6.0; OS: 8.2 *vs* 10.5 |
| Koeberle *et al*[60] | 2016 | Sorafenib | Sorafenib + everolimus | PFS | PFS: Final; OS: Final | PFS: 6.6 *vs* 5.7; OS: 10.0 *vs* 12 |
| Bi *et al*[17] | 2020 | Donafinib | Sorafenib | OS | PFS: Final; OS: Final | PFS: 3.7 *vs* 3.6; OS: 21.1 *vs* 10.3 |
| Qin *et al*[61] | 2013 | FOLFOX4 | Doxorubicin | OS | PFS: Final; OS: Final | PFS: 2.9 *vs* 1.8; OS: 6.4 *vs* 5.0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Yeo *et al*[62] | 2005 | Doxorubicin | PIAF | OS | PFS: No; OS: Final | OS: 6.8 *vs* 8.7 |

FOLFOX4: Oxaliplatin/folinic acid/5-fluorouracil; GEMOX: Gemcitabine and oxaliplatin; NE, Not reported; OS, Overall survival; PFS, Progression-free survival; PIAF, Cisplatin/interferon α-2b/doxorubicin/5-fluorouracil; TTP, Time to progression.

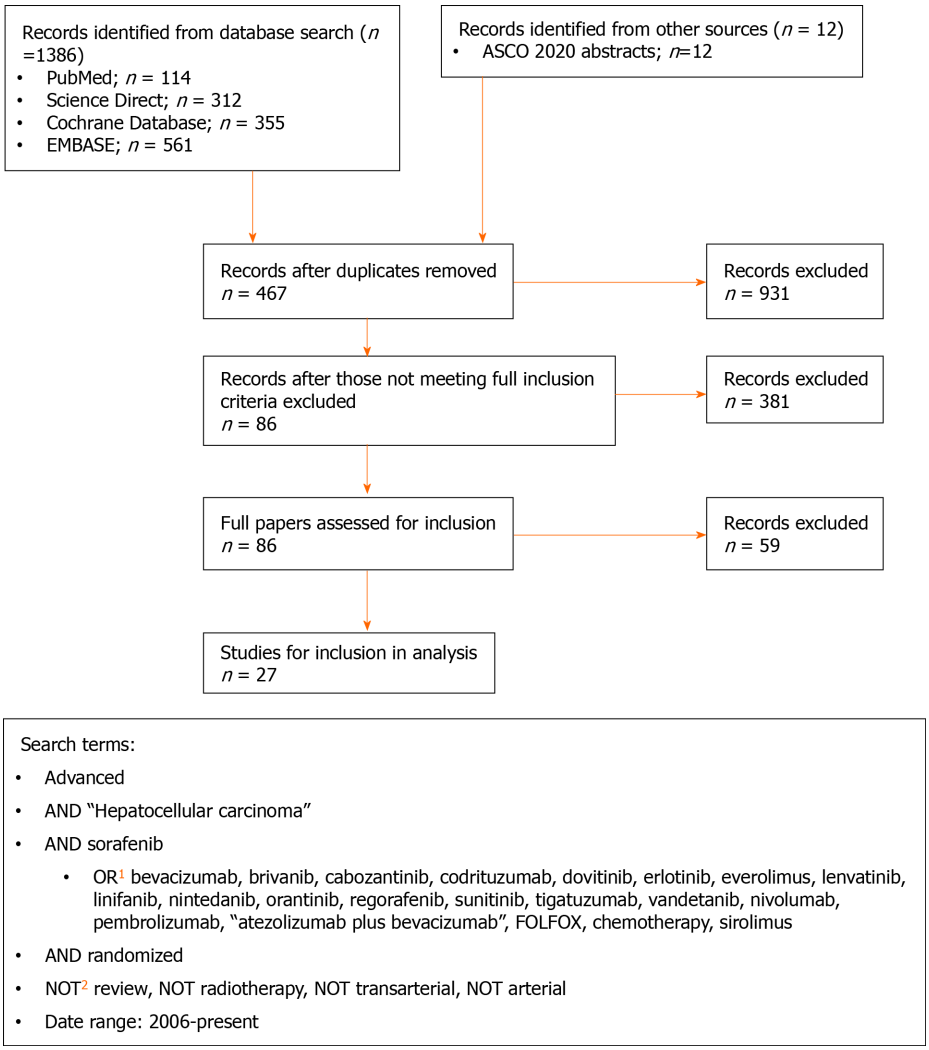



**Figure 1 Flow chart of study selection and search terms.** Different therapeutics were searched for using individual searches to allow easier processing of the results; NOT was used for databases allowing use of this operator. ASCO: American Society of Clinical Oncology.

bevacizumab + erlotinib, sorafenib + doxorubicin, sorafenib + resminostat, sorafenib + pravastatin, AEG35156 (a second-generation synthetic antisense oligonucleotide inhibitor of cellular expression of the X-linked inhibitor of apoptosis protein) + sorafenib, sorafenib + gemcitabine and cisplatin, sorafenib + tegafur–uracil, sorafenib + everolimus, and donafinib) and two investigated combination chemotherapy regimens [oxaliplatin/folinic acid/5-fluorouracil (FOLFOX4) and cisplatin/interferon α-2b/doxorubicin/5-fluorouracil] (Table 1). Twenty-one of the included studies used sorafenib as the comparator treatment, three used doxorubicin, and three studies were placebo controlled (including the two Phase III studies of sorafenib). The majority of the studies had OS ($n$ = 12) or PFS/TTP ($n$ = 10) as the primary endpoint, and almost all had reported final data for these endpoints.

### *Quality assessment*

Study design characteristics are summarized in Supplementary Table 1. In brief, all 27 studies selected for inclusion were randomized controlled studies (20 provided details of the randomization scheme used and seven articles did not specify), seven of the studies used double blinding and 20 were open label, and 24 included a data flow

chart. Overall, the quality of the included studies was considered relatively high (Figure 1).

### Patient description

All of the studies included patients with advanced HCC who had not received previous treatment. Overall, the total of 10256 patients included in the analysis were predominantly male and had median ages ranging from 49 to 68 years, and most of the studies included > 50% of patients with extrahepatic metastasis (Table 2).

### Evaluation of efficacy

**Overall response rate:** A total of 18 studies reported ORR, including 19 interventions and allowing 20 comparisons (Figure 2A). No significant heterogeneity was detected between the studies (tau-squared = 0; $I^2$ = 0%; $P$ = 0.9502) and a fixed effect model was selected. $P$ value for ORR showed that lenvatinib was associated with the best ORR among all treatments included in the analysis ($P$ = 0.9042) (Figure 2B). Atezolizumab + bevacizumab ranked second ($P$ = 0.8045) and nivolumab ranked third ($P$ = 0.7834). Using lenvatinib as the comparator, all treatments included in the analysis had an estimated risk ratio for ORR ($RR_{ORR}$) of < 1, except for AEG35156 + sorafenib, which had an estimated $RR_{ORR}$ of 1.3451 [95% confidence interval (CI): 0.07-25.21] (Figure 2B).

**Progression-free survival:** A total of 15 studies reported PFS, including 15 interventions and allowing 15 comparisons (Figure 3A). No significant heterogeneity was detected (tau-squared = 0; $I^2$ = 0%; $P$ = 0.7361) and a fixed effect model was selected. Atezolizumab + bevacizumab was ranked first for PFS ($P$ = 0.9501), followed by lenvatinib ($P$ = 0.9041). Nivolumab ranked sixth ($P$ = 0.558) (Figure 3B). With the exception of atezolizumab + bevacizumab ($HR_{PFS}$ = 0.90; 95%CI: 0.64-1.25), the estimated HRs for PFS for all included treatments *vs* lenvatinib were > 1; however, the associated 95%CI passed through unity for bevacizumab plus erlotinib, linifanib, and FOLFOX4.

**Time to progression:** A total of 17 studies reported TTP, including 17 interventions and allowing 19 comparisons (Figure 3C). No significant heterogeneity was detected between studies (tau-squared = 0; $I^2$ = 0%; $P$ = 0.9028) and a fixed effect model was selected. Lenvatinib was ranked first for TTP ($P$ = 0.9888) followed by linifanib ($P$ = 0.9067) and sorafenib + doxorubicin ($P$ = 0.7344) (Figure 3D). When compared with lenvatinib, all other treatments in the analysis had an estimated $HR_{TTP}$ > 1, although the associated 95%CI passed through unity for linifanib and sorafenib plus tegafur–uracil.

