

World Journal of *Gastroenterology*

World J Gastroenterol 2019 January 14; 25(2): 151-281



**REVIEW**

- 151** Role of diet and gut microbiota on colorectal cancer immunomodulation
De Almeida CV, de Camargo MR, Russo E, Amedei A
- 163** Current status, problems, and perspectives of non-alcoholic fatty liver disease research
Tanaka N, Kimura T, Fujimori N, Nagaya T, Komatsu M, Tanaka E

MINIREVIEWS

- 178** Prevention of overuse: A view on upper gastrointestinal endoscopy
de Jong JJ, Lantinga MA, Drenth JP
- 190** Adverse events related to colonoscopy: Global trends and future challenges
Kim SY, Kim HS, Park HJ

ORIGINAL ARTICLE**Basic Study**

- 205** Human antigen R mediated post-transcriptional regulation of inhibitors of apoptosis proteins in pancreatic cancer
Lukosiute-Urboniene A, Jasukaitiene A, Silkuniene G, Barauskas V, Gulbinas A, Dambrasas Z
- 220** Six-long non-coding RNA signature predicts recurrence-free survival in hepatocellular carcinoma
Gu JX, Zhang X, Miao RC, Xiang XH, Fu YN, Zhang JY, Liu C, Qu K
- 233** Biomarker identification and trans-regulatory network analyses in esophageal adenocarcinoma and Barrett's esophagus
Lv J, Guo L, Wang JH, Yan YZ, Zhang J, Wang YY, Yu Y, Huang YF, Zhao HP

Retrospective Study

- 245** Submucosal tunneling endoscopic resection: An effective and safe therapy for upper gastrointestinal submucosal tumors originating from the muscularis propria layer
Du C, Chai NL, Ling-Hu EQ, Li ZJ, Li LS, Zou JL, Jiang L, Lu ZS, Meng JY, Tang P
- 258** Novel abdominal negative pressure lavage-drainage system for anastomotic leakage after R0 resection for gastric cancer
Zheng ZF, Lu J, Zhang PY, Xu BB, Zheng CH, Li P, Xie JW, Wang JB, Lin JX, Chen QY, Huang CM

Observational Study

- 269** Increased expression of brain-derived neurotrophic factor is correlated with visceral hypersensitivity in patients with diarrhea-predominant irritable bowel syndrome
Zhang Y, Qin G, Liu DR, Wang Y, Yao SK

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Andreas Larentzakis, MD, MSc, PhD, Academic Fellow, Attending Doctor, Doctor, Surgeon, Surgical Oncologist, Department of Surgery, Athens Medical School, National and Kapodistrian University of Athens, Athens 11527, Greece

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, etc. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology (*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 Directory of Open Access Journals. The 2018 edition of Journal Citation Report® cites the 2017 impact factor for *WJG* as 3.300 (5-year impact factor: 3.387), ranking *WJG* as 35th among 80 journals in gastroenterology and hepatology (quartile in category Q2).

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Shu-Yu Yin*

Proofing 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

EDITORIAL BOARD MEMBERS

<http://www.wjgnet.com/1007-9327/editorialboard.htm>

EDITORIAL OFFICE

Ze-Mao Gong, Director

PUBLICATION DATE

January 14, 2019

COPYRIGHT

© 2019 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 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>



Current status, problems, and perspectives of non-alcoholic fatty liver disease research

Naoki Tanaka, Takefumi Kimura, Naoyuki Fujimori, Tadanobu Nagaya, Michiharu Komatsu, Eiji Tanaka

ORCID number: Naoki Tanaka (0000-0002-0606-2101); Takefumi Kimura (0000-0002-1481-1029); Naoyuki Fujimori (0000-0001-8744-8139); Tadanobu Nagaya (0000-0002-0091-8343); Michiharu Komatsu (0000-0002-7860-2816); Eiji Tanaka (0000-0002-0724-2104).

Author contributions: All authors have approved the submission of this manuscript. Tanaka N wrote the manuscript; Tanaka N, Kimura T, Fujimori N, Nagaya T and Komatsu M have conducted long-term NAFLD/NASH research and discussed the perspectives; Tanaka E supervised the manuscript.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (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

Received: October 5, 2018

Peer-review started: October 5, 2018

First decision: November 14, 2018

Naoki Tanaka, Department of Metabolic Regulation, Shinshu University School of Medicine, Matsumoto 390-8621, Japan

Naoki Tanaka, International Research Center for Agricultural Food Industry, Shinshu University, Matsumoto 390-8621, Japan

Takefumi Kimura, Naoyuki Fujimori, Tadanobu Nagaya, Michiharu Komatsu, Eiji Tanaka, Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto 390-8621, Japan

Corresponding author: Naoki Tanaka, MD, PhD, Associate Professor, Doctor, Department of Metabolic Regulation, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan. naopi@shinshu-u.ac.jp

Telephone: +81-263-372634

Fax: +81-263-329412

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a major chronic liver disease that can lead to liver cirrhosis, liver cancer, and ultimately death. NAFLD is pathologically classified as non-alcoholic fatty liver (NAFL) or non-alcoholic steatohepatitis (NASH) based on the existence of ballooned hepatocytes, although the states have been known to transform into each other. Moreover, since the detection of ballooned hepatocytes may be difficult with limited biopsied specimens, its clinical significance needs reconsideration. Repeated liver biopsy to assess histological NAFLD activity for therapeutic response is also impractical, creating the need for body fluid biomarkers and less invasive imaging modalities. Recent longitudinal observational studies have emphasized the importance of advanced fibrosis as a determinant of NAFLD outcome. Thus, identifying predictors of fibrosis progression and developing better screening methods will enable clinicians to isolate high-risk NAFLD patients requiring early intensive intervention. Despite the considerable heterogeneity of NAFLD with regard to underlying disease, patient age, and fibrosis stage, several clinical trials are underway to develop a first-in-class drug. In this review, we summarize the present status and future direction of NAFLD/NASH research towards solving unmet medical needs.

Key words: Non-alcoholic steatohepatitis; Fibrosis; Steatosis; Ballooning; Biomarker; Outcome; Treatment

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

Revised: December 24, 2018

Accepted: December 27, 2018

Article in press: December 28, 2018

Published online: January 14, 2019

Core tip: Recent trends in diet and lifestyle have increased the prevalence of non-alcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) worldwide. Although advances in non-invasive biomarkers and imaging modalities have improved disease detection and follow-up, considerable work is needed to identify individuals with low fibrosis stages or at risk of rapid disease progression. In the future, earlier detection will enable prompt single or combination treatment with new-line drugs that have been optimized for maximum benefit and fewer adverse events. Only with a concerted effort across multi-disciplinary fields can clinicians begin to halt the rapid spread of NAFLD/NASH.

Citation: Tanaka N, Kimura T, Fujimori N, Nagaya T, Komatsu M, Tanaka E. Current status, problems, and perspectives of non-alcoholic fatty liver disease research. *World J Gastroenterol* 2019; 25(2): 163-177

URL: <https://www.wjgnet.com/1007-9327/full/v25/i2/163.htm>

DOI: <https://dx.doi.org/10.3748/wjg.v25.i2.163>

INTRODUCTION

With the worldwide spread of sedentary lifestyle and diet westernization, the prevalence of non-alcoholic fatty liver disease (NAFLD) has increased in many countries among children and the elderly alike^[1,2]. Approximately 25% of adults in the United States have fatty liver in the absence of excessive ethanol consumption. In Japan, roughly a third of individuals were found to have NAFLD in annual health checkups^[3], translating to an estimated 20 million NAFLD patients. In China, fatty liver disease is increasing at a rate of 0.594% per year and is expected to afflict 20% of Chinese by 2020^[4]. NAFLD is becoming the most common liver disease worldwide.

NAFLD was originally considered as non-progressive and fundamentally benign until Dr. Jurgen Ludwig, a pathologist at the Mayo Clinic, proposed the concept of non-alcoholic steatohepatitis (NASH) in 1980^[5]. They observed that 20 patients without a drinking habit displayed histological findings similar to those in alcoholic steatohepatitis, such as fatty changes, focal hepatocyte necrosis, ballooned hepatocytes with Mallory-Denk inclusion bodies, lobular inflammation, and perisinusoidal/perivenular fibrosis. These patients frequently had diabetes, dyslipidemia, hypertension, and/or obesity. Thereafter, worldwide increases in obesity have led to a rapid spread of the concept of NASH, with many cases of fatty liver progressing to liver cirrhosis and hepatocellular carcinoma (HCC) being reported^[6-8]. At present, NAFLD is classified into two categories according to liver pathology: non-alcoholic fatty liver (NAFL), also designated as simple steatosis or isolated steatosis, and NASH. Whereas NASH is defined as the presence of macrovesicular steatosis in addition to hepatocyte ballooning degeneration, lobular inflammation, and/or fibrosis, NAFL is characterized as macrovesicular steatosis without ballooned hepatocytes^[9-12].

NAFLD is frequently associated with increased visceral adiposity (obesity) and ensuing metabolic abnormalities, including insulin resistance, diabetes, hypertension, dyslipidemia, atherosclerosis, and systemic micro-inflammation. A recent meta-analysis involving over 8.5 million individuals from 22 countries showed that more than 80% of NASH patients were overweight or obese, 72% had dyslipidemia, and 44% had type 2 diabetes mellitus^[13]. Therefore, NAFLD can also be regarded as a hepatic manifestation of metabolic syndrome. Although it remains controversial whether NAFLD is a cause or a result of glucose intolerance and insulin resistance, a prospective study has demonstrated higher risks of diabetes and cardiovascular events in non-diabetic NAFLD patients than non-NAFLD ones^[14]. Therefore, NAFLD is a detrimental condition necessitating appropriate interventions.

NAFLD also occurs in children and adolescents. The prevalence of NAFLD among junior high school students was estimated at approximately 4% in certain areas of Japan, and one student had obesity, diabetes, dyslipidemia, and NASH with mild fibrosis^[15]. A study of more than 250000 Danish children showed that high body mass index (BMI) in childhood increased the risk of HCC in adulthood^[16]. The above findings suggest that chronic obesity and NAFLD from childhood may produce a higher risk of liver fibrosis, cancer, and decompensation requiring liver transplantation at older ages. The economic burden of NAFLD in the United States is already enormous, with more than \$100 billion in annual direct medical costs

primarily for NASH and its sequelae^[13].

