**Name of Journal:** *World Journal of Hepatology*

**Manuscript NO:** 89551

**Manuscript Type:** EDITORIAL

**Metabolomics in liver diseases: A novel alternative for liver biopsy?**

Tanaka Y. Metabolomics: Novel alternative for liver biopsy

Yasuo Tanaka

**Yasuo Tanaka,** Department of Gastroenterology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan

**Author contributions:** Tanaka Y contributed to the writing, and editing the manuscript, and review of literature.

**Supported by** JSPS KAKENHI, No. JP21K07906.

**Corresponding author: Yasuo Tanaka, MD, PhD, Chief Doctor,** Department of Gastroenterology, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. ytanaka@hosp.ncgm.go.jp

**Received:** November 9, 2023

**Revised:** December 5, 2023

**Accepted:** December 19, 2023

**Published online:**

**Abstract**

Hepatitis C virus (HCV) remains a significant public health problem as it can cause acute and chronic hepatitis. Chronic HCV infection is a major cause of liver fibrosis, and evaluation of liver fibrosis is essential because the prognosis of patients with chronic HCV infection is closely related to the stage of fibrosis. Liver fibrosis is traditionally evaluated based on pathological analysis of biopsy specimens, which is considered the gold standard. Nevertheless, liver biopsy is invasive and susceptible to sampling error and inter- and intraobserver variation in pathological interpretation; it is also costly. Therefore, noninvasive diagnostic investigations have been developed, including the use of fibrotic markers, scoring systems based on routine blood tests, and transient elastography with magnetic resonance imaging or ultrasonography. Recently, metabolomics, an emerging technology, has been used to detect the fibrosis stage. In this editorial, I comment on the article titled “Metabolomics in chronic hepatitis C: Decoding fibrosis grading and underlying pathways” by Ferrasi *et al* published in the recent issue of the *World Journal of Hepatology*. I discuss previous studies on the use of metabolome analysis for the diagnosis of HCV-related liver fibrosis and the potential development of biopsy-free diagnostic techniques.

**Key Words:** Metabolomics; Hepatitis C virus; Liver fibrosis; Liver cirrhosis; Serum biomarker

Tanaka Y. Metabolomics in liver diseases: A novel alternative for liver biopsy? *World J Hepatol* 2023; In press

**Core Tip:** Metabolomics, a rapidly emerging technology, offers a non-invasive alternative to conventional blood tests and transient elastography with magnetic resonance imaging or ultrasonography for fibrosis staging. I consider the article titled “Metabolomics in chronic hepatitis C: Decoding fibrosis grading and underlying pathways” by Ferrasi *et al,* published in the latest issue of the *World J Hepatol*. I review prior studies concerning the role of metabolomics in diagnosing HCV-related liver fibrosis and establishing a foundation for non-invasive diagnostic techniques.

**INTRODUCTION**

Hepatitis C virus (HCV) remains a significant public health concern as it can lead to acute and chronic hepatitis. The development of direct-acting antiviral therapy has substantially improved the rate of sustained virologic response and has generated interest in the goal of HCV elimination. In 2016, the World Health Organization called for the elimination of HCV infection by the year 2030[1].

Chronic HCV infection is a major cause of liver fibrosis, which is characterized by the formation of fibrous scar tissue resulting from the accumulation of extracellular matrix proteins, primarily cross-linked collagens. This tissue replaces injured liver tissue[2] and can lead to liver cirrhosis, defined as the histological development of regenerative nodules surrounded by fibrous bands. In turn, liver cirrhosis can lead to portal hypertension and end-stage liver disease[3].

Assessing the stage of liver fibrosis is essential because the prognosis of patients with liver fibrosis is closely linked to the stage of fibrosis, with those having advanced fibrosis being at higher risk for experiencing liver disease-related clinical events, such as hepatic failure and hepatocellular carcinoma[4]. Physicians require accurate methods to evaluate the progression of liver fibrosis to guide patient management and predict long-term outcomes.

Liver biopsy has traditionally been considered the gold-standard investigation for evaluating such disease. Nevertheless, it has several limitations. It is an invasive procedure that is associated with potential sampling error, inter- and intraobserver variability in pathological interpretation, and high cost[5]. To address these limitations, noninvasive diagnostic investigations have been developed.