**Overall survival:** A total of 24 studies reported OS, including 25 interventions and allowing 28 comparisons (Figure 3E). No significant heterogeneity was detected between studies (tau-squared = 0; $I^2$ = 0%; $P$ = 0.9802) and a fixed effect model was selected. Atezolizumab + bevacizumab was ranked highest for OS ($P$ = 0.9651) followed by vandetanib 100 mg/d ($P$ = 0.8653), donafinib ($P$ = 0.7958), and nivolumab ($P$ = 0.7701) (Figure 3F). Lenvatinib ranked sixth ($P$ = 0.6675). Atezolizumab + bevacizumab was associated with a lower risk of death *vs* lenvatinib ($HR_{os}$ = 0.63; 95%CI: 0.44-0.89), and the $HR_{os}$ for most other treatments *vs* lenvatinib had associated 95%CIs that passed through unity.

**Outcomes in patients with HBV infection:** Ten studies included sub-analyses of patients with HBV infection, including data on the following treatments: atezolizumab + bevacizumab[11], brivanib[22], nivolumab[23], lenvatinib[10], linifanib[24], sorafenib[25], sorafenib + erlotinib[26], sorafenib + resminostat[27], tigatuzumab + sorafenib[28], and sunitinib[29]. A total of three studies reported PFS in patients with HBV infection, including four interventions and three comparisons (Supplementary Figure 2A). A fixed effect model was selected for the analysis. Lenvatinib ranked first for PFS ($P$ = 0.8786) followed by atezolizumab + bevacizumab ($P$ = 0.7746) and donafinib ($P$ = 0.2972) (Figure 2B). A comparison of HRs for PFS *vs* lenvatinib is shown in Supplementary Figure 2B. A total of nine studies reported OS, including ten interventions and allowing nine comparisons (Supplementary Figure 2C). A random effect model was selected for the analysis. Atezolizumab + bevacizumab ranked first ( $P$ = 0.9751), followed by lenvatinib ($P$ = 0.8308) and nivolumab ($P$ = 0.7732) (Supplementary Figure 2D). Comparison with lenvatinib revealed that atezolizumab + bevacizumab had an estimated $HR_{OS}$ < 1 and all other interventions had an $HR_{OS}$ > 1 ( Supplementary Figure 2D).

**Table 2 Patient characteristics in the included studies**

| Ref. | Year | Treatments | *n* | Age, median | Males, % | ECOG 0/1–2, % | Extrahepatic disease, % |
|---|---|---|---|---|---|---|---|
| Yen *et al*[47] | 2018 | Nintedanib | 63 | 58 | 91 | 55.6/44.5 | 68.3 |
| | | Sorafenib | 32 | 62 | 81 | 56.3/43.8 | 68.3 |
| Ciuleanu *et al*[48] | 2016 | Mapatumumab + sorafenib | 50 | 60 | 52 | 36.0/64.0 | 66.0 |
| | | Placebo + sorafenib | 51 | 61 | 77 | 33.3/66.6 | 49.0 |
| Finn *et al*[11] | 2020 | Atezolizumab + bevacizumab | 336 | 64 | 82 | 62.0/38.0 | 63.0 |
| | | Sorafenib | 165 | 66 | 83 | 62.0/38.0 | 56.0 |
| Abou-Alfa *et al*[49] | 2010 | Doxorubicin + sorafenib | 47 | 66 | 66 | - | 51.1 |
| | | Doxorubicin + placebo | 49 | 65 | 86 | - | 79.6 |
| Cheng *et al*[50] | 2016 | Dovitinib | 82 | 56 | 89 | 63.0/37.0 | - |
| | | Sorafenib | 83 | 56 | 81 | 64.0/35.0 | - |
| Cheng *et al*[28] | 2015 | Tigatuzumab (6 + 2) + sorafenib | 53 | 63 | 85 | 60.4/39.6 | - |
| | | Tigatuzumab (6 + 6) + sorafenib | 54 | 63 | 83 | 57.4/42.6 | - |
| | | Sorafenib | 55 | 66 | 80 | 54.5/45.5 | - |
| Hsu *et al*[51] | 2012 | Vandetanib 300 mg/d | 19 | 55 | 95 | - | - |
| | | Vandetanib 100 mg/d | 25 | 61 | 68 | - | - |
| | | Placebo | 23 | 56 | 87 | - | - |
| Johnson *et al*[22] | 2013 | Sorafenib | 578 | 60 | 84 | 61.0/39.0 | 62.0 |
| | | Brivanib | 577 | 61 | 84 | 64.0/36.0 | 63.0 |
| Cainap *et al*[24] | 2015 | Linifanib | 514 | 59 | 86 | 62.8/37.2 | 59.7 |
| | | Sorafenib | 521 | 60 | 84 | 66.2/33.8 | 56.8 |
| Kudo *et al*[10] | 2018 | Lenvatinib | 478 | 63 | 85 | - | - |
| | | Sorafenib | 476 | 62 | 84 | - | - |
| Yau *et al*[23] | 2019 | Nivolumab | 371 | 65 | 85 | - | - |
| | | Sorafenib | 372 | 65 | 85 | - | - |
| Cheng *et al*[29] | 2013 | Sunitinib | 530 | 59 | 82 | 52.5/46.8 | 78.9 |
| | | Sorafenib | 544 | 59 | 84 | 52.9/46.7 | 76.3 |
| Zhu *et al*[26] | 2015 | Sorafenib + erlotinib | 362 | 60 | 82 | 61.3/38.7 | 56.6 |
| | | Sorafenib + placebo | 358 | 61 | 80 | 60.3/39.7 | 61.2 |
| Llovet *et al*[52] | 2008 | Sorafenib | 299 | 65 | 87 | 54.0/46.0 | 53.0 |
| | | Placebo | 303 | 66 | 87 | 54.0/46.0 | 50.0 |
| Cheng *et al*[25] | 2009 | Sorafenib | 150 | 51 | 85 | 25.3/74.6 | 68.7 |
| | | Placebo | 76 | 52 | 87 | 27.6/72.4 | 68.4 |
| Palmer *et al*[53] | 2018 | Nintedanib | 62 | 66 | 77 | 51.6/48.4 | 64.5 |
| | | Sorafenib | 31 | 64 | 84 | 58.1/33.0 | 67.7 |
| Thomas *et al*[54] | 2018 | Bevacizumab + erlotinib | 47 | 61 | NR | 32.0/68.0 | 40.0 |
| | | Sorafenib | 43 | 61 | NR | 40.0/60.0 | 25.0 |
| Abou-Alfa *et al*[55] | 2019 | Sorafenib + doxorubicin | 180 | 62 | 85 | 36.1/63.9 | - |
| | | Sorafenib | 176 | 62 | 87 | 39.8/60.2 | - |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Tak *et al*[27] | 2018 | Sorafenib | 84 | 62 | 87 | - | 56.0 |
| | | Sorafenib + resminostat | 86 | 65 | 80 | - | 51.8 |
| Jouve *et al*[56] | 2019 | Sorafenib + pravastatin | 162 | 68 | 96 | - | 29.0 |
| | | Sorafenib | 161 | 68 | 88 | - | 30.4 |
| Lee *et al*[57] | 2016 | AEG35156 + sorafenib | 31 | 61 | 87 | 3.2/96.8 | - |
| | | Sorafenib | 17 | 54 | 88 | 11.8/88.3 | - |
| Assenat *et al*[58] | 2019 | Sorafenib + GEMOX | 39 | 62 | 86 | - | 77.0 |
| | | Sorafenib | 44 | 65 | 92 | - | 61.0 |
| Azim *et al*[59] | 2018 | Sorafenib + tegafur–uracil | 36 | 59 | 86 | 69.4/30.6 | 52.8 |
| | | Sorafenib | 38 | 59 | 90 | 65.8/34.2 | 47.4 |
| Koeberle *et al*[60] | 2016 | Sorafenib | 46 | 65 | 87 | 72.0/28.0 | 57.0 |
| | | Sorafenib + everolimus | 59 | 66 | 81 | 59.0/41.0 | 54.0 |
| Bi *et al*[17] | 2020 | Donafinib | 328 | 53 | 86 | 61.3/38.7 | - |
| | | Sorafenib | 331 | 53 | 88 | 66.8/33.2 | - |
| Qin *et al*[61] | 2013 | FOLFOX4 | 184 | 50 | 90 | - | - |
| | | Doxorubicin | 187 | 49 | 87 | - | - |
| Yeo *et al*[62] | 2005 | Doxorubicin | 94 | 54 | 90 | 87.2/12.8 | - |
| | | PIAF | 94 | 49 | 93 | 92.6/7.4 | - |

ECOG: Eastern Co-operative Oncology Group; FOLFOX4: Oxaliplatin/folinic acid/5-fluorouracil; GEMOX: Gemcitabine and oxaliplatin; NR: Not reported; PIAF: Cisplatin/interferon α-2b/doxorubicin/5-fluorouracil.