NAFLD/NASH currently stands at the crossroads of gastroenterology, cardiovascular disease, endocrinology/metabolism, and oncology. The path of NAFLD/NASH research appears long and diverse, but progress on improved screening techniques and therapeutic agents is ongoing. In this review, we consolidate the broad clinical picture of NAFLD/NASH and outline the unresolved problems surrounding NAFLD/NASH research and treatment.

CURRENT STATUS OF NAFLD/NASH

Pathogenesis

Understanding the pathogenesis of NAFLD/NASH is essential to establish proper therapeutic interventions. However, disease development is so complicated that it has been designated as “multiple hit and organ theory”^[17].

Mechanism of steatogenesis: An initial step in NAFLD onset is triacylglycerol (TAG) accumulation in hepatocytes. TAG is synthesized from fatty acid (FA) and glycerol. FA is usually absorbed from the circulation into the hepatocytes or produced from glucose in the liver *via de novo* lipogenesis. FA is catabolized primarily by β -oxidation in the mitochondria or peroxisomes, and excess amounts are converted into TAG and stored as lipid droplets in hepatocytes. The TAG in lipid droplets is hydrolyzed or secreted into the circulation as very-low-density lipoprotein particles. Disruption of those pathways can result in hepatosteatosis^[18].

FA β -oxidation in hepatocytes is mainly regulated by the nuclear receptor peroxisome proliferator-activated receptor (PPAR) α . PPAR α down-regulation has been associated with NAFLD/NASH^[18]. Hyperglycemia enhances *de novo* lipogenesis, which is strongly regulated by insulin through activation of transcriptional factor sterol regulatory element-binding protein 1c (SREBP-1c). This mechanism may partially explain the close relationship between NAFLD/NASH and insulin resistance.

Dynamic changes in hepatocyte lipid droplets are also important considerations in the mechanism of hepatic steatosis. Indeed, hepatocyte-specific disruption of fat-specific protein 27 [FSP27, human cell death-inducing DFF45-like effector C (CIDE)], a lipid-coating protein stabilizing TAG in lipid droplets, in *ob/ob* mice attenuates fatty liver through increased TAG hydrolysis and FA utilization^[19].

As approximately 60% of FA in the liver originates from white adipose tissue^[20], adipocyte dysfunction may lead to the FA overflow and NAFLD/NASH. Adipocyte-specific FSP27-disrupted mice aggravate high-fat diet-induced hepatic steatosis because of impaired fat storage and enhanced lipolysis in white adipose tissue^[21]. Enhanced white adipose lipolysis to steatotic mice induced by choline-deficient diet promotes FA mobilization from adipose to liver and increases hepatic oxidative stress, leading to development of steatohepatitis^[22]. Additionally, NAFLD/NASH is frequently accompanied in lipodystrophic patients^[23] and humans having CIDE mutation exhibit lipodystrophy, marked insulin resistance, and NASH with advanced fibrosis^[24]. These findings indicate the importance of liver-adipose axis for the occurrence of NAFLD/NASH.

Mechanisms promoting hepatocyte injury and inflammation: TAG stored as lipid droplets are not strongly toxic to hepatocytes. Several studies have demonstrated the absence of a correlation between the degree of TAG accumulation and NAFLD severity, and hepatosteatosis is known to attenuate with fibrosis progression. Therefore, TAG precursors and intermediates, such as palmitate, diacylglycerol (DAG), and ceramide, are likely detrimental for hepatocytes. Palmitate increases oxidative and endoplasmic reticulum (ER) stress, leading to c-jun N-terminal kinase (JNK) activation and lipopoptosis^[25-27]. DAG activates protein kinase C and disrupts insulin signaling. Ceramide up-regulates the expression of SREBP-1c and promotes the production of palmitate^[28]. Moreover, fat-rich cells are prone to lipid peroxidation, leading to mitochondrial and ER dysfunction. Increased free cholesterol also causes mitochondrial dysfunction and inflammasome activation^[29]. Cytotoxicities mediated by these specific lipids (*i.e.*, lipotoxicity) are one of the major causes of hepatocyte injury in NASH.

Damaged hepatocytes release several pro-inflammatory mediators that include damage-associated molecular patterns and pathogen-associated molecular patterns to recruit immune cells and activate Kupffer cells. Activated immune cells release bioactive molecules that further damage hepatocytes or render them more sensitive to various substances, such as microbiome-derived lipopolysaccharides and secondary bile acids as well as food contaminants from gut, thus amplifying cell death and

inflammation^[30,31].

Mechanisms of fibrogenesis: Damaged hepatocytes and activated immune cells also promote hepatic stellate cell (HSC) activation. During the normal repair/regeneration process, healthy hepatocytes occupy voids created by sporadic hepatocyte death. Chronic hepatocyte death or impaired hepatocyte regeneration leads to alternative replacement by fibers and extracellular matrix, causing significant scarring and remodeling of the normal architecture of hepatic lobules^[32]. Although HSC activation is a key event in liver fibrogenesis regardless of etiology, fibrogenesis mainly in the perisinusoidal space is relatively specific to steatohepatitis.

Mechanism of hepatocarcinogenesis: Several epidemiological studies have revealed obesity and diabetes as risk factors for HCC. In the context of Asian populations, the impact of occult hepatitis B virus (HBV) infection should also be taken into consideration when discussing NAFLD-related HCC since HBV has carcinogenic properties due to its DNA integration^[33]. Kimura *et al*^[34] analyzed 77 Japanese patients with HCC who underwent surgical resection and were negative for serum anti-HBV core/surface antibodies, HBV surface antigen, and anti-hepatitis C virus (HCV) antibody. The NAFLD-related HCC subjects had a higher BMI and prevalence of diabetes, although 30%-40% had none-to-mild fibrosis in non-cancerous tissue. Multivariate analysis revealed that the presence of diabetes was associated with NAFLD-HCC with none-to-mild fibrosis. In agreement with other reports^[8,35], NAFLD-HCC may occur not only in fibrotic/cirrhotic livers (*i.e.*, through the classical inflammation-fibrosis-HCC sequence), but also from none-to-mild hepatic fibrosis even in the absence of past HBV infection. Although the mechanism of how obesity and diabetes influence hepatocarcinogenesis is not fully clarified, insulin resistance, increased circulating advanced glycation end products and ensuing disruption of cell proliferation signals, and genetic background including PNPLA3, TERT, and MBOAT may have prominent roles in HCC development^[36].

Diagnosis

Diagnosis of NAFLD: NAFLD is often asymptomatic and detected only by abnormal liver function or imaging results in health checkups or during follow-up for other diseases. Patients with persistent elevation of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and fatty change on ultrasonography (US) or computed tomography (CT) without a history of habitual drug/ethanol intake or positive hepatitis virus markers or autoantibodies can be suspected as having NAFLD. NAFLD/NASH may also develop after gastrointestinal surgery, including pancreaticoduodenectomy and intestinal bypass^[37,38]. Since some genetic diseases, including Wilson's disease, citrin deficiency^[39-41], and cholesteryl ester storage disease^[42], exhibit hepatic steatosis mimicking NAFLD, careful exclusion of these disorders is important. In cirrhotic NASH, serum AST/ALT levels and hepatic TAG accumulation are markedly reduced, such as in burned-out NASH, which may be diagnosed as cryptogenic liver cirrhosis^[7,43]. It should be noted that normal ALT levels cannot exclude the possibility of NASH with advanced fibrosis; the combination of liver function testing with fibrosis markers and/or imaging modalities is indispensable for an accurate diagnosis.

Borderline between non-alcoholic and alcoholic status: The threshold of ethanol consumption amount for differentiating between NAFLD and alcoholic liver disease is problematic because the impact of ethanol on the liver differs among individuals with regards to race, sex, aldehyde dehydrogenase 2 gene polymorphisms, mode of drinking (binging or persistent small amounts), and lifestyle. The precise amount of ethanol intake is also sometimes hard to estimate. Some reports have shown that mild drinking attenuates hepatic steatosis, while in our cohort, NAFLD patients with a mild drinking habit (< 20 g/d) had a higher male prevalence, increased gamma-glutamyl transpeptidase, and more frequent liver cirrhosis^[44]. Furthermore, the occurrence rate of HCC in advanced fibrosis was higher in those patients. More attention is necessary on the impact of mild drinking on NAFLD outcome.

Evaluation of hepatic steatosis using imaging modalities: US is a simple method to detect fatty liver, but the lack of quantitative performance guidelines causes inter-observer differences and complicates the monitoring of fat accumulation changes during interventions^[45,46]. As calculated by non-enhanced abdominal CT liver/spleen Hounsfield unit (HU) and liver HU scores of < 40 or a liver/spleen HU ratio of < 0.9 indicates the presence of hepatic steatosis. Although CT has greater quantitative performance, objectivity, and reproducibility compared with US, it also carries the disadvantages of radiation exposure, cost, HU variability depending devices set-up, and inaccuracy from accompanying iron/copper depositions^[45,46]. Magnetic resonance

imaging (MRI) can quantify hepatic fat accumulation almost perfectly. The correlation coefficient between the area of lipid droplets in liver biopsy sections and the amount of fat estimated by MR is reportedly more than 0.9^[47]. Although such equipment is prohibitively expensive for many primary care clinics and other facilities. The recently introduced Fibroscan® can quantify the degree of hepatic fat accumulation and fibrosis as controlled attenuation parameter (CAP) and E values, respectively. However, CAP values correlated with the area of lipid droplets in liver histology only in NAFLD patients with BMI < 28 kg/m² and none-to-mild fibrosis, suggesting limited applicability outside those parameters^[48]. Further improvements in diagnostic performance are needed, especially for severely obese patients.