Direct fibrotic markers, such as hyaluronic acid[6] and tissue inhibitor of metalloproteinase-1[7], and scoring systems based on routine blood tests, such as the Fibrosis-4 Index based on four factors[8] and the Aspartate Transaminase to Platelet Ratio Index[9], are cost-effective and easily accessible alternatives to liver biopsy.

Transient elastography using magnetic resonance imaging[10] or ultrasonography[11] is another option. However, their availability is limited due to the high cost of equipment.

Recently, novel diagnostic investigations based on emerging technologies, such as metabolomics, have been developed. Metabolomics involves comprehensive profiling and comparison of metabolites in biological samples, including plasma, serum, urine, and cell and tissue extracts[12]. The collected samples undergo pretreatment, and metabolites are measured using nuclear magnetic resonance or mass spectrometry (MS) combined with liquid chromatography (LC-MS), gas chromatography (GC-MS), or electrospray ionization (ESI-MS). Metabolomics offers a unique advantage because it represents the current physiological "state" of an individual, allowing exploration of factors that influence the human phenotype. The data obtained from these analyses are analyzed to determine the signatures of cellular biochemical activity. This approach is relatively novel; therefore, few studies have evaluated the associations between the metabolome and HCV-related liver disease and even fewer related to HCV-related liver fibrosis (Table 1).

Fitian *et al*[13] performed a comprehensive analysis of the global serum metabolomes of 30 patients with hepatocellular carcinoma, 27 patients with HCV-related cirrhosis, and 30 healthy controls using GC-MS and ultrahigh-performance LC-MS-MS. They found a strong association between elevated levels of bile acids (such as taurochenodeoxycholate and taurocholate) and dicarboxylic acids (such as azelate, undecanedioate, and sebacate) and cirrhosis.

Sarfaraz *et al*[14] evaluated noninvasive biomarkers for liver fibrosis, steatosis, and inflammation in patients with chronic HCV, and found that the upregulated metabolites in severe fibrosis included 1,7 dimethylxanthine, caffeine, methylsuccinate tyrosine, histidine, 2-hydroxyisovalerate, propionate, methionine, methylguanidine, 2-oxoisocaproate, and formate. Conversely, the downregulated metabolites included N-acetylaspartate, creatinine, urea, threonine, glycine, methylhistidine, adenosine, N-acetylglycine, glutamine, and asparagine.

Cano *et al*[15] examined serum metabolomics and fibrosis progression in HCV patients 1 year after transplantation. Patients at fibrosis stages F0–F1 were categorized as slow “fibrosers,” whereas those at stages F2–F4 were categorized as rapid fibrosers. The investigators found that the levels of glycocholic acid, taurochenodeoxycholic acid, and sphingomyelins (SMs) (d18:0/18:0) were increased in rapid fibrosers. Conversely, the ratio of branched-chain amino acids to aromatic amino acids was reduced in rapid fibrosers. Furthermore, they developed a model to discriminate between rapid and slow fibrosers using an algorithm consisting of four lipid metabolites: two SMs [SM (d18:2/16:0) and SM (38:1)] and two phosphatidylcholines (PCs) [PC (16:0/16:0) and PC (16:0/18:0)]. This model accurately classifies rapid and slow fibrosers after transplantation.

Gaggini *et al*[16] analyzed the sera collected at baseline from 75 HCV patients using GC-MS and LC-MS, and revealed that low ceramide (18:1/22:0), ceramide (18:1/24:0), and diacylglycerol (42:6) levels and a high phosphocholine (40:6) level were associated with greater fibrosis.

Shanmuganathan *et al*[17] demonstrated that serum levels of choline and histidine were consistently higher in HCV patients with late-stage (F2–F4) liver fibrosis compared to early-stage (F0–F1) fibrosis.