### *Safety*

**Grade ≥ 3 adverse events:** In total, 17 studies reported data on the incidence of Grade ≥ 3 AEs, including 19 interventions and allowing 21 comparisons (Supplementary Figure 3A). No significant heterogeneity was detected between studies (tau-squared = 0; $I^2$ = 0%; $P$ = 0.4493) and a fixed effect model was selected. Nivolumab ranked 2/19 ($P$ = 0.9351), sorafenib ranked 8/19 ($P$ = 0.5040), atezolizumab + bevacizumab ranked 11/19 ($P$ = 0.4167), and lenvatinib ranked 16/19 ($P$ = 0.2468) for incidence of Grade ≥ 3 AEs (higher ranking indicated a lower incidence of AEs) (Supplementary Figure 3B).

**Treatment termination due to adverse events:** A total of 13 studies reported the incidence of treatment termination due to AEs, including 13 interventions and allowing 15 comparisons (Supplementary Figure 3C). A degree of heterogeneity was detected between studies (tau-squared = 0.1536; $I^2$ = 65%; $P$ = 0.0573) and a random effect model was selected. After ranking all interventions from the lowest to highest incidence of terminations due to AEs, vandetanib 300 mg/d and 100 mg/d were ranked first and second ($P$ = 0.8036 and 0.7252, respectively), sorafenib ranked 5/13 ($P$ = 0.5372), nintedanib ranked 8/13 ($P$ = 0.4251), lenvatinib ranked 10/13 ($P$ = 0.3907), and atezolizumab + bevacizumab ranked 13/13 ($P$ = 0.2584) (Supplementary Figure 3D).

## DISCUSSION

Following an expansion of first-line systemic treatment options for HCC over the past decade, international treatment guidelines now recommend sorafenib, lenvatinib, and atezolizumab plus bevacizumab in this setting, as well as nivolumab and FOLFOX (off-label use in many countries, but approved by the China National Medical Products Administration) for selected patients[4,12,13]. Numerous other therapies and combinations of therapies have also been unsuccessfully investigated in first-line advanced HCC management. However, most trials of systemic therapy for HCC used sorafenib as the comparator, as it was the only approved systemic therapy available at the time, and this limits the clinicians' ability to compare currently available treatment options. The present study represents one of the most comprehensive systematic

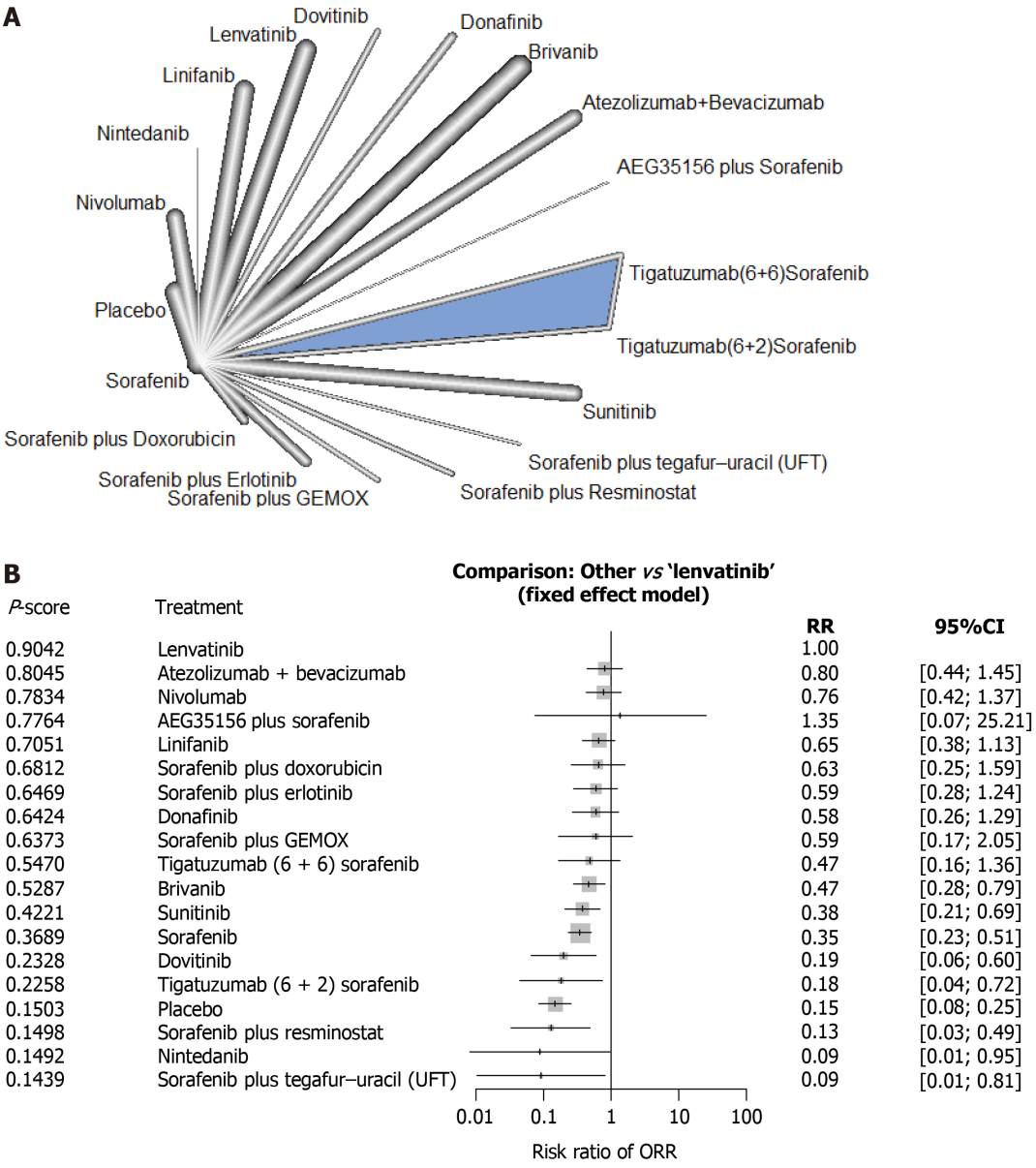
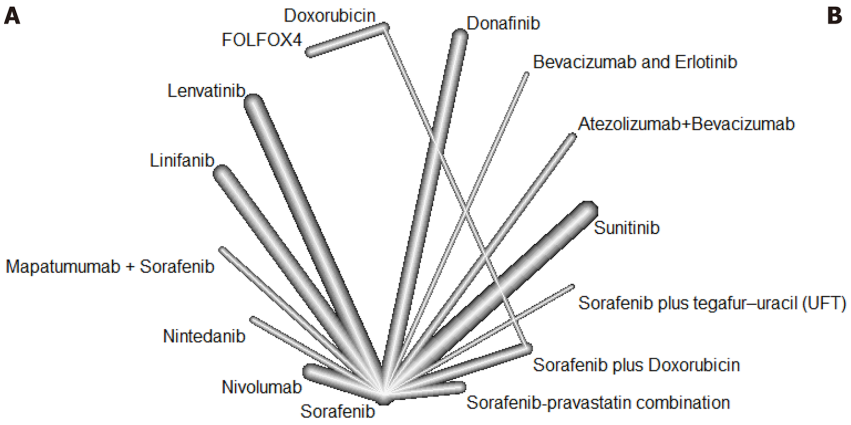



**Figure 2 Response rates of first-line systemic therapy in patients with advanced hepatocellular carcinoma.** A: Network diagram; B: Interventions ranked by *P* value with risk ratios and 95% confidence interval for overall response rate for each treatment *vs* lenvatinib. CI: Confidence interval; GEMOX: Gemcitabine and oxaliplatin; ORR: Overall response rate; RR: Risk ratio.

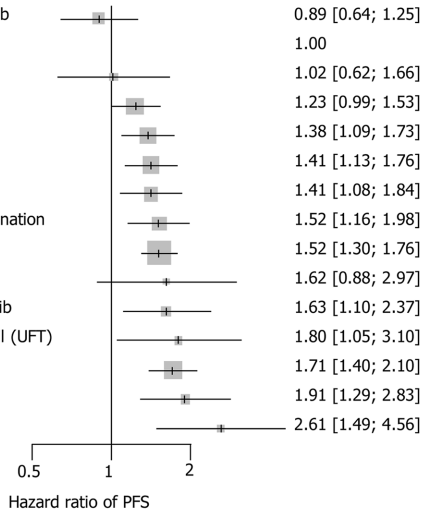
reviews and meta-analyses of first-line systemic treatments for advanced unresectable HCC conducted to date, and compares the treatment outcomes and safety of lenvatinib with multiple other systemic therapies, including immunotherapy (nivolumab) and combined therapy with immunotherapy and a TKI (atezolizumab + bevacizumab).