Clinical significance of liver biopsy and detection of ballooned hepatocytes: The discrimination of NASH from NAFL and assessment of the histological severity of NAFLD are routinely performed using pathological findings of the liver, but repeated liver biopsy is somewhat invasive, costly, and ultimately unrealistic. Sampling error and inter-rater discrepancies in pathological diagnosis are also problematic^[12,49,50]. In the search for less invasive and more accurate methods to assess NAFLD pathology, several serum biomarkers to detect the presence of ballooned hepatocytes have been evaluated^[51-55]. For instance, cytokeratin 18 (CK18) accumulates in ballooned hepatocytes with Mallory-Denk body-like inclusion bodies. Circulating CK18 fragment concentrations were significantly increased in NASH compared with NAFL and healthy controls and correlated with the incidence of ballooned hepatocytes and histological NAFLD activity score (NAS)^[51].

Multiple studies have emphasized the importance of ballooned hepatocytes in NAFLD/NASH. The need to discriminate NASH from NAFL stems from the notion that the prognosis of the former (steatosis plus ballooned hepatocytes) is poorer than that of the latter (steatosis without ballooned hepatocytes). However, ballooned hepatocytes sometimes disappear and NASH may transform into NAFL and vice versa; some NAFL cases have progressed to liver cirrhosis presumably through NASH. We earlier described a NAFLD patient who underwent careful 27-year follow-up^[7]. The patient was diagnosed as having NAFL at the first liver biopsy, which gradually progressed to cirrhosis and HCC over 20 years. This case teaches us that NAFL is not always benign. Moreover, HCC may develop from NAFL regardless of the absence of advanced fibrosis, past HBV infection, or regular ethanol consumption^[34]. More importantly, recent studies have demonstrated that the presence of advanced fibrosis, but not ballooned hepatocytes, was a determinant of poor prognosis in NAFLD patients^[56,57]. Taken together, it appears that the clinical significance of ballooned hepatocytes has given way to that of fibrosis in NAFLD/NASH.

Evaluation of liver fibrosis: Considering recent trends, the need for less invasive, more accurate methods of assessing liver fibrosis has produced several potential fibrosis indicators. Platelet count and serum levels of hyaluronic acid, type 4 collagen 7S, Mac2-binding protein, and autotaxin are promising biomarkers that predict advanced fibrosis (Table 1)^[58-63]. Although those single indicators are simple, convenient, and useful for busy clinicians, it should be emphasized that results may be influenced by underlying conditions, such as co-existing collagen disease, systemic inflammation, and renal dysfunction. NAFLD fibrosis score, AST-to-platelet ratio index (APRI), FIB-4 index, BARD, CA index, ELF, and FibroTest have also been proposed as indices to predict advanced fibrosis in NAFLD patients (Table 2)^[64-71]. ELF and FibroTest use direct markers of collagen synthesis and degradation, but such measurements are uncommon in clinical situations. In contrast, NAFLD fibrosis score, APRI, and FIB-4 exploit the biochemical test components of age, AST, ALT, glucose, BMI, platelets and albumin, all of which are routinely obtained in clinical practice. However, the scores of these indices tend to be increased in the elderly, and it is also unclear whether changes in AST, ALT, and BMI are correlated with the degree of actual fibrosis. For more global applicability, the cut-off values of single biomarkers and panels will require optimization according to country, race, sex, age, and other factors.

In addition to serum biochemical analysis, repeated quantification of the severity of liver fibrosis using imaging modalities is considered ideal for monitoring NAFLD progression and assessing therapeutic response. MR elastography has a significantly higher diagnostic accuracy than does transient elastography for the detection of fibrosis stage^[72]. Since US is more widespread than MRI, two-dimensional shear wave elastography might compensate for an inability for MR elastography at some institutions.

Treatment

Table 1 Biomarkers predicting \geq F3 fibrosis in Japanese non-alcoholic fatty liver disease patients

| Biomarker | Fibrosis stage | Cut-off value | AUC | Ref. |
|---------------------------------------|----------------|--------------------------------|------|------|
| Platelet count | F4 | $15.3 \times 10^4/\mu\text{L}$ | 0.92 | [58] |
| | F4 | $16 \times 10^4/\mu\text{L}$ | 0.98 | [59] |
| Hyaluronic acid | \geq F3 | 42 ng/mL | 0.97 | [59] |
| Type 4 collagen 7S | \geq F3 | 6.0 ng/mL | 0.88 | [60] |
| Mac2-binding protein | \geq F3 | 2.24 $\mu\text{g/mL}$ | 0.78 | [61] |
| WFA ⁺ Mac2-binding protein | \geq F3 | 0.83 COI | 0.82 | [60] |
| Autotaxin | \geq F3 | 1.23 COI | 0.83 | [62] |
| | \geq F3 | 1.19 mg/L | 0.75 | [63] |
| | F4 | 1.20 mg/L | 0.87 | |

AUC: Area under the receiver operating characteristic curve.

Body weight reduction: Since early-stage NAFLD/NASH is basically resolved by weight loss, lifestyle modifications geared towards weight reduction are routinely prescribed. Vilar-Gomez *et al.*[73] analyzed NASH patients who received repeated biopsy and revealed that weight loss, the absence of diabetes, ALT normalization, young age, and baseline NAS ≤ 5 as independent predictors of NASH resolution without fibrosis worsening after 1-year of lifestyle intervention. Weight loss of 5% and 7%-10% attenuated steatosis and steatohepatitis, respectively[74,75]. However, it is sometimes difficult for diet and exercise regimens to achieve and maintain a 10% weight loss. To facilitate this, close multi-disciplinary cooperation between doctors (gastroenterologists, cardiologists, endocrinologists, *etc.*), nurses, dietitians, and exercise therapists is needed. In cases of morbid obesity with unsuccessful weight reduction, bariatric surgery is a promising option. Although it was documented that bariatric surgery can significantly improve NASH[76], its long-term safety and effectiveness remain under debate.

Pharmacological interventions for underlying disorders: Since NAFLD/NASH is accompanied by dyslipidemia, hyperglycemia, and insulin resistance, the correction of these disorders is beneficial for disease management. The therapeutic agents recommended by the Japan Society of Gastroenterology (JSG) and the Japan Society of Hepatology (JSH) are vitamin E, pioglitazone (for NAFLD/NASH with diabetes), and statin (for NAFLD/NASH with dyslipidemia)[77]. The American Association for the Study of Liver Disease (AASLD) guidelines 2017 have included these substances as well[78]. On the contrary, metformin and ursodeoxycholic acid are not recommended. Vitamin E is a lipid-soluble vitamin that scavenges free radicals to reduce oxidative stress in NAFLD/NASH livers. In PIVENS trials, vitamin E significantly improved NASH histology in non-diabetic and non-cirrhotic adult NASH patients compared with a placebo[79]. However, the safety of long-term, high-dose vitamin E treatment has not been confirmed. A PPAR γ activator, pioglitazone increases circulating adiponectin and attenuates insulin resistance, steatosis, lobular inflammation, and fibrosis in diabetic/pre-diabetic NASH patients (ClinicalTrials.gov Identifier: NCT00994682)[80]. PPAR γ agonist might also reduce HCC prevalence in diabetic patients[81], but fluid retention (edema, heart failure) and osteoporosis were observed as major adverse effects[82]. For all pharmacological agents, the balance of long-term benefits and risks along with improvements to minimize adverse effects remain a constant challenge.

Novel agents under clinical trials: Several promising agents undergoing clinical trials are listed in Table 3. Among them, obeticholic acid, elafibranor, selonsertib, and cenicriviroc are now in phase III trials[83]. It is noteworthy that these trials evaluate not only histological improvement of NASH, but also the benefit of long-term outcome for NASH patients, such as prevention of progression into cirrhosis, hepatic decompensation, and death.

Obeticholic acid is a potent whole-body farnesoid X receptor (FXR) agonist[18]. The drug improved necroinflammation without worsening fibrosis compared with a placebo in the large-scale FLINT trial of NASH patients[84]. At present, an international phase III trial is ongoing (REGENERATE study, NCT02548351). However, obeticholic acid significantly increased blood triglyceride and low-density-lipoprotein-cholesterol levels and decreased high-density-lipoprotein-cholesterol concentrations, which

Table 2 Representative indices predicting \geq F3 fibrosis in non-alcoholic fatty liver disease patients

| Score/Index | Formula | Ref. |
|----------------------|---|------|
| NAFLD fibrosis score | $1.675 + 0.037 \times \text{Age} + 0.094 \times \text{BMI} + 1.13 \times \text{IFG/DM (with = 1, without = 0)} + 0.99 \times \text{AST/ALT} - 0.013 \times \text{PLT} - 0.66 \times \text{Alb}$ | [64] |
| APRI | $[(\text{AST}/\text{upper limit of normal AST})/\text{PLT}] \times 100$ | [65] |
| FIB-4 index | $[\text{Age} \times \text{AST}]/[\text{PLT} \times \text{ALT}^{1/2}]$ | [66] |
| BARD score | BMI ≥ 28 (1 point) AST/ALT ≥ 0.8 (2 points) The presence of DM (1 point) | [67] |
| CA index-fibrosis | $1.5 \times 4\text{C7S} + 0.0264 \times \text{AST}$ | [68] |
| ELF score | $2.494 + \ln(\text{hyaluronic acid}) + \ln(\text{P-III-P}) + \ln(\text{TIMP-1})$ | [69] |

BMI: Body mass index; IFG: Impaired fasting glucose; DM: Diabetes mellitus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; PLT: Platelet count; Alb: Albumin; 4C7S: Type 4 collagen 7S; P-III-P: Procollagen type III amino-terminal peptide; TIMP-1: Tissue inhibitor of metalloproteinase-1; NAFLD: Non-alcoholic fatty liver disease.

might raise the risk of cardiovascular diseases.

Elafibranor (GFT-505) is a PPAR α/δ dual agonist. While PPAR α activation attenuates hepatic steatosis and inflammation, PPAR δ stimulation can ameliorate hepatic inflammation and fibrosis^[18]. In the GOLDEN-505 trial, 120 mg elafibranor resolved NASH and improved liver enzymes, glucose, and lipid profiles in larger proportions of NASH patients with NAS ≥ 4 compared with a placebo. Patients with NASH resolution after receiving the drug exhibited lower liver fibrosis stages compared with those without resolution^[85]. A phase III trial verifying the effect of 120 mg elafibranor is underway for NASH patients with NAS ≥ 4 and stage 2/3 fibrosis (RESOLVE-IT study, NCT02704403).