Khalil *et al*[18] found that changes in serum levels of several bile acids exhibit a linear trend across hepatocellular carcinoma, cirrhosis, non-cirrhosis, and healthy controls, potentially reflecting disease progression. Furthermore, receiver operating characteristic (ROC) curve analysis identified five conjugated acids (taurocholic acid, glycocholic acid, glycoursodeoxycholic acid, taurochenodeoxycholic acid, and glycochenodeoxycholic acid) that effectively distinguished hepatocellular carcinoma (HCC) from patients with non-cirrhotic livers.

Ferrasi *et al*[19] provided new insights into the pathogenesis and progression of liver fibrosis in HCV infection through metabolite analyses. They analyzed sera from 46 HCV patients and 50 healthy controls using ESI-MS. ESI is a soft-ionization technique that limits ion excitation, resulting in minimal or no analyte fragmentation[20]. This ionization technique has revolutionized the analysis of large biomolecules, such as the detection of coenzyme A in the present study. Statistical analysis was performed using partial least squares discriminant analysis and the variable importance score. The six most important ions were selected for each group, encompassing various metabolites categorized as sterols, lipids (glycerolipids, eicosanoids, sphingolipids, prenol lipid, and glycerophospholipids), coenzyme A, polypeptide, methyladenosine, amino acid derivatives, and acylcarnitines. The investigators performed ROC curve analysis to determine the diagnostic accuracy of metabolites associated with each grade of fibrosis. The metabolites demonstrated high sensitivity and specificity for each fibrosis grade except for F2. Consistent with the findings by Cano *et al*[15], detection of sterols, such as 18:0 and 20:5 cholesteryl esters, among patients with F1 fibrosis revealed downregulation of cholesteryl esters in rapid “fibrosers.” Furthermore, the detection of diacylglycerols among patients with F1 fibrosis supported previous results that diacylglycerols were downregulated in patients with severe fibrosis[16]. Conversely, the significant upregulation of acylcarnitines among patients with F4 fibrosis mirrored the hyper-carcinogenic state observed in HCC patients[13]. These studies have provided useful information regarding detection of the fibrosis grade and underlying pathways in HCV infection.

However, the aforementioned results raise concerns about whether these metabolites are specific to HCV-related liver fibrosis or if they may also be caused by other etiologies, such as hepatitis B virus infection, alcohol consumption, and nonalcoholic steatohepatitis.

Given the absence of overlap between each fibrosis stage, the changes in metabolites with fibrosis progression remain unclear. In particular, it remains to be explored whether the metabolite levels exhibit a linear relationship with fibrosis stage. Furthermore, the biological significance of each metabolite is not yet known. Further studies with larger sample sizes are needed to verify these results.

**CONCLUSION**

Metabolomics is a newly developed technology that has several limitations due to the influence of several factors, including sampling time, collection protocol, and measurement methods. Furthermore, it is more time-consuming and expensive compared to other methods. However, this novel approach offers valuable information for diagnosis, prognosis, and treatment of liver disease. The role of metabolomics in HCV requires further investigation. In the future, metabolomics may enable the diagnosis of liver diseases without the need for biopsy.

**REFERENCES**

1 **World Health Organization.** Global hepatitis report 2017. World Health Organization 2017; ISBN: 978-92-4-156545-5

2 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

3 **Ginès P**, Krag A, Abraldes JG, Solà E, Fabrellas N, Kamath PS. Liver cirrhosis. *Lancet* 2021; **398**: 1359-1376 [PMID: 34543610 DOI: 10.1016/S0140-6736(21)01374-X]

4 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]

5 **Bedossa P**, Carrat F. Liver biopsy: the best, not the gold standard. *J Hepatol* 2009; **50**: 1-3 [PMID: 19017551 DOI: 10.1016/j.jhep.2008.10.014]

6 **McHutchison JG**, Blatt LM, de Medina M, Craig JR, Conrad A, Schiff ER, Tong MJ. Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J Gastroenterol Hepatol* 2000; **15**:945-951 [DOI: 10.1046/j.1440-1746.2000.02233.x]

7 **Leroy V**, Monier F, Bottari S, Trocme C, Sturm N, Hilleret MN, Morel F, Zarski JP. Circulating matrix metalloproteinases 1, 2, 9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: comparison with PIIINP and hyaluronic acid. *Am J Gastroenterol* 2004; **99**: 271-279 [PMID: 15046217 DOI: 10.1111/j.1572-0241.2004.04055.x]