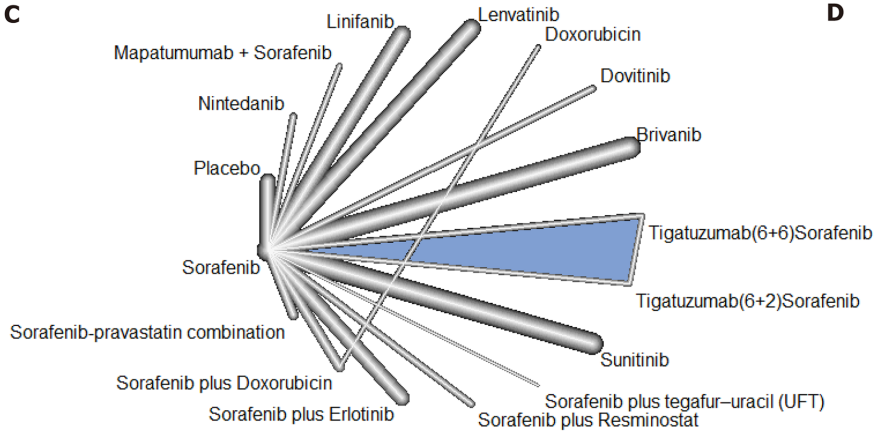
Our results show that atezolizumab plus bevacizumab is associated with the best OS outcomes of all therapies included in the analysis. This result is supported by findings from a recent meta-analysis that investigated optimal treatment sequencing for HCC and also reported that atezolizumab plus bevacizumab had a higher OS benefit *vs* lenvatinib ($HR_{OS}$ = 0.63; 95%CI: 0.44-0.89), nivolumab ($HR_{OS}$ = 0.68; 95%CI: 0.48-0.98), and sorafenib ($HR_{OS}$ = 0.58; 95%CI: 0.42-0.80)[16]. Atezolizumab plus bevacizumab is the first combined immunotherapy and vascular-targeted regimen to be recommended as a first-line treatment option in the National Comprehensive Cancer Network HCC guidelines[4]. The long OS associated with atezolizumab plus bevacizumab may be related to the 'long tail' effect characteristic of immune checkpoint inhibitors, which was also observed in the Phase III Checkmate 459 study of nivolumab. A number of studies have identified several mechanisms by which angiogenesis-related processes can enhance immune checkpoint inhibitors therapy, including vascular normalization, reduction of hypoxia, and increasing tumor infilt-




B

| *P*-score | Treatment | HR [95%CI] |
|---|---|---|
| 0.9501 | Atezolizumab + bevacizumab | 0.89 [0.64; 1.25] |
| 0.9041 | Lenvatinib | 1.00 |
| 0.8490 | Bevacizumab and erlotinib | 1.02 [0.62; 1.66] |
| 0.7411 | Linifanib | 1.23 [0.99; 1.53] |
| 0.5899 | Donafinib | 1.38 [1.09; 1.73] |
| 0.5548 | Nivolumab | 1.41 [1.13; 1.76] |
| 0.5511 | Sorafenib plus doxorubicin | 1.41 [1.08; 1.84] |
| 0.4347 | Sorafenib-pravastatin combination | 1.52 [1.16; 1.98] |
| 0.4246 | Sorafenib | 1.52 [1.30; 1.76] |
| 0.3951 | FOLFOX4 | 1.62 [0.88; 2.97] |
| 0.3599 | Mapatumumab plus sorafenib | 1.63 [1.10; 2.37] |
| 0.2738 | Sorafenib plus tegafur–uracil (UFT) | 1.80 [1.05; 3.10] |
| 0.2422 | Sunitinib | 1.71 [1.40; 2.10] |
| 0.1883 | Nintedanib | 1.91 [1.29; 2.83] |
| 0.0414 | Doxorubicin | 2.61 [1.49; 4.56] |

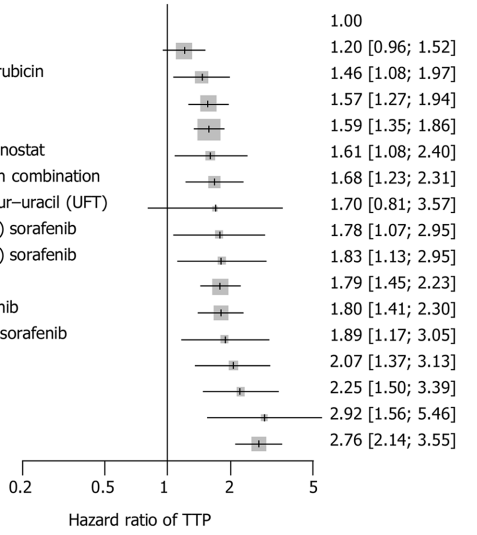



D

**Comparison: Other *vs* 'lenvatinib' (fixed effect model)**

| *P*-score | Treatment | HR [95%CI] |
|---|---|---|
| 0.9888 | Lenvatinib | 1.00 |
| 0.9067 | Linifanib | 1.20 [0.96; 1.52] |
| 0.7344 | Sorafenib plus doxorubicin | 1.46 [1.08; 1.97] |
| 0.6562 | Brivanib | 1.57 [1.27; 1.94] |
| 0.6484 | Sorafenib | 1.59 [1.35; 1.86] |
| 0.5944 | Sorafenib plus resminostat | 1.61 [1.08; 2.40] |
| 0.5426 | Sorafenib-pravastatin combination | 1.68 [1.23; 2.31] |
| 0.5265 | Sorafenib plus tegafur–uracil (UFT) | 1.70 [0.81; 3.57] |
| 0.4797 | Tigatuzumab (6 + 2) sorafenib | 1.78 [1.07; 2.95] |
| 0.4473 | Tigatuzumab (6 + 6) sorafenib | 1.83 [1.13; 2.95] |
| 0.4370 | Sunitinib | 1.79 [1.45; 2.23] |
| 0.4360 | Sorafenib plus erlotinib | 1.80 [1.41; 2.30] |
| 0.4081 | Mapatumumab plus sorafenib | 1.89 [1.17; 3.05] |
| 0.3013 | Nintedanib | 2.07 [1.37; 3.13] |
| 0.2222 | Dovitinib | 2.25 [1.50; 3.39] |
| 0.1003 | Doxorubicin | 2.92 [1.56; 5.46] |
| 0.0702 | Placebo | 2.76 [2.14; 3.55] |