Selonsertib (GS-4997) is an apoptosis signal-regulating kinase 1 (ASK1) inhibitor. ASK1 is activated by various stimuli, including hyperglycemia, transforming growth factor- β , and oxidative stimulus to induce apoptosis and fibrosis through p38 and JNK. Up-regulated ASK1-JNK1 axis aggravates insulin resistance, steatosis, and inflammation and further activates ASK1, resulting in a vicious cycle. In an animal NASH model, ASK1 inhibition reduced body weight along with hepatic fat and fibrosis and improved insulin resistance^[86]. In a phase II study, selonsertib ameliorated NASH activity and fibrosis^[87]. An international phase III trial is ongoing for stage 3 fibrosis and cirrhotic NASH patients (STELLAR3 study, NCT03053050 and STELLAR4 study, NCT03053063, respectively).

Lastly, cenicriviroc[®] is a C-C motif chemokine receptor (CCR) 2/5 antagonist. In a phase IIb trial (CENTAUR study), the agent attenuated liver fibrosis without worsening NASH compared with a placebo^[88]. Currently, a phase III clinical trial evaluating the effect of cenicriviroc[®] for NASH patients with stage 2/3 fibrosis is underway (AURORA study, NCT03028740). Other clinical trials are listed in [Table 3](#)^[89-93].

PROMBLEMS AND PERSPECTIVES IN NAFLD/NASH RESEARCH

Despite the dramatic gains in detection and treatment, there remain many unsolved issues in the field of NAFLD/NASH.

Pathogenesis

How animal data apply to humans? The pathogenesis of NASH/NAFLD is multifactorial and complicated. Still, reducing intracellular FA and free cholesterol while correcting obesity and insulin resistance may be fundamentally beneficial across species to attenuate NAFLD/NASH. Since NASH is both a metabolic disease and an inflammatory condition, strategies to inhibit inflammatory signaling and reduce oxidative and ER stress will be useful. However, lipid metabolism and immune mechanisms differ between rodents and humans, so any application of murine findings needs caution. Animal models that can precisely reproduce the human NASH condition are desired.

How do organs and cells crosstalk in NAFLD/NASH? Crosstalk among normal/steatotic hepatocytes, immune cells, HSCs and the organ network is an

Table 3 Novel therapeutic agents for non-alcoholic fatty liver disease/ non-alcoholic steatohepatitis under clinical trials

| Target | Agent | Action | Clinical trial |
|------------------------------|------------------|--|----------------|
| Diabetes, insulin resistance | Semaglutide | DPP4 inhibitor | Phase II |
| | Gliflozin | SGLT2 inhibitor ^[89] | Pilot |
| | BMS-986036 | Recombinant FGF21 ^[90] | Phase II |
| Dyslipidemia | Aramchol | SCD inhibitor | Phase II |
| | GS-0976 | ACC inhibitor | Phase II |
| Nuclear receptor | Obeticholic acid | FXR agonist ^[84] | Phase III |
| | Elafibranor | PPAR α / δ agonist ^[85] | Phase III |
| | MGL-3196 | TR β agonist | Phase II |
| Apoptosis | Emricasan | Pan-caspase inhibitor ^[91] | Phase II |
| Inflammation, fibrosis | Selonsertib | ASK1 inhibitor ^[87] | Phase III |
| | Cenicriviroc | CCR2/5 antagonist ^[88] | Phase III |
| | JKB-121 | TLR4 antagonist | Phase II |
| | GR-MD-02 | Galectin 3 inhibitor ^[92] | Phase II |
| | ND-LO2-s0201 | HSP47 siRNA ^[93] | Phase I |

intriguing theme in the context of NAFLD/NASH pathogenesis. Especially, contribution of gut-liver axis and microbiota to NASH development is drawing much attention (Table 4)^[94-96]. Although the merits of direct manipulation of the intestinal microbiota with antibiotics, prebiotics, or probiotics are debatable for human NAFLD/NASH due to the sheer diversity of microbiota, modulation of the gut-liver axis to target microbiota-derived metabolites is considered an attractive option. For instance, microbiota-derived deoxycholate evoked senescence-associated secretory phenotypes in HSCs and facilitated HCC development in hepatocarcinogen-primed genetically obese or high fat diet-fed mice^[97]. Microbiota-derived taurocholate stimulated ceramide synthesis in enterocytes through FXR in mice, after which circulating ceramide promotes hepatic steatosis^[28]. However, the applicability of these mechanisms to humans remains unclear. Recently, it was reported that microbiota-derived short-chain fatty acids lowered resting regulatory T-cells and associated with systemic T-cell activation in humans^[96]. As such, discovering the pathogenic molecules that mediate organ/cell crosstalk and contribute to NAFLD/NASH development in humans will provide much needed insights into disease management^[31,98].

What should we learn from human genome analysis? Although a genome-wide association study clarified the genetic predisposition of NAFLD/NASH, the mechanism and functional changes by gene mutation/polymorphism require more precise assessment in genetically modified animals and human cells. Research is underway on the key genes involved in the development of NAFLD-related fibrosis and HCC.

Diagnosis

What is the simplest and most accurate surrogate marker of liver pathology in NAFLD/NASH? At present, NASH improvement is defined as the reduction of NAS and fibrosis stage as well as of scores for steatosis, ballooning, and lobular inflammation compared with baseline liver histology. There are several shortcomings to assessing NAFLD/NASH activity by liver biopsy only, the biggest of which is the impracticality of multiple procedures during follow-up. MRI can accurately quantify liver fat and stiffness, but is limited to large hospitals. Therefore, surrogate biomarkers that closely reflect liver pathology are needed for clinical trials and eventual adoption in monitoring clinical course and therapeutic response in real-world clinical situations. Moreover, the clinical significance of the conventional and widely-used biomarkers AST and ALT are in need of reconsideration in NAFLD/NASH.

How should we detect early-stage NAFLD/NASH? With the development of modern imaging modalities and biomarkers, it has become easier to detect advanced fibrosis stage of NAFLD/NASH. New and more powerful anti-fibrosis agents are on the horizon, but complete reversion from a cirrhotic liver to a soft one may prove difficult. Thus, strategies to detect early-stage NASH with mild-to-moderate fibrosis and

Table 4 Dysbiosis in human non-alcoholic fatty liver disease/ non-alcoholic steatohepatitis

| Ref. | Patients | Changes in microbiota | Related phenotypic changes |
|---------------------------------------|--|---|---|
| Boursier <i>et al</i> ^[94] | F0/1 NAFLD 30 (NASH 10), ≥ F2 NAFLD 27 (NASH 25) | <i>Bacteroides</i> ↑ in NASH, <i>Ruminococcus</i> ↑ in ≥ F2 | Not assessed |
| Da Silva <i>et al</i> ^[95] | NAFLD 39 (NASH 24, NAFL 15); healthy control 28 | <i>Lactobacillus</i> ↑, <i>Lactobacillaceae</i> ↑, <i>Bacteroidetes</i> ↓, <i>Firmicutes</i> ↓, <i>Ruminococcus</i> ↓, <i>Faecalibacterium prausnitzii</i> ↓, <i>Coprococcus</i> ↓ in NAFLD compared to control; no differences between NASH and NAFL | Fecal propionate↑, isobutyric acid↑, Serum 2-hydroxy-butyrate↑, L-lactate↑ in NAFLD |
| Rau <i>et al</i> ^[96] | NAFLD 32 (NASH 18, NAFL 14); healthy control 27 | <i>Fusobacteria</i> ↑, <i>Fusobacteriaceae</i> ↑ in NASH compared to NAFL and control | Fecal propionate↑, acetate↑, Treg↓ in NASH |

NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

prevent fibrosis progression are required as well, leading to preemptive and precise medicine.

Who diagnoses and follows NAFLD/NASH? NAFLD/NASH has become a common liver disease worldwide. Many NAFLD/NASH patients are followed by clinicians other than hepatologists, such as by cardiologists, endocrinologists, and primary care doctors. Therefore, hepatologists should aim to establish clear and simple guidelines on strategies to find, diagnose, follow/assess, and treat NAFLD/NASH for non-specialists.

Treatment

What is the goal of NAFLD/NASH treatment? The ultimate goal in NAFLD/NASH is the extension of overall survival and improvement of quality of life. Since the disease is frequently accompanied by obesity, atherosclerosis, and diabetes, not only hepatic complications (HCC, portal hypertension, and liver decompensation), but also extrahepatic conditions (cerebrocardiovascular disease, renal failure, and cancer) should be considered. Therapies will require careful adjustment according to underlying risk and NAFLD/NASH stage, and multi-disciplinary cooperation among caregivers will be key. Indicators of adequate disease control are currently lacking in NAFLD/NASH. In diabetic patients, the correction of hemoglobin A1c values prevents diabetic complications and improves outcome. Hepatologists require more of such indicators of NAFLD/NASH control^[99].

Who should be treated intensively? It is important that NAFLD/NASH patients with advanced fibrosis should be intensively followed and treated due to the high risk of liver failure and HCC. The speed of fibrosis progression may differ among NAFLD/NASH patients, even in the early stage of fibrosis. Finding clinical determinants to detect rapid fibrosis candidates and high-risk HCC group at fibrosis stage 0-1 NAFLD/NASH will enable clinicians to better identify patients requiring careful monitoring and early intensive treatment^[100-102].

How is the efficacy of pharmacotherapies improving? Although several new agents will be available in a near future^[83,103-105], the efficacy of any single drug may vary widely for each patient. NAFLD/NASH is a complex syndrome, and its main pathogenesis likely differs among individuals; for example, hepatic lipid metabolism might depend on the underlying diseases (diabetes *vs* hypercholesterolemia) or fibrosis stage (stage 0-1 *vs* stage 3-4)^[106]. Accordingly, patient stratification and careful selection of therapies, such as dual/triple agonists and combinations of several agents, may improve efficacy. In addition, enhancing target tissue/cell specificity (*e.g.*, adipose-specific PPAR γ activators and intestine-specific FXR agonists/antagonists) will help achieve higher efficacy and reduced adverse effects^[18].