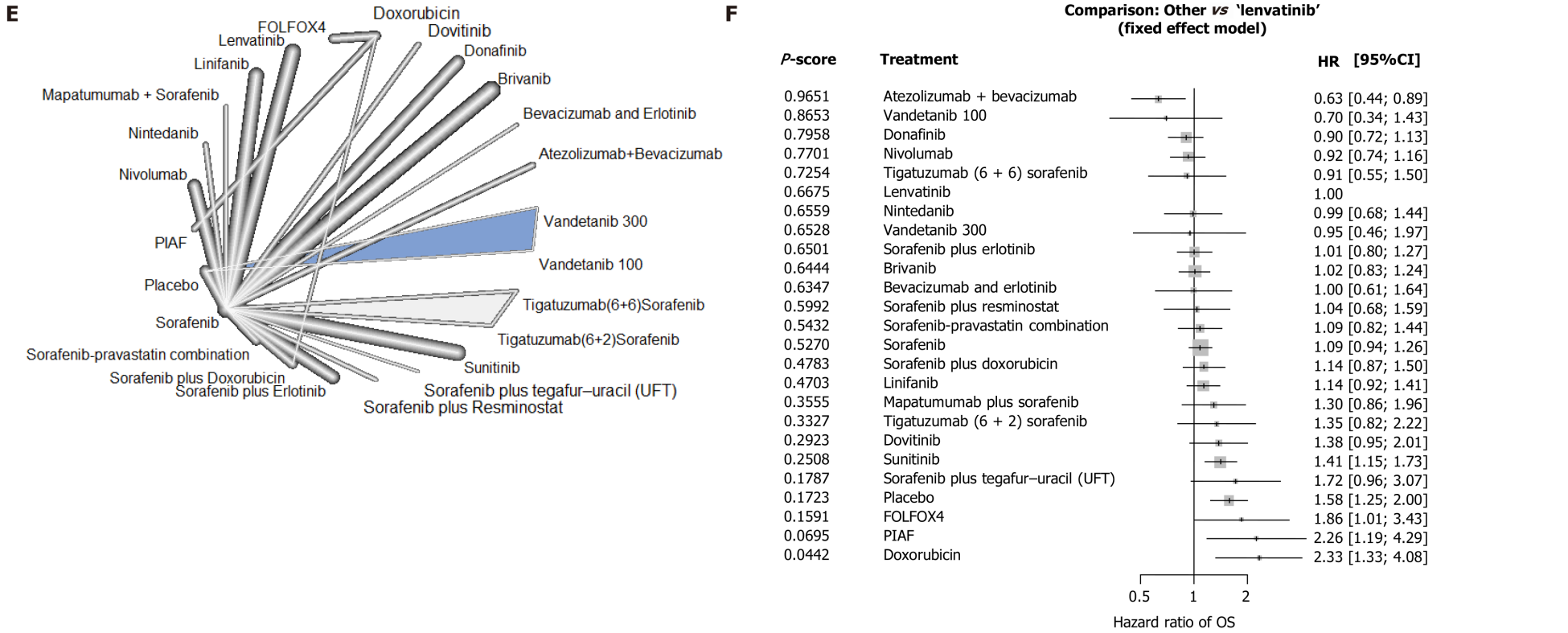



**Figure 3 Survival outcomes in patients with advanced hepatocellular carcinoma following first-line systemic therapy.** A, C, and E: Network diagrams; B: Interventions ranked by *P* value with hazard ratios for progression-free survival, D: Time to progression and F: overall survival for each treatment *vs* lenvatinib. CI: Confidence interval; FOLFOX4: Oxaliplatin/folinic acid/5-fluorouracil; HCC: Hepatocellular carcinoma; HR: Hazard ratio; OS: Overall survival; PIAF: Cisplatin/interferon α-2b/doxorubicin/5-fluorouracil; TTP: Time to progression.

rating lymphocytes[30]. Although bevacizumab monotherapy failed Phase II trials in unresectable HCC, in combination with atezolizumab it led to superior efficacy compared with bevacizumb monotherapy[31]. However, consideration of treatment safety and tolerability is also an important factor in clinical decision-making. Our analysis revealed that atezolizumab plus bevacizumab was associated with the highest incidence of discontinuation due to AEs. This may be associated with the relatively long time to progression and duration of treatment reported for atezolizumab plus bevacizumab, but as treatment discontinuations due to AEs usually involve uncontrolled Grade ≥ 3 AEs, this would likely be a weak association. In addition, the prescribing information for bevacizumab highlights a possible risk of bleeding, and requires termination of bevacizumab at least 4 wk before surgery[32]. Therefore, in

patients with high risk of gastric esophageal varices and patients with the potential to undergo any surgical procedures, atezolizumab plus bevacizumab should be used carefully, to manage the risk of bleeding events. Atezolizumab plus bevacizumab may be more suitable for patients who are unsuitable for surgery but with good liver function and limited cirrhosis, who have the potential to achieve a long-term survival benefit with systemic therapy.

The results of this meta-analysis show that there is currently not one single systemic treatment for advanced HCC associated with superior outcomes across all outcome measurements (ORR, OS, PFS, and safety). This highlights the importance of individualized treatment selection based on specific treatment goals. For example, a number of studies have shown that lenvatinib or lenvatinib combination therapy[33] can allow patients to achieve downstaging and become eligible for surgery[34-36]. For patients with HCC ineligible for surgical intervention at diagnosis, we are of the opinion that treatment selection should be objective based. In patients without serious underlying liver disease and for whom surgery may be possible, systemic treatments with the highest ORR are the optimal choice. Conversely, for patients with poor liver function, underlying liver disease, or local advanced HCC, selection of therapies based on longer OS may provide the most benefit.

In our analysis, lenvatinib had superior short-term efficacy compared with all other systemic therapies investigated. Lenvatinib ranked first for ORR and TTP, and second for PFS after atezolizumab plus bevacizumab. This finding is supported by the results of another recent network meta-analysis presented at the ASCO Gastrointestinal Symposium 2021 that also ranked atezolizumab plus bevacizumab first for OS but lenvatinib first for ORR[37]. In addition, although direct comparison of the ORRs (RECIST v1.1) reported for atezolizumab plus bevacizumab and lenvatinib in the IMbrave 100 and REFLECT studies appears to show a moderately higher ORR for atezolizumab plus bevacizumab (27% *vs* 18%)[10,11], our network analysis provides a more robust comparison of the two therapies by comparing both to sorafenib. There are several possible mechanistic explanations for this finding. First, preclinical studies show that lenvatinib has multiple targets including VEGFR1-3, FGFR1-4, PDGFRα, RET, and KIT, and this broad spectrum of activity may be one factor explaining the high response rates associated with this therapy[38]. Furthermore, lenvatinib is a type V TKI with fast binding and relatively slow dissociation compared with other TKIs[39]. In addition to anti-vascular effects, lenvatinib also has a regulatory effect on the immune microenvironment of liver cancer[40]. Preclinical research has shown that, compared with sorafenib, lenvatinib has a significant anti-tumor effect in immunodeficient mice, suggesting that lenvatinib may activate immune function by decreasing the number of tumor-associated macrophages, increasing the proportion of activated CD8+ cells[40], and increasing activation and infiltration of natural killer cells[41].

HBV-related liver cancer is particularly prevalent in Asian populations, especially in China where 69%-80% of liver cancers have an HBV etiology[42,43]. Our meta-analysis of data from patients with HCC and HBV infection suggested that, in terms of OS, atezolizumab plus bevacizumab, lenvatinib, and nivolumab are the three most effective treatments in this patient population. For PFS, lenvatinib ranked first over atezolizumab plus bevacizumab. This finding supports previous meta-analyses that have shown lenvatinib to have a particularly strong anti-tumor effect *vs* sorafenib in patients with HBV-related HCC[44,45]. It is unclear why lenvatinib may have a particularly good anti-tumor effect in HBV-related HCC, but it may be due to the impact of lenvatinib on the immune microenvironment, as described above. In addition, the China National Health and Health Commission guidelines for diagnosis and treatment of primary liver cancer in China (2019 edition) recommend lenvatinib as a systemic therapy with good efficacy in patients with HBV-related liver cancer[46].

This network meta-analysis had several possible limitations. First, the quality of studies included in the analysis had some heterogeneity; for example, the analysis included both large Phase III clinical trials, such as REFLECT and Checkmate 459, and smaller Phase II clinical studies. Second, there was also heterogeneity in the patient populations included in the analysis, including patients from different geographic regions, of different races, and different proportions of patients with HBV infection. Additionally, it should be noted that second-line therapeutic options for HCC have greatly improved over the past decade. As a result, estimates of first-line OS from older studies are generally shorter than those from more recent studies. However, among the therapies included in this analysis that are currently approved for first-line HCC, only sorafenib has OS data old enough to potentially be biased by this phenomenon. Fortunately, our analysis was based on pooled data from the pivotal study of sorafenib in 2008 and comparator arms of trials conducted between 2008 and 2020, which limits the potential effect of bias from improvements in second-line

therapies[47-62]. Finally, because multiple interventions were included in the analysis, several had data from only one study and therefore a relatively small sample size, which may have led to bias.

## CONCLUSION

This network meta-analysis of first-line systemic therapies for advanced HCC revealed that atezolizumab plus bevacizumab is associated with the best OS and PFS, but also with a high incidence of discontinuation due to AEs. The results also showed that lenvatinib is associated with the best ORR of all systemic therapies included in the analysis, as well as a relatively high PFS, particularly in patients with HBV-related liver cancer in whom lenvatinib ranked first for PFS, over atezolizumab plus bevacizumab. Therefore, in patients with unresectable advanced HCC, systemic treatment should be selected based on the individualized treatment goals of each patient.

## ARTICLE HIGHLIGHTS

### *Research background*

The recent expansion of first-line systemic therapy options for patients with advanced hepatocellular carcinoma represents a significant advance in the treatment of this disease. However, the majority of clinical trials in first-line hepatocellular carcinoma management used placebo or sorafenib as comparators, and there are limited data providing a cross comparison of the efficacy and safety of treatments in this setting, especially for newly-approved immune checkpoint inhibitor and vascular endothelial growth factor inhibitor combination treatments.

### *Research motivation*

Clinical trials of recently-approved therapies for hepatocellular carcinoma have revealed differing profiles of efficacy and safety, and comparative data to inform selection of first-line treatments for individual patients are limited. Furthermore, although lenvatinib is widely seen as a standard of care in real clinical practice, and is a recommended first-line therapy in most international treatment guidelines, there are limited head-to-head data comparing lenvatinib with other systemic therapies.

### *Research objectives*

The objectives of this network meta-analysis were to systematically review and compare the response rates, survival outcomes, and safety of first-line systemic therapies for advanced hepatocellular carcinoma, and to provide a comparison between lenvatinib and other systemic therapies in this setting. The study also included a sub-group analysis of patients with hepatitis B virus infection, which is an important population in the Asia-Pacific region and has not been covered by other current meta-analyses.

### *Research methods*

We searched PubMed, Science Direct, the Cochrane Database, Excerpta Medica Database, and abstracts from the American Society of Clinical Oncology 2020 annual congress. Eligible studies were randomized controlled trials of systemic therapy enrolling adults with advanced/unresectable hepatocellular carcinoma. A network meta-analysis was used to synthesize data and perform direct and indirect comparisons between treatments for endpoints including (where available) overall response rate, overall survival, progression-free survival, time-to-progression, incidence of Grade ≥ 3 adverse events, incidence of treatment interruptions due to adverse events, and incidence of dose reductions due to adverse events. *P* value, a frequentist analog to the surface under the cumulative ranking curve, was used to rank treatments.

### *Research results*

Treatments included in the analysis were atezolizumab plus bevacizumab, brivanib, donafenib, dovitinib, FOLFOX4, lenvatinib, linifanib, nintedanib, nivolumab, sorafenib, sunitinib, vandetanib, 11 sorafenib combination therapies, and three other

combination therapies. Atezolizumab plus bevacizumab was ranked first for progression-free survival and overall survival but also had the highest rate of discontinuations due to adverse events. Lenvatinib ranked first for overall response rate and second for progression-free survival. Our findings show that first-line systemic treatment should be selected based on individualized treatment goals and provide valuable comparative data that can help to inform treatment decisions.

### Research conclusions

Our findings suggest that there is no one single first-line treatment for advanced hepatocellular carcinoma associated with superior outcomes across all outcome measurements. Therefore, first-line systemic treatment should be selected based on individualized treatment goals.

### Research perspectives

Future research should continue to evaluate new therapeutic strategies for hepatocellular carcinoma in the context of existing treatments, and provide further information to support treatment selection for individual patients.

## REFERENCES


1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Zhou M**, Wang H, Zhu J, Chen W, Wang L, Liu S, Li Y, Liu Y, Yin P, Liu J, Yu S, Tan F, Barber RM, Coates MM, Dicker D, Fraser M, González-Medina D, Hamavid H, Hao Y, Hu G, Jiang G, Kan H, Lopez AD, Phillips MR, She J, Vos T, Wan X, Xu G, Yan LL, Yu C, Zhao Y, Zheng Y, Zou X, Naghavi M, Wang Y, Murray CJ, Yang G, Liang X. Cause-specific mortality for 240 causes in China during 1990-2013: a systematic subnational analysis for the Global Burden of Disease Study 2013. *Lancet* 2016; **387**: 251-272 [PMID: 26510778 DOI: 10.1016/S0140-6736(15)00551-6]

3 **Allemann P**, Demartines N, Bouzourene H, Tempia A, Halkic N. Long-term outcome after liver resection for hepatocellular carcinoma larger than 10 cm. *World J Surg* 2013; **37**: 452-458 [PMID: 23188527 DOI: 10.1007/s00268-012-1840-5]

4 **National Comprehensive Cancer Network**. Hepatobiliary cancers 2020 [cited 20 March 2021]. Available from: https://www.abc-directory.com/site/1034756

5 **Park JW**, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, Sherman M. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015; **35**: 2155-2166 [PMID: 25752327 DOI: 10.1111/Liv.12818]

6 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]

7 **Li D**, Sedano S, Allen R, Gong J, Cho M, Sharma S. Current Treatment Landscape for Advanced Hepatocellular Carcinoma: Patient Outcomes and the Impact on Quality of Life. *Cancers (Basel)* 2019; **11** [PMID: 31216701 DOI: 10.3390/cancers11060841]

8 **Bruix J**, Raoul JL, Sherman M, Mazzaferro V, Bolondi L, Craxi A, Galle PR, Santoro A, Beaugrand M, Sangiovanni A, Porta C, Gerken G, Marrero JA, Nadel A, Shan M, Moscovici M, Voliotis D, Llovet JM. Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma: subanalyses of a phase III trial. *J Hepatol* 2012; **57**: 821-829 [PMID: 22727733 DOI: 10.1016/j.jhep.2012.06.014]

9 **Bruix J**, da Fonseca LG, Reig M. Insights into the success and failure of systemic therapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 617-630 [PMID: 31371809 DOI: 10.1038/s41575-019-0179-x]

10 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib *vs* sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

11 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020; **382**: 1894-1905 [PMID: 32402160 DOI: 10.1056/NEJMoa1915745]

12 **Heimbach JK**. Overview of the Updated AASLD Guidelines for the Management of HCC. *Gastroenterol Hepatol (N Y)* 2017; **13**: 751-753 [PMID: 29339953]

13 **Vogel A**, Cervantes A, Chau I, Daniele B, Llovet JM, Meyer T, Nault JC, Neumann U, Ricke J, Sangro B, Schirmacher P, Verslype C, Zech CJ, Arnold D, Martinelli E; ESMO Guidelines Committee. Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; **29**: iv238-iv255 [PMID: 30285213 DOI: 10.1093/annonc/mdy308]

14 **Kudo M**. Systemic Therapy for Hepatocellular Carcinoma: Latest Advances. *Cancers (Basel)* 2018; **10** [PMID: 30380773 DOI: 10.3390/cancers10110412]

15 **Ding W**, Tan Y, Qian Y, Xue W, Wang Y, Jiang P, Xu X. First-line targ veted therapies of advanced hepatocellular carcinoma: A Bayesian network analysis of randomized controlled trials. *PLoS One* 2020; **15**: e0229492 [PMID: 32134981 DOI: 10.1371/journal.pone.0229492]

16 **Sonbol MB**, Riaz IB, Naqvi SAA, Almquist DR, Mina S, Almasri J, Shah S, Almader-Douglas D, Uson Junior PLS, Mahipal A, Ma WW, Jin Z, Mody K, Starr J, Borad MJ, Ahn DH, Murad MH, Bekaii-Saab T. Systemic Therapy and Sequencing Options in Advanced Hepatocellular Carcinoma: A Systematic Review and Network Meta-analysis. *JAMA Oncol* 2020; **6**: e204930 [PMID: 33090186 DOI: 10.1001/jamaoncol.2020.4930]

17 **Bi F**, Qin SK, Gu SZ, Bai YX, Chen ZD, Wang ZS, Ying J, Lu YY, Meng ZHQ, Pan HM, Yang P, Zhang HL, Chen X, Xu AB, Liu XF, Meng Q, Wu LQ, Chen F. Donafenib *vs* Sorafenib as first-line therapy in advanced hepatocellular carcinoma: an open-label, randomized, multicentre phase II/III trial. *J Clin Oncol* 2020; **38**: 4506-4506 [DOI: 10.1200/JCO.2020.38.15_suppl.4506]

18 **Higgins JP**, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA; Cochrane Bias Methods Group; Cochrane Statistical Methods Group. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011; **343**: d5928 [PMID: 22008217 DOI: 10.1136/bmj.d5928]

19 **Rücker G**. Network meta-analysis, electrical networks and graph theory. *Res Synth Methods* 2012; **3**: 312-324 [PMID: 26053424 DOI: 10.1002/jrsm.1058]

20 **Rücker G**, Schwarzer G. Reduce dimension or reduce weights? *Stat Med* 2014; **33**: 4353-4369 [PMID: 24942211 DOI: 10.1002/sim.6236]

21 **Rücker G**, Schwarzer G. Ranking treatments in frequentist network meta-analysis works without resampling methods. *BMC Med Res Methodol* 2015; **15**: 58 [PMID: 26227148 DOI: 10.1186/s12874-015-0060-8]

22 **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL. Brivanib *vs* sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]

23 **Yau T**, Park JW, Finn RS, Cheng AL, Mathurin P, Edeline J, Kudo M, Han KH, Harding JJ, Merle P, Rosmorduc O, Wyrwicz L, Schott E, Choo SP, Kelley RK, Begic D, Chen G, Neely J, Anderson J, Sangro B. CheckMate 459: A randomized, multi-center phase III study of nivolumab (NIVO) *vs* sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). *Ann Oncol* 2019; **30**: 874 [DOI: 10.1093/annonc/mdz394.029]

24 **Cainap C**, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens FA, Qian J, McKee MD, Ricker JL, Carlson DM, El-Nowiem S. Linifanib *vs* Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2015; **33**: 172-179 [PMID: 25488963 DOI: 10.1200/JCO.2013.54.3298]