CONCLUSION

Recent trends in diet and lifestyle have increased the prevalence of NAFLD/NASH worldwide. Although advances in non-invasive biomarkers and imaging modalities have improved disease detection and follow-up, considerable work is needed to identify individuals with low fibrosis stages or at risk of rapid disease progression. In the future, earlier detection will enable prompt single or combination treatment with

new-line drugs that have been optimized for maximum benefit and fewer adverse events. Only with a concerted effort across multi-disciplinary fields can clinicians begin to halt the rapid spread of NAFLD/NASH.

ACKNOWLEDGMENTS

The authors thank the following collaborators for their invaluable helps, advice, instruction, and encouragement throughout our fatty liver disease studies: Dr. Kenji Sano, Dr. Wataru Okiyama, Dr. Goro Tsuruta, Dr. Hiroyuki Kitabatake, Prof. Masahide Yazaki, Dr. Yasunari Fujinaga, Dr. Akira Kobayashi, Dr. Takahiro Yamaura, Dr. Ayumi Sugiura, Dr. Tomoo Yamazaki, Dr. Satoru Joshita, Dr. Takeji Umemura, Dr. Tetsuya Ichijo, Dr. Akihiro Matsumoto, Dr. Kaname Yoshizawa, and Emeritus Prof. Kendo Kiyosawa (Shinshu University School of Medicine); Dr. Akira Horiuchi (Showa Inan General Hospital); Dr. Makoto Nakamuta (Kyushu Medical Center); Dr. Frank J. Gonzalez (National Institutes of Health); and Prof. Etsuko Hashimoto (Tokyo Women's Medical University). The authors also appreciate Mr. Trevor Ralph for his English editorial assistance.

REFERENCES

- 1 Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, Colombo M, Craxi A, Crespo J, Day CP, Eguchi Y, Geier A, Kondili LA, Kroy DC, Lazarus JV, Loomba R, Manns MP, Marchesini G, Nakajima A, Negro F, Petta S, Ratziu V, Romero-Gomez M, Sanyal A, Schattenberg JM, Tacke F, Tanaka J, Trautwein C, Wei L, Zeuzem S, Razavi H. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. *J Hepatol* 2018; **69**: 896-904 [PMID: 29886156 DOI: 10.1016/j.jhep.2018.05.036]
- 2 Perumpail BJ, Khan MA, Yoo ER, Cholaneril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol* 2017; **23**: 8263-8276 [PMID: 29307986 DOI: 10.3748/wjg.v23.i47.8263]
- 3 Eguchi Y, Hyogo H, Ono M, Mizuta T, Ono N, Fujimoto K, Chayama K, Saibara T; JSG-NAFLD. Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *J Gastroenterol* 2012; **47**: 586-595 [PMID: 22328022 DOI: 10.1007/s00535-012-0533-z]
- 4 Zhu JZ, Zhou QY, Wang YM, Dai YN, Zhu J, Yu CH, Li YM. Prevalence of fatty liver disease and the economy in China: A systematic review. *World J Gastroenterol* 2015; **21**: 5695-5706 [PMID: 25987797 DOI: 10.3748/wjg.v21.i18.5695]
- 5 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]
- 6 Marrero JA, Fontana RJ, Su GL, Conjeevaram HS, Emick DM, Lok AS. NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology* 2002; **36**: 1349-1354 [PMID: 12447858 DOI: 10.1053/jhep.2002.36939]
- 7 Nagaya T, Tanaka N, Komatsu M, Ichijo T, Sano K, Horiuchi A, Joshita S, Umemura T, Matsumoto A, Yoshizawa K, Aoyama T, Kiyosawa K, Tanaka E. Development from simple steatosis to liver cirrhosis and hepatocellular carcinoma: a 27-year follow-up case. *Clin J Gastroenterol* 2008; **1**: 116-121 [PMID: 26193649 DOI: 10.1007/s12328-008-0017-0]
- 8 Yasui K, Hashimoto E, Komorizono Y, Koike K, Arai S, Imai Y, Shima T, Kanbara Y, Saibara T, Mori T, Kawata S, Uto H, Takami S, Sumida Y, Takamura T, Kawanaka M, Okanoue T; Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan/Japan NASH Study Group, Ministry of Health, Labour, and Welfare of Japan. Characteristics of patients with nonalcoholic steatohepatitis who develop hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2011; **9**: 428-433; quiz e50 [PMID: 21320639 DOI: 10.1016/j.cgh.2011.01.023]
- 9 Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; **332**: 1519-1523 [PMID: 21700865 DOI: 10.1126/science.1204265]
- 10 Watanabe S, Hashimoto E, Ikejima K, Uto H, Ono M, Sumida Y, Seike M, Takei Y, Takehara T, Tokushige K, Nakajima A, Yoneda M, Saibara T, Shiota G, Sakaida I, Nakamuta M, Mizuta T, Tsubouchi H, Sugano K, Shimosegawa T. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatol Res* 2015; **45**: 363-377 [PMID: 25832328 DOI: 10.1111/hepr.12511]
- 11 Hashimoto E, Tokushige K, Ludwig J. Diagnosis and classification of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Current concepts and remaining challenges. *Hepatol Res* 2015; **45**: 20-28 [PMID: 24661406 DOI: 10.1111/hepr.12333]
- 12 Sakamoto M, Tsujikawa H, Effendi K, Ojima H, Harada K, Zen Y, Kondo F, Nakano M, Kage M, Sumida Y, Hashimoto E, Yamada G, Okanoue T, Koike K. Pathological findings of nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. *Pathol Int* 2017; **67**: 1-7 [PMID: 27995687 DOI: 10.1111/pin.12485]
- 13 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]
- 14 Heianza Y, Arase Y, Tsuji H, Fujihara K, Saito K, Hsieh SD, Tanaka S, Kodama S, Hara S, Sone H. Metabolically healthy obesity, presence or absence of fatty liver, and risk of type 2 diabetes in Japanese individuals: Toranomon Hospital Health Management Center Study 20 (TOPICS 20). *J Clin Endocrinol Metab* 2014; **99**: 2952-2960 [PMID: 24823457 DOI: 10.1210/jc.2013-4427]
- 15 Tsuruta G, Tanaka N, Hongo M, Komatsu M, Horiuchi A, Hamamoto K, Iguchi C, Nakayama Y,

- Umamura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E. Nonalcoholic fatty liver disease in Japanese junior high school students: its prevalence and relationship to lifestyle habits. *J Gastroenterol* 2010; **45**: 666-672 [PMID: [20084525](#) DOI: [10.1007/s00535-009-0198-4](#)]
- 16 **Berentzen TL**, Gamborg M, Holst C, Sørensen TI, Baker JL. Body mass index in childhood and adult risk of primary liver cancer. *J Hepatol* 2014; **60**: 325-330 [PMID: [24076363](#) DOI: [10.1016/j.jhep.2013.09.015](#)]
- 17 **Buzzetti E**, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 2016; **65**: 1038-1048 [PMID: [26823198](#) DOI: [10.1016/j.metabol.2015.12.012](#)]
- 18 **Tanaka N**, Aoyama T, Kimura S, Gonzalez FJ. Targeting nuclear receptors for the treatment of fatty liver disease. *Pharmacol Ther* 2017; **179**: 142-157 [PMID: [28546081](#) DOI: [10.1016/j.pharmthera.2017.05.011](#)]
- 19 **Matsusue K**, Kusakabe T, Noguchi T, Takiguchi S, Suzuki T, Yamano S, Gonzalez FJ. Hepatic steatosis in leptin-deficient mice is promoted by the PPARgamma target gene Fsp27. *Cell Metab* 2008; **7**: 302-311 [PMID: [18396136](#) DOI: [10.1016/j.cmet.2008.03.003](#)]
- 20 **Donnelly KL**, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1343-1351 [PMID: [15864352](#) DOI: [10.1172/JCI23621](#)]
- 21 **Tanaka N**, Takahashi S, Matsubara T, Jiang C, Sakamoto W, Chanturiya T, Teng R, Gavrilova O, Gonzalez FJ. Adipocyte-specific disruption of fat-specific protein 27 causes hepatosteatosis and insulin resistance in high-fat diet-fed mice. *J Biol Chem* 2015; **290**: 3092-3105 [PMID: [25477509](#) DOI: [10.1074/jbc.M114.605980](#)]
- 22 **Tanaka N**, Takahashi S, Fang ZZ, Matsubara T, Krausz KW, Qu A, Gonzalez FJ. Role of white adipose lipolysis in the development of NASH induced by methionine- and choline-deficient diet. *Biochim Biophys Acta* 2014; **1841**: 1596-1607 [PMID: [25178843](#) DOI: [10.1016/j.bbali.2014.08.015](#)]
- 23 **Safar Zadeh E**, Lungu AO, Cochran EK, Brown RJ, Ghany MG, Heller T, Kleiner DE, Gorden P. The liver diseases of lipodystrophy: the long-term effect of leptin treatment. *J Hepatol* 2013; **59**: 131-137 [PMID: [23439261](#) DOI: [10.1016/j.jhep.2013.02.007](#)]
- 24 **Rubio-Cabezas O**, Puri V, Murano I, Saudek V, Semple RK, Dash S, Hyden CS, Bottomley W, Vigouroux C, Magré J, Raymond-Barker P, Murgatroyd PR, Chawla A, Skepper JN, Chatterjee VK, Suliman S, Patch AM, Agarwal AK, Garg A, Barroso I, Cinti S, Czech MP, Argente J, O'Rahilly S, Savage DB; LD Screening Consortium. Partial lipodystrophy and insulin resistant diabetes in a patient with a homozygous nonsense mutation in CIDEC. *EMBO Mol Med* 2009; **1**: 280-287 [PMID: [20049731](#) DOI: [10.1002/emmm.200900037](#)]
- 25 **Tanaka N**, Takahashi S, Zhang Y, Krausz KW, Smith PB, Patterson AD, Gonzalez FJ. Role of fibroblast growth factor 21 in the early stage of NASH induced by methionine- and choline-deficient diet. *Biochim Biophys Acta* 2015; **1852**: 1242-1252 [PMID: [25736301](#) DOI: [10.1016/j.bbadi.2015.02.012](#)]
- 26 **Tanaka N**, Takahashi S, Hu X, Lu Y, Fujimori N, Golla S, Fang ZZ, Aoyama T, Krausz KW, Gonzalez FJ. Growth arrest and DNA damage-inducible 45α protects against nonalcoholic steatohepatitis induced by methionine- and choline-deficient diet. *Biochim Biophys Acta Mol Basis Dis* 2017; **1863**: 3170-3182 [PMID: [28844958](#) DOI: [10.1016/j.bbadi.2017.08.017](#)]
- 27 **Akazawa Y**, Nakao K. To die or not to die: death signaling in nonalcoholic fatty liver disease. *J Gastroenterol* 2018; **53**: 893-906 [PMID: [29574534](#) DOI: [10.1007/s00535-018-1451-5](#)]
- 28 **Jiang C**, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, Cai J, Qi Y, Fang ZZ, Takahashi S, Tanaka N, Desai D, Amin SG, Albert I, Patterson AD, Gonzalez FJ. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest* 2015; **125**: 386-402 [PMID: [25500885](#) DOI: [10.1172/JCI76738](#)]
- 29 **Gan LT**, Van Rooyen DM, Koina ME, McCuskey RS, Teoh NC, Farrell GC. Hepatocyte free cholesterol lipotoxicity results from JNK1-mediated mitochondrial injury and is HMGB1 and TLR4-dependent. *J Hepatol* 2014; **61**: 1376-1384 [PMID: [25064435](#) DOI: [10.1016/j.jhep.2014.07.024](#)]
- 30 **Zhang L**, Nichols RG, Correll J, Murray IA, Tanaka N, Smith PB, Hubbard TD, Sebastian A, Albert I, Hatzakis E, Gonzalez FJ, Perdew GH, Patterson AD. Persistent Organic Pollutants Modify Gut Microbiota-Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation. *Environ Health Perspect* 2015; **123**: 679-688 [PMID: [25768209](#) DOI: [10.1289/ehp.1409055](#)]
- 31 **Chu H**, Duan Y, Yang L, Schnabl B. Small metabolites, possible big changes: a microbiota-centered view of non-alcoholic fatty liver disease. *Gut* 2018 [PMID: [30171065](#) DOI: [10.1136/gutjnl-2018-316307](#)]
- 32 **Lee YA**, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. *Gut* 2015; **64**: 830-841 [PMID: [25681399](#) DOI: [10.1136/gutjnl-2014-306842](#)]
- 33 **Saitta C**, Tripodi G, Barbera A, Bertuccio A, Smedile A, Ciancio A, Raffa G, Sangiovanni A, Navarra G, Raimondo G, Pollicino T. Hepatitis B virus (HBV) DNA integration in patients with occult HBV infection and hepatocellular carcinoma. *Liver Int* 2015; **35**: 2311-2317 [PMID: [25677098](#) DOI: [10.1111/liv.12807](#)]
- 34 **Kimura T**, Kobayashi A, Tanaka N, Sano K, Komatsu M, Fujimori N, Yamazaki T, Shibata S, Ichikawa Y, Joshita S, Umamura T, Matsumoto A, Horiuchi A, Mori H, Wada S, Kiyosawa K, Miyagawa SI, Tanaka E. Clinicopathological characteristics of non-B non-C hepatocellular carcinoma without past hepatitis B virus infection. *Hepatol Res* 2017; **47**: 405-418 [PMID: [27288988](#) DOI: [10.1111/hepr.12762](#)]
- 35 **Wong CR**, Nguyen MH, Lim JK. Hepatocellular carcinoma in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2016; **22**: 8294-8303 [PMID: [27729736](#) DOI: [10.3748/wjg.v22.i37.8294](#)]
- 36 **Younes R**, Bugianesi E. Should we undertake surveillance for HCC in patients with NAFLD? *J Hepatol* 2018; **68**: 326-334 [PMID: [29122695](#) DOI: [10.1016/j.jhep.2017.10.006](#)]
- 37 **Tanaka N**, Horiuchi A, Yokoyama T, Kaneko G, Horigome N, Yamaura T, Nagaya T, Komatsu M, Sano K, Miyagawa S, Aoyama T, Tanaka E. Clinical characteristics of de novo nonalcoholic fatty liver disease following pancreaticoduodenectomy. *J Gastroenterol* 2011; **46**: 758-768 [PMID: [21267748](#) DOI: [10.1007/s00535-011-0370-5](#)]
- 38 **Nagaya T**, Tanaka N, Kimura T, Kitabatake H, Fujimori N, Komatsu M, Horiuchi A, Yamaura T,

- Umemura T, Sano K, Gonzalez FJ, Aoyama T, Tanaka E. Mechanism of the development of nonalcoholic steatohepatitis after pancreaticoduodenectomy. *BBA Clin* 2015; **3**: 168-174 [PMID: 26674248 DOI: 10.1016/j.bbacli.2015.02.001]
- 39 **Tanaka N**, Yazaki M, Kobayashi K. A lean man with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2007; **5**: A32 [PMID: 16901765 DOI: 10.1016/j.cgh.2006.06.014]
- 40 **Komatsu M**, Yazaki M, Tanaka N, Sano K, Hashimoto E, Takei Y, Song YZ, Tanaka E, Kiyosawa K, Saheki T, Aoyama T, Kobayashi K. Citrin deficiency as a cause of chronic liver disorder mimicking non-alcoholic fatty liver disease. *J Hepatol* 2008; **49**: 810-820 [PMID: 18620775 DOI: 10.1016/j.jhep.2008.05.016]
- 41 **Komatsu M**, Kimura T, Yazaki M, Tanaka N, Yang Y, Nakajima T, Horiuchi A, Fang ZZ, Joshita S, Matsumoto A, Umemura T, Tanaka E, Gonzalez FJ, Ikeda S, Aoyama T. Steatogenesis in adult-onset type II citrullinemia is associated with down-regulation of PPAR α . *Biochim Biophys Acta* 2015; **1852**: 473-481 [PMID: 25533124 DOI: 10.1016/j.bbadis.2014.12.011]
- 42 **Pericleous M**, Kelly C, Wang T, Livingstone C, Ala A. Wolman's disease and cholesteryl ester storage disorder: the phenotypic spectrum of lysosomal acid lipase deficiency. *Lancet Gastroenterol Hepatol* 2017; **2**: 670-679 [PMID: 28786388 DOI: 10.1016/S2468-1253(17)30052-3]
- 43 **Tanaka N**, Tanaka E, Sheena Y, Komatsu M, Okiyama W, Misawa N, Muto H, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Horiuchi A, Kiyosawa K. Useful parameters for distinguishing nonalcoholic steatohepatitis with mild steatosis from cryptogenic chronic hepatitis in the Japanese population. *Liver Int* 2006; **26**: 956-963 [PMID: 16953836 DOI: 10.1111/j.1478-3231.2006.01338.x]
- 44 **Kimura T**, Tanaka N, Fujimori N, Sugiura A, Yamazaki T, Joshita S, Komatsu M, Umemura T, Matsumoto A, Tanaka E. Mild drinking habit is a risk factor for hepatocarcinogenesis in non-alcoholic fatty liver disease with advanced fibrosis. *World J Gastroenterol* 2018; **24**: 1440-1450 [PMID: 29632425 DOI: 10.3748/wjg.v24.i13.1440]
- 45 **Li Q**, Dhyani M, Grajo JR, Sirlin C, Samir AE. Current status of imaging in nonalcoholic fatty liver disease. *World J Hepatol* 2018; **10**: 530-542 [PMID: 30190781 DOI: 10.4254/wjgh.v10.i8.530]
- 46 **Zhang YN**, Fowler KJ, Hamilton G, Cui JY, Sy EZ, Balanay M, Hooker JC, Szevenyeni N, Sirlin CB. Liver fat imaging-a clinical overview of ultrasound, CT, and MR imaging. *Br J Radiol* 2018; **91**: 20170959 [PMID: 29722568 DOI: 10.1259/bjr.20170959]
- 47 **Hatta T**, Fujinaga Y, Kadoya M, Ueda H, Murayama H, Kurozumi M, Ueda K, Komatsu M, Nagaya T, Joshita S, Kodama R, Tanaka E, Uehara T, Sano K, Tanaka N. Accurate and simple method for quantification of hepatic fat content using magnetic resonance imaging: a prospective study in biopsy-proven nonalcoholic fatty liver disease. *J Gastroenterol* 2010; **45**: 1263-1271 [PMID: 20625773 DOI: 10.1007/s00535-010-0277-6]
- 48 **Fujimori N**, Tanaka N, Shibata S, Sano K, Yamazaki T, Sekiguchi T, Kitabatake H, Ichikawa Y, Kimura T, Komatsu M, Umemura T, Matsumoto A, Tanaka E. Controlled attenuation parameter is correlated with actual hepatic fat content in patients with non-alcoholic fatty liver disease with none-to-mild obesity and liver fibrosis. *Hepatol Res* 2016; **46**: 1019-1027 [PMID: 27183219 DOI: 10.1111/hepr.12649]
- 49 **Tanaka N**, Ichijo T, Okiyama W, Mutou H, Misawa N, Matsumoto A, Yoshizawa K, Tanaka E, Kiyosawa K. Laparoscopic findings in patients with nonalcoholic steatohepatitis. *Liver Int* 2006; **26**: 32-38 [PMID: 16420508 DOI: 10.1111/j.1478-3231.2005.01198.x]
- 50 **Brunt EM**. Nonalcoholic fatty liver disease and the ongoing role of liver biopsy evaluation. *Hepatol Commun* 2017; **1**: 370-378 [PMID: 29404465 DOI: 10.1002/hep4.1055]
- 51 **Tsutsui M**, Tanaka N, Kawakubo M, Sheena Y, Horiuchi A, Komatsu M, Nagaya T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E, Sano K. Serum fragmented cytokeratin 18 levels reflect the histologic activity score of nonalcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. *J Clin Gastroenterol* 2010; **44**: 440-447 [PMID: 20104187 DOI: 10.1097/MCG.0b013e3181bdefe2]
- 52 **Tanaka N**, Matsubara T, Krausz KW, Patterson AD, Gonzalez FJ. Disruption of phospholipid and bile acid homeostasis in mice with nonalcoholic steatohepatitis. *Hepatology* 2012; **56**: 118-129 [PMID: 22290395 DOI: 10.1002/hep.25630]
- 53 **Matsubara T**, Tanaka N, Krausz KW, Manna SK, Kang DW, Anderson ER, Luecke H, Patterson AD, Shah YM, Gonzalez FJ. Metabolomics identifies an inflammatory cascade involved in dioxin- and diet-induced steatohepatitis. *Cell Metab* 2012; **16**: 634-644 [PMID: 23140643 DOI: 10.1016/j.cmet.2012.10.006]
- 54 **Kitabatake H**, Tanaka N, Fujimori N, Komatsu M, Okubo A, Kakegawa K, Kimura T, Sugiura A, Yamazaki T, Shibata S, Ichikawa Y, Joshita S, Umemura T, Matsumoto A, Koinuma M, Sano K, Aoyama T, Tanaka E. Association between endotoxemia and histological features of nonalcoholic fatty liver disease. *World J Gastroenterol* 2017; **23**: 712-722 [PMID: 28216979 DOI: 10.3748/wjg.v23.i4.712]
- 55 **Enomoto H**, Bando Y, Nakamura H, Nishiguchi S, Koga M. Liver fibrosis markers of nonalcoholic steatohepatitis. *World J Gastroenterol* 2015; **21**: 7427-7435 [PMID: 26139988 DOI: 10.3748/wjg.v21.i24.7427]
- 56 **Angulo P**, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Keach JC, Lafferty HD, Stahler A, Haflidadottir S, Bendtsen F. Liver Fibrosis, but No Other Histologic Features, Is Associated With Long-term Outcomes of Patients With Nonalcoholic Fatty Liver Disease. *Gastroenterology* 2015; **149**: 389-397.e10 [PMID: 25935633 DOI: 10.1053/j.gastro.2015.04.043]
- 57 **Loomba R**, Chalasani N. The Hierarchical Model of NAFLD: Prognostic Significance of Histologic Features in NASH. *Gastroenterology* 2015; **149**: 278-281 [PMID: 26116800 DOI: 10.1053/j.gastro.2015.06.016]
- 58 **Yoneda M**, Fujii H, Sumida Y, Hyogo H, Itoh Y, Ono M, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Imajo K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T; Japan Study Group of Nonalcoholic Fatty Liver Disease/Japan Study Group of Nonalcoholic Fatty Liver Disease. Platelet count for predicting fibrosis in nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 1300-1306 [PMID: 21750883 DOI: 10.1007/s00535-011-0436-4]
- 59 **Kaneda H**, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. *J Gastroenterol Hepatol* 2006; **21**: 1459-1465 [PMID: 16911693 DOI: 10.1016/j.jhep.2006.06.014]