25 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

26 **Zhu AX**, Rosmorduc O, Evans TR, Ross PJ, Santoro A, Carrilho FJ, Bruix J, Qin S, Thuluvath PJ, Llovet JM, Leberre MA, Jensen M, Meinhardt G, Kang YK. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015; **33**: 559-566 [PMID: 25547503 DOI: 10.1200/JCO.2013.53.7746]

27 **Tak WY**, Ryoo BY, Lim HY, Kim DY, Okusaka T, Ikeda M, Hidaka H, Yeon JE, Mizukoshi E, Morimoto M, Lee MA, Yasui K, Kawaguchi Y, Heo J, Morita S, Kim TY, Furuse J, Katayama K, Aramaki T, Hara R, Kimura T, Nakamura O, Kudo M. Phase I/II study of first-line combination therapy with sorafenib plus resminostat, an oral HDAC inhibitor, *vs* sorafenib monotherapy for advanced hepatocellular carcinoma in east Asian patients. *Invest New Drugs* 2018; **36**: 1072-1084 [PMID: 30198057 DOI: 10.1007/s10637-018-0658-x]

28 **Cheng AL**, Kang YK, He AR, Lim HY, Ryoo BY, Hung CH, Sheen IS, Izumi N, Austin T, Wang Q, Greenberg J, Shiratori S, Beckman RA, Kudo M; Investigators' Study Group. Safety and efficacy of tigatuzumab plus sorafenib as first-line therapy in subjects with advanced hepatocellular carcinoma: A phase 2 randomized study. *J Hepatol* 2015; **63**: 896-904 [PMID: 26071796 DOI: 10.1016/j.jhep.2015.06.001]

29 **Cheng AL**, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib *vs* sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/JCO.2012.45.8372]

30 **Yi M**, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. *Mol Cancer* 2019; **18**: 60 [PMID: 30925919 DOI: 10.1186/s12943-019-0974-6]

31 **Lee MS**, Ryoo BY, Hsu CH, Numata K, Stein S, Verret W, Hack SP, Spahn J, Liu B, Abdullah H,

Wang Y, He AR, Lee KH; GO30140 investigators. Atezolizumab with or without bevacizumab in unresectable hepatocellular carcinoma (GO30140): an open-label, multicentre, phase 1b study. *Lancet Oncol* 2020; **21**: 808-820 [PMID: 32502443 DOI: 10.1016/S1470-2045(20)30156-X]

32 **ten Doesschate T**, van Haren E, Wijma RA, Koch BCP. The effectiveness of nitrofurantoin, fosfomycin and trimethoprim for the treatment of cystitis in relation to renal function. *Clin Microbiol Infec* 2020; **26** [DOI: 10.1016/j.cmi.2020.03.001]

33 **Kudo M**, Ueshima K, Chan S, Minami T, Chishina H, Aoki T, Takita M, Hagiwara S, Minami Y, Ida H, Takenaka M, Sakurai T, Watanabe T, Morita M, Ogawa C, Wada Y, Ikeda M, Ishii H, Izumi N, Nishida N. Lenvatinib as an Initial Treatment in Patients with Intermediate-Stage Hepatocellular Carcinoma Beyond Up-To-Seven Criteria and Child-Pugh A Liver Function: A Proof-Of-Concept Study. *Cancers (Basel)* 2019; **11** [PMID: 31370183 DOI: 10.3390/cancers11081084]

34 **Sun HC**, Zhu XD, Huang C, Shen YH, Ge NL, Chen Y, Tan CJ, Zhou J, Fan J. Combination therapy with lenvatinib and anti-PD-1 antibodies for unresectable or advanced hepatocellular carcinoma: A real-world study. *J Clin Oncol* 2020; **38**: e16610-e16610 [DOI: 10.1200/JCO.2020.38.15_suppl.e16610]

35 **Chen X**, Zhang Y, Zhang N, Ge Y, Jia W. Lenvatinib combined nivolumab injection followed by extended right hepatectomy is a feasible treatment for patients with massive hepatocellular carcinoma: a case report. *Onco Targets Ther* 2019; **12**: 7355-7359 [PMID: 31686845 DOI: 10.2147/OTT.S217123]

36 **He M**, Li Q, Zou R, Shen J, Fang W, Tan G, Zhou Y, Wu X, Xu L, Wei W, Le Y, Zhou Z, Zhao M, Guo Y, Guo R, Chen M, Shi M. Sorafenib Plus Hepatic Arterial Infusion of Oxaliplatin, Fluorouracil, and Leucovorin *vs* Sorafenib Alone for Hepatocellular Carcinoma With Portal Vein Invasion: A Randomized Clinical Trial. *JAMA Oncol* 2019; **5**: 953-960 [PMID: 31070690 DOI: 10.1001/jamaoncol.2019.0250]

37 **Park R**, Silva L, Nissaisorakarn V, Riano I, Saeed A. Comparison of systemic therapy efficacy in advanced hepatocellular carcinoma: Systematic review and frequentist network meta-analysis of randomized controlled trials. *J Clin Oncol* 2021; **39**: 293-293 [DOI: 10.1200/JCO.2021.39.3_suppl.293]

38 **Yamamoto Y**, Matsui J, Matsushima T, Obaishi H, Miyazaki K, Nakamura K, Tohyama O, Semba T, Yamaguchi A, Hoshi SS, Mimura F, Haneda T, Fukuda Y, Kamata JI, Takahashi K, Matsukura M, Wakabayashi T, Asada M, Nomoto KI, Watanabe T, Dezso Z, Yoshimatsu K, Funahashi Y, Tsuruoka A. Lenvatinib, an angiogenesis inhibitor targeting VEGFR/FGFR, shows broad antitumor activity in human tumor xenograft models associated with microvessel density and pericyte coverage. *Vasc Cell* 2014; **6**: 18 [PMID: 25197551 DOI: 10.1186/2045-824X-6-18]

39 **Okamoto K**, Ikemori-Kawada M, Jestel A, von König K, Funahashi Y, Matsushima T, Tsuruoka A, Inoue A, Matsui J. Distinct binding mode of multikinase inhibitor lenvatinib revealed by biochemical characterization. *ACS Med Chem Lett* 2015; **6**: 89-94 [PMID: 25589937 DOI: 10.1021/mL500394m]

40 **Kato Y**, Tabata K, Kimura T, Yachie-Kinoshita A, Ozawa Y, Yamada K, Ito J, Tachino S, Hori Y, Matsuki M, Matsuoka Y, Ghosh S, Kitano H, Nomoto K, Matsui J, Funahashi Y. Lenvatinib plus anti-PD-1 antibody combination treatment activates CD8+ T cells through reduction of tumor-associated macrophage and activation of the interferon pathway. *PLoS One* 2019; **14**: e0212513 [PMID: 30811474 DOI: 10.1371/journal.pone.0212513]

41 **Zhang Q**, Liu H, Wang H, Lu M, Miao Y, Ding J, Li H, Gao X, Sun S, Zheng J. Lenvatinib promotes antitumor immunity by enhancing the tumor infiltration and activation of NK cells. *Am J Cancer Res* 2019; **9**: 1382-1395 [PMID: 31392076]

42 **Zhu RX**, Seto WK, Lai CL, Yuen MF. Epidemiology of Hepatocellular Carcinoma in the Asia-Pacific Region. *Gut Liver* 2016; **10**: 332-339 [PMID: 27114433 DOI: 10.5009/gnl15257]

43 **Fan JH**, Wang JB, Jiang Y, Xiang W, Liang H, Wei WQ, Qiao YL, Boffetta P. Attributable causes of liver cancer mortality and incidence in china. *Asian Pac J Cancer Prev* 2013; **14**: 7251-7256 [PMID: 24460283 DOI: 10.7314/apjcp.2013.14.12.7251]

44 **Casadei Gardini A**, Puzzoni M, Montagnani F, Marisi G, Tamburini E, Cucchetti A, Solaini L, Foschi FG, Conti F, Ercolani G, Cascinu S, Scartozzi M. Profile of lenvatinib in the treatment of hepatocellular carcinoma: design, development, potential place in therapy and network meta-analysis of hepatitis B and hepatitis C in all Phase III trials. *Onco Targets Ther* 2019; **12**: 2981-2988 [PMID: 31118665 DOI: 10.2147/OTT.S192572]

45 **Park J**, Cho J, Lim JH, Lee MH, Kim J. Relative Efficacy of Systemic Treatments for Patients with Advanced Hepatocellular Carcinoma According to Viral Status: A Systematic Review and Network Meta-Analysis. *Target Oncol* 2019; **14**: 395-403 [PMID: 31290003 DOI: 10.1007/s11523-019-00651-7]