- 10.1111/j.1440-1746.2006.04447.x]
- 60 **Ogawa Y**, Honda Y, Kessoku T, Tomeno W, Imajo K, Yoneda M, Kawanaka M, Kirikoshi H, Ono M, Taguri M, Saito S, Yamanaka T, Wada K, Nakajima A. Wisteria floribunda agglutinin-positive Mac-2-binding protein and type 4 collagen 7S: useful markers for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2018; **33**: 1795-1803 [PMID: 29633352 DOI: 10.1111/jgh.14156]
 - 61 **Kamada T**, Ono M, Hyogo H, Fujii H, Sumida Y, Yamada M, Mori K, Tanaka S, Maekawa T, Ebisutani Y, Yamamoto A, Takamatsu S, Yoneda M, Kawada N, Chayama K, Saibara T, Takehara T, Miyoshi E; Japan Study Group of Nonalcoholic Fatty Liver Disease (JSGNAFLD)/Japan Study Group of Nonalcoholic Fatty Liver Disease (JSGNAFLD). Use of Mac-2 binding protein as a biomarker for nonalcoholic fatty liver disease diagnosis. *Hepatol Commun* 2017; **1**: 780-791 [PMID: 29404494 DOI: 10.1002/hep4.1080]
 - 62 **Atsukawa M**, Tsubota A, Okubo T, Arai T, Nakagawa A, Itokawa N, Kondo C, Kato K, Hatori T, Hano H, Oikawa T, Emoto N, Abe M, Kage M, Iwakiri K. Serum Wisteria floribunda agglutinin-positive Mac-2 binding protein more reliably distinguishes liver fibrosis stages in non-alcoholic fatty liver disease than serum Mac-2 binding protein. *Hepatol Res* 2018; **48**: 424-432 [PMID: 29274190 DOI: 10.1111/hepr.13046]
 - 63 **Fujimori N**, Umemura T, Kimura T, Tanaka N, Sugiura A, Yamazaki T, Joshita S, Komatsu M, Usami Y, Sano K, Igarashi K, Matsumoto A, Tanaka E. Serum autotaxin levels are correlated with hepatic fibrosis and ballooning in patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2018; **24**: 1239-1249 [PMID: 29568204 DOI: 10.3748/wjg.v24.i11.1239]
 - 64 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Sakkena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Thorneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]
 - 65 **Peleg N**, Issachar A, Sneha-Arbib O, Shlomai A. AST to Platelet Ratio Index and fibrosis 4 calculator scores for non-invasive assessment of hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Dig Liver Dis* 2017; **49**: 1133-1138 [PMID: 28572039 DOI: 10.1016/j.dld.2017.05.002]
 - 66 **Sumida Y**, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Fujita K, Chayama K, Saibara T, Kawada N, Fujimoto K, Kohgo Y, Yoshikawa T, Okanoue T; Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD)/Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD). Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012; **12**: 2 [PMID: 2221544 DOI: 10.1186/1471-230X-12-2]
 - 67 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]
 - 68 **Okanoue T**, Ebise H, Kai T, Mizuno M, Shima T, Ichihara J, Aoki M. A simple scoring system using type IV collagen 7S and aspartate aminotransferase for diagnosing nonalcoholic steatohepatitis and related fibrosis. *J Gastroenterol* 2018; **53**: 129-139 [PMID: 28589339 DOI: 10.1007/s00535-017-1355-9]
 - 69 **Guha IN**, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, Kaye P, Burt AD, Ryder SD, Aithal GP, Day CP, Rosenberg WM. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: Validating the European Liver Fibrosis Panel and exploring simple markers. *Hepatology* 2008; **47**: 455-460 [PMID: 18038452 DOI: 10.1002/hep.21984]
 - 70 **Ratzliff V**, Massard J, Charlotte F, Messous D, Imbert-Bismut F, Bonyhay L, Tahiri M, Munteanu M, Thabut D, Cadranet JF, Le Bail B, de Ledinghen V, Poynard T; LIDO Study Group; CYTOL study group; LIDO Study Group; CYTOL study group. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. *BMC Gastroenterol* 2006; **6**: 6 [PMID: 16503961 DOI: 10.1186/1471-230X-6-6]
 - 71 **Sumida Y**, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2014; **20**: 475-485 [PMID: 24574716 DOI: 10.3748/wjg.v20.i2.475]
 - 72 **Hsu C**, Caussy C, Imajo K, Chen J, Singh S, Kaulback K, Le MD, Hooker J, Tu X, Bettencourt R, Yin M, Sirlin CB, Ehman RL, Nakajima A, Loomba R. Magnetic Resonance vs Transient Elastography Analysis of Patients With Nonalcoholic Fatty Liver Disease: A Systematic Review and Pooled Analysis of Individual Participants. *Clin Gastroenterol Hepatol* 2018 [PMID: 29908362 DOI: 10.1016/j.cgh.2018.05.059]
 - 73 **Vilar-Gomez E**, Yasells-Garcia A, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Villa-Jimenez O, Friedman SL, Diago M, Romero-Gomez M. Development and validation of a noninvasive prediction model for nonalcoholic steatohepatitis resolution after lifestyle intervention. *Hepatology* 2016; **63**: 1875-1887 [PMID: 26849287 DOI: 10.1002/hep.28484]
 - 74 **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]
 - 75 **Marchesini G**, Petta S, Dalle Grave R. Diet, weight loss, and liver health in nonalcoholic fatty liver disease: Pathophysiology, evidence, and practice. *Hepatology* 2016; **63**: 2032-2043 [PMID: 26663351 DOI: 10.1002/hep.28392]
 - 76 **von Schönfels W**, Beckmann JH, Ahrens M, Hendricks A, Röcken C, Szymczak S, Hampe J, Schafmayer C. Histologic improvement of NAFLD in patients with obesity after bariatric surgery based on standardized NAS (NAFLD activity score). *Surg Obes Relat Dis* 2018; **14**: 1607-1616 [PMID: 30146425 DOI: 10.1016/j.soard.2018.07.012]
 - 77 **Watanabe S**, Hashimoto E, Ikejima K, Uto H, Ono M, Sumida Y, Seike M, Takei Y, Takehara T, Tokushige K, Nakajima A, Yoneda M, Saibara T, Shiota G, Sakaida I, Nakamura M, Mizuta T, Tsubouchi H, Sugano K, Shimosegawa T; Japanese Society of Gastroenterology; Japan Society of Hepatology; Japanese Society of Gastroenterology; Japan Society of Hepatology. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *J Gastroenterol* 2015; **50**: 364-377 [PMID: 25708290 DOI: 10.1007/s00535-015-1050-7]
 - 78 **Younossi Z**, Tacke F, Arrese M, Sharma BC, Mostafa I, Bugianesi E, Wong VW, Yilmaz Y, George J, Fan J, Vos MB. Global Perspectives on Non-alcoholic Fatty Liver Disease and Non-alcoholic