46 **Department of Medical Administration**, National Health and Health Commission of the People's Republic of China. [Guidelines for diagnosis and treatment of primary liver cancer in China (2019 edition)]. *Zhonghua Gan Zang Bing Za Zhi* 2020; **28**: 112-128 [PMID: 32164061 DOI: 10.3760/cma.j.issn.1007-3418.2020.02.004]

47 **Yen CJ**, Kim TY, Feng YH, Chao Y, Lin DY, Ryoo BY, Huang DC, Schnell D, Hocke J, Loembé AB, Cheng AL. A Phase I/Randomized Phase II Study to Evaluate the Safety, Pharmacokinetics, and Efficacy of Nintedanib *vs* Sorafenib in Asian Patients with Advanced Hepatocellular Carcinoma. *Liver Cancer* 2018; **7**: 165-178 [PMID: 29888206 DOI: 10.1159/000486460]

48 **Ciuleanu T**, Bazin I, Lungulescu D, Miron L, Bondarenko I, Deptala A, Rodriguez-Torres M, Giantonio B, Fox NL, Wissel P, Egger J, Ding M, Kalyani RN, Humphreys R, Gribbin M, Sun W. A

randomized, double-blind, placebo-controlled phase II study to assess the efficacy and safety of mapatumumab with sorafenib in patients with advanced hepatocellular carcinoma. *Ann Oncol* 2016; **27**: 680-687 [PMID: 26802147 DOI: 10.1093/annonc/mdw004]

49 **Abou-Alfa GK**, Johnson P, Knox JJ, Capanu M, Davidenko I, Lacava J, Leung T, Gansukh B, Saltz LB. Doxorubicin plus sorafenib *vs* doxorubicin alone in patients with advanced hepatocellular carcinoma: a randomized trial. *JAMA* 2010; **304**: 2154-2160 [PMID: 21081728 DOI: 10.1001/jama.2010.1672]

50 **Cheng AL**, Thongprasert S, Lim HY, Sukeepaisarnjaroen W, Yang TS, Wu CC, Chao Y, Chan SL, Kudo M, Ikeda M, Kang YK, Pan H, Numata K, Han G, Balsara B, Zhang Y, Rodriguez AM, Wang Y, Poon RT. Randomized, open-label phase 2 study comparing frontline dovitinib *vs* sorafenib in patients with advanced hepatocellular carcinoma. *Hepatology* 2016; **64**: 774-784 [PMID: 27082062 DOI: 10.1002/hep.28600]

51 **Hsu C**, Yang TS, Huo TI, Hsieh RK, Yu CW, Hwang WS, Hsieh TY, Huang WT, Chao Y, Meng R, Cheng AL. Vandetanib in patients with inoperable hepatocellular carcinoma: a phase II, randomized, double-blind, placebo-controlled study. *J Hepatol* 2012; **56**: 1097-1103 [PMID: 22245891 DOI: 10.1016/j.jhep.2011.12.013]

52 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J; SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

53 **Palmer DH**, Ma YT, Peck-Radosavljevic M, Ross P, Graham J, Fartoux L, Deptala A, Studeny M, Schnell D, Hocke J, Loembé AB, Meyer T. A multicentre, open-label, phase-I/randomised phase-II study to evaluate safety, pharmacokinetics, and efficacy of nintedanib vs. sorafenib in European patients with advanced hepatocellular carcinoma. *Br J Cancer* 2018; **118**: 1162-1168 [PMID: 29563636 DOI: 10.1038/s41416-018-0051-8]

54 **Thomas MB**, Garrett-Mayer E, Anis M, Anderton K, Bentz T, Edwards A, Brisendine A, Weiss G, Siegel AB, Bendell J, Baron A, Duddalwar V, El-Khoueiry A. A Randomized Phase II Open-Label Multi-Institution Study of the Combination of Bevacizumab and Erlotinib Compared to Sorafenib in the First-Line Treatment of Patients with Advanced Hepatocellular Carcinoma. *Oncology* 2018; **94**: 329-339 [PMID: 29719302 DOI: 10.1159/000485384]

55 **Abou-Alfa GK**, Shi Q, Knox JJ, Kaubisch A, Niedzwiecki D, Posey J, Tan BR Jr, Kavan P, Goel R, Lammers PE, Bekaii-Saab TS, Tam VC, Rajdev L, Kelley RK, El Dika I, Zemla T, Potaracke RI, Balletti J, El-Khoueiry AB, Harding JH, Suga JM, Schwartz LH, Goldberg RM, Bertagnolli MM, Meyerhardt J, O'Reilly EM, Venook AP. Assessment of Treatment With Sorafenib Plus Doxorubicin *vs* Sorafenib Alone in Patients With Advanced Hepatocellular Carcinoma: Phase 3 CALGB 80802 Randomized Clinical Trial. *JAMA Oncol* 2019 [PMID: 31486832 DOI: 10.1001/jamaoncol.2019.2792]

56 **Jouve JL**, Lecomte T, Bouché O, Barbier E, Khemissa Akouz F, Riachi G, Nguyen Khac E, Ollivier-Hourmand I, Debette-Gratien M, Faroux R, Villing AL, Vergniol J, Ramee JF, Bronowicki JP, Seitz JF, Legoux JL, Denis J, Manfredi S, Phelip JM; PRODIGE-11 investigators/collaborators. Pravastatin combination with sorafenib does not improve survival in advanced hepatocellular carcinoma. *J Hepatol* 2019; **71**: 516-522 [PMID: 31125576 DOI: 10.1016/j.jhep.2019.04.021]

57 **Lee FA**, Zee BC, Cheung FY, Kwong P, Chiang CL, Leung KC, Siu SW, Lee C, Lai M, Kwok C, Chong M, Jolivet J, Tung S. Randomized Phase II Study of the X-linked Inhibitor of Apoptosis (XIAP) Antisense AEG35156 in Combination With Sorafenib in Patients With Advanced Hepatocellular Carcinoma (HCC). *Am J Clin Oncol* 2016; **39**: 609-613 [PMID: 24977690 DOI: 10.1097/coc.0000000000000099]

58 **Assenat E**, Pageaux GP, Thézenas S, Peron JM, Bécouarn Y, Seitz JF, Merle P, Blanc JF, Bouché O, Ramdani M, Poujol S, de Forges H, Ychou M, Boige V. Sorafenib alone vs. sorafenib plus GEMOX as 1st-line treatment for advanced HCC: the phase II randomised PRODIGE 10 trial. *Br J Cancer* 2019; **120**: 896-902 [PMID: 30944458 DOI: 10.1038/s41416-019-0443-4]

59 **Azim HA**, Omar A, Atef H, Zawahry H, Shaker MK, Abdelmaksoud AK, EzzElarab M, Abdel-Rahman O, Ismail M, Kassem L, Waked I. Sorafenib plus tegafur-uracil (UFT) *vs* sorafenib as first line systemic treatment for patients with advanced stage HCC: a Phase II trial (ESLC01 study). *J Hepatocell Carcinoma* 2018; **5**: 109-119 [PMID: 30510922 DOI: 10.2147/JHC.S169285]

60 **Koeberle D**, Dufour JF, Demeter G, Li Q, Ribi K, Samaras P, Saletti P, Roth AD, Horber D, Buehlmann M, Wagner AD, Montemurro M, Lakatos G, Feilchenfeldt J, Peck-Radosavljevic M, Rauch D, Tschanz B, Bodoky G; Swiss Group for Clinical Cancer Research (SAKK). Sorafenib with or without everolimus in patients with advanced hepatocellular carcinoma (HCC): a randomized multicenter, multinational phase II trial (SAKK 77/08 and SASL 29). *Ann Oncol* 2016; **27**: 856-861 [PMID: 26884590 DOI: 10.1093/annonc/mdw054]

61 **Qin S**, Bai Y, Lim HY, Thongprasert S, Chao Y, Fan J, Yang TS, Bhudhisawasdi V, Kang WK, Zhou Y, Lee JH, Sun Y. Randomized, multicenter, open-label study of oxaliplatin plus fluorouracil/Leucovorin *vs* doxorubicin as palliative chemotherapy in patients with advanced hepatocellular carcinoma from Asia. *J Clin Oncol* 2013; **31**: 3501-3508 [PMID: 23980077 DOI: 10.1200/JCO.2012.44.5643]

62 **Yeo W**, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin

*vs* cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538 [PMID: 16234567 DOI: 10.1093/jnci/dji315]

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

© 2021 Baishideng Publishing Group Inc. All rights reserved.