- Steatohepatitis. *Hepatology* 2018 [PMID: 30179269 DOI: 10.1002/hep.30251]
- 79 **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]
 - 80 **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]
 - 81 **Huang MY**, Chung CH, Chang WK, Lin CS, Chen KW, Hsieh TY, Chien WC, Lin HH. The role of thiazolidinediones in hepatocellular carcinoma risk reduction: a population-based cohort study in Taiwan. *Am J Cancer Res* 2017; **7**: 1606-1616 [PMID: 28744408]
 - 82 **Portillo-Sanchez P**, Bril F, Lomonaco R, Barb D, Orsak B, Bruder JM, Cusi K. Effect of pioglitazone on bone mineral density in patients with nonalcoholic steatohepatitis: A 36-month clinical trial. *J Diabetes* 2018 [PMID: 30073778 DOI: 10.1111/1753-0407.12833]
 - 83 **Sumida Y**, Yoneda M. Current and future pharmacological therapies for NAFLD/NASH. *J Gastroenterol* 2018; **53**: 362-376 [PMID: 29247356 DOI: 10.1007/s00535-017-1415-1]
 - 84 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarthy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]
 - 85 **Ratzliff V**, Harrison SA, Francque S, Bedossa P, Leher P, Serfaty L, Romero-Gomez M, Boursier J, Abdelmalek M, Caldwell S, Drenth J, Anstee QM, Hum D, Hanf R, Megnier S, Staels B, Sanyal A; GOLDEN-505 Investigator Study Group. GOLDEN-505 Investigator Study Group. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor- α and - δ , Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. *Gastroenterology* 2016; **150**: 1147-1159.e5 [PMID: 26874076 DOI: 10.1053/j.gastro.2016.01.038]
 - 86 **Wang PX**, Ji YX, Zhang XJ, Zhao LP, Yan ZZ, Zhang P, Shen LJ, Yang X, Fang J, Tian S, Zhu XY, Gong J, Zhang X, Wei QF, Wang Y, Li J, Wan L, Xie Q, She ZG, Wang Z, Huang Z, Li H. Targeting CASP8 and FADD-like apoptosis regulator ameliorates nonalcoholic steatohepatitis in mice and nonhuman primates. *Nat Med* 2017; **23**: 439-449 [PMID: 28218919 DOI: 10.1038/nm.4290]
 - 87 **Schuster S**, Feldstein AE. NASH: Novel therapeutic strategies targeting ASK1 in NASH. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 329-330 [PMID: 28377639 DOI: 10.1038/nrgastro.2017.42]
 - 88 **Friedman SL**, Ratzliff V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, Francque S, Farrell G, Kowdley KV, Craxi A, Simon K, Fischer L, Melchor-Khan L, Vest J, Wiens BL, Vig P, Seyedkazemi S, Goodman Z, Wong VW, Loomba R, Tacke F, Sanyal A, Lefebvre E. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. *Hepatology* 2018; **67**: 1754-1767 [PMID: 28833331 DOI: 10.1002/hep.29477]
 - 89 **Akuta N**, Watanabe C, Kawamura Y, Arase Y, Saitoh S, Fujiyama S, Sezaki H, Hosaka T, Kobayashi M, Kobayashi M, Suzuki Y, Suzuki F, Ikeda K, Kumada H. Effects of a sodium-glucose cotransporter 2 inhibitor in nonalcoholic fatty liver disease complicated by diabetes mellitus: Preliminary prospective study based on serial liver biopsies. *Hepatol Commun* 2017; **1**: 46-52 [PMID: 29404432 DOI: 10.1002/hep4.1019]
 - 90 **Sanyal A**, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, Lawitz EJ, Halegoua-DeMarzio D, Kundu S, Noviello S, Luo Y, Christian R. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *Lancet* 2018 [PMID: 30554783 DOI: 10.1016/S0140-6736(18)31785-9]
 - 91 **Barreyro FJ**, Holod S, Finocchietto PV, Camino AM, Aquino JB, Avagnina A, Carreras MC, Poderoso JJ, Gores GJ. The pan-caspase inhibitor Emricasan (IDN-6556) decreases liver injury and fibrosis in a murine model of non-alcoholic steatohepatitis. *Liver Int* 2015; **35**: 953-966 [PMID: 24750664 DOI: 10.1111/liv.12570]
 - 92 **Harrison SA**, Marri SR, Chalasani N, Kohli R, Aronstein W, Thompson GA, Irish W, Miles MV, Xanthakos SA, Lawitz E, Noureddin M, Schiano TD, Siddiqui M, Sanyal A, Neuschwander-Tetri BA, Traber PG. Randomised clinical study: GR-MD-02, a galectin-3 inhibitor, vs. placebo in patients having non-alcoholic steatohepatitis with advanced fibrosis. *Aliment Pharmacol Ther* 2016; **44**: 1183-1198 [PMID: 27778367 DOI: 10.1111/apt.13816]
 - 93 **Birukawa NK**, Murase K, Sato Y, Kosaka A, Yoneda A, Nishita H, Fujita R, Nishimura M, Ninomiya T, Kajiwara K, Miyazaki M, Nakashima Y, Ota S, Murakami Y, Tanaka Y, Minomi K, Tamura Y, Niitsu Y. Activated hepatic stellate cells are dependent on self-collagen, cleaved by membrane type 1 matrix metalloproteinase for their growth. *J Biol Chem* 2014; **289**: 20209-20221 [PMID: 24867951 DOI: 10.1074/jbc.M113.544494]
 - 94 **Boursier J**, Mueller O, Barret M, Machado M, Fizzanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016; **63**: 764-775 [PMID: 26600078 DOI: 10.1002/hep.28356]
 - 95 **Da Silva HE**, Teterina A, Comelli EM, Taibi A, Arendt BM, Fischer SE, Lou W, Allard JP. Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep* 2018; **8**: 1466 [PMID: 29362454 DOI: 10.1038/s41598-018-19753-9]
 - 96 **Rau M**, Rehman A, Dittrich M, Groen AK, Hermanns HM, Seyfried F, Beyersdorf N, Dandekar T, Rosenstiel P, Geier A. Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United European Gastroenterol J* 2018; **6**: 1496-1507 [PMID: 30574320 DOI: 10.1177/2050640618804444]
 - 97 **Yoshimoto S**, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, Honda K, Ishikawa Y, Hara E, Ohtani N. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 2013; **499**: 97-101 [PMID: 23803760 DOI: 10.1038/nature12347]
 - 98 **Tripathi A**, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, Knight R. The gut-liver axis

- and the intersection with the microbiome. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 397-411 [PMID: 29748586 DOI: 10.1038/s41575-018-0011-z]
- 99 **Alkhoury N**, Poordad F, Lawitz E. Management of nonalcoholic fatty liver disease: Lessons learned from type 2 diabetes. *Hepatol Commun* 2018; **2**: 778-785 [PMID: 30027137 DOI: 10.1002/hep4.1195]
 - 100 **Nasr P**, Ignatova S, Kechagias S, Ekstedt M. Natural history of nonalcoholic fatty liver disease: A prospective follow-up study with serial biopsies. *Hepatol Commun* 2017; **2**: 199-210 [PMID: 29404527 DOI: 10.1002/hep4.1134]
 - 101 **Loomba R**, Sanyal AJ, Kowdley KV, Terrault N, Chalasani NP, Abdelmalek MF, McCullough AJ, Shringarpure R, Ferguson B, Lee L, Chen J, Liberman A, Shapiro D, Neuschwander-Tetri BA. Factors Associated With Histologic Response in Adult Patients With Nonalcoholic Steatohepatitis. *Gastroenterology* 2019; **156**: 88-95.e5 [PMID: 30222962 DOI: 10.1053/j.gastro.2018.09.021]
 - 102 **Eslam M**, Hashem AM, Romero-Gomez M, Berg T, Dore GJ, Mangia A, Chan HLY, Irving WL, Sheridan D, Abate ML, Adams LA, Weltman M, Bugianesi E, Spengler U, Shaker O, Fischer J, Mollison L, Cheng W, Nattermann J, Riordan S, Miele L, Kelaeng KS, Ampuero J, Ahlenstiel G, McLeod D, Powell E, Liddle C, Douglas MW, Booth DR, George J; International Liver Disease Genetics Consortium (ILDGC) International Liver Disease Genetics Consortium (ILDGC). FibroGENE: A gene-based model for staging liver fibrosis. *J Hepatol* 2016; **64**: 390-398 [PMID: 26592354 DOI: 10.1016/j.jhep.2015.11.008]
 - 103 **Tanaka N**, Sano K, Horiuchi A, Tanaka E, Kiyosawa K, Aoyama T. Highly purified eicosapentaenoic acid treatment improves nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2008; **42**: 413-418 [PMID: 18277895 DOI: 10.1097/MCG.0b013e31815591aa]
 - 104 **Sumida Y**, Murotani K, Saito M, Tamasawa A, Osonoi Y, Yoneda M, Osonoi T. Effect of luseogliflozin on hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease: A prospective, single-arm trial (LEAD trial). *Hepatol Res* 2018 [PMID: 30051943 DOI: 10.1111/hepr.13236]
 - 105 **Komatsu M**, Tanaka N, Kimura T, Fujimori N, Sano K, Horiuchi A, Sugiyama A, Yamazaki T, Shibata S, Joshita S, Umemura T, Matsumoto A, Tanaka E. Miglitol attenuates non-alcoholic steatohepatitis in diabetic patients. *Hepatol Res* 2018; **48**: 1092-1098 [PMID: 29935004 DOI: 10.1111/hepr.13223]
 - 106 **Nagaya T**, Tanaka N, Suzuki T, Sano K, Horiuchi A, Komatsu M, Nakajima T, Nishizawa T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Nakayama J, Tanaka E, Aoyama T. Down-regulation of SREBP-1c is associated with the development of burned-out NASH. *J Hepatol* 2010; **53**: 724-731 [PMID: 20655124 DOI: 10.1016/j.jhep.2010.04.033]

P- Reviewer: Angelico F, Kadayifci A, Kahraman A

S- Editor: Ma RY **L- Editor:** A **E- Editor:** Yin SY





Published By Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjgnet.com>