8 **Sterling RK,** Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology* 2006; **43**:1317-1325 [DOI: 10.1002/hep.21178]

9 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

10 **Singh S**, Venkatesh SK, Wang Z, Miller FH, Motosugi U, Low RN, Hassanein T, Asbach P, Godfrey EM, Yin M, Chen J, Keaveny AP, Bridges M, Bohte A, Murad MH, Lomas DJ, Talwalkar JA, Ehman RL. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. *Clin Gastroenterol Hepatol* 2015; **13**: 440-451.e6 [PMID: 25305349 DOI: 10.1016/j.cgh.2014.09.046]

11 **Nakagomi R**, Tateishi R, Masuzaki R, Soroida Y, Iwai T, Kondo M, Fujiwara N, Sato M, Minami T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Kondo Y, Tanaka Y, Otsuka M, Kato N, Moriya K, Ikeda H, Koike K. Liver stiffness measurements in chronic hepatitis C: Treatment evaluation and risk assessment. *J Gastroenterol Hepatol* 2019; **34**: 921-928 [PMID: 30393960 DOI: 10.1111/jgh.14530]

12 **Masoodi M**, Gastaldelli A, Hyötyläinen T, Arretxe E, Alonso C, Gaggini M, Brosnan J, Anstee QM, Millet O, Ortiz P, Mato JM, Dufour JF, Orešič M. Metabolomics and lipidomics in NAFLD: biomarkers and non-invasive diagnostic tests. *Nat Rev Gastroenterol Hepatol* 2021; **18**: 835-856 [PMID: 34508238 DOI: 10.1038/s41575-021-00502-9]

13 **Fitian AI**, Nelson DR, Liu C, Xu Y, Ararat M, Cabrera R. Integrated metabolomic profiling of hepatocellular carcinoma in hepatitis C cirrhosis through GC/MS and UPLC/MS-MS. *Liver Int* 2014; **34**: 1428-1444 [PMID: 24661807 DOI: 10.1111/liv.12541]

14 **Sarfaraz MO**, Myers RP, Coffin CS, Gao ZH, Shaheen AA, Crotty PM, Zhang P, Vogel HJ, Weljie AM. A quantitative metabolomics profiling approach for the noninvasive assessment of liver histology in patients with chronic hepatitis C. *Clin Transl Med* 2016; **5**: 33 [PMID: 27539580 DOI: 10.1186/s40169-016-0109-2]

15 **Cano A**, Mariño Z, Millet O, Martínez-Arranz I, Navasa M, Falcón-Pérez JM, Pérez-Cormenzana M, Caballería J, Embade N, Forns X, Bosch J, Castro A, Mato JM. A Metabolomics Signature Linked To Liver Fibrosis In The Serum Of Transplanted Hepatitis C Patients. *Sci Rep* 2017; **7**: 10497 [PMID: 28874799 DOI: 10.1038/s41598-017-10807-y]

16 **Gaggini M**, Carli F, Rosso C, Younes R, D'Aurizio R, Bugianesi E, Gastaldelli A. Altered Metabolic Profile and Adipocyte Insulin Resistance Mark Severe Liver Fibrosis in Patients with Chronic Liver Disease. *Int J Mol Sci* 2019; **20** [PMID: 31888144 DOI: 10.3390/ijms20246333]

17 **Shanmuganathan M**, Sarfaraz MO, Kroezen Z, Philbrick H, Poon R, Don-Wauchope A, Puglia M, Wishart D, Britz-McKibbin P. A Cross-Platform Metabolomics Comparison Identifies Serum Metabolite Signatures of Liver Fibrosis Progression in Chronic Hepatitis C Patients. *Front Mol Biosci* 2021; **8**: 676349 [PMID: 34414211 DOI: 10.3389/fmolb.2021.676349]

18 **Khalil A**, ElSheashaey A, Abdelsameea E, Obada M, Bayomy F F M, El-Said H. Value of Bile Acids in Diagnosing Hepatitis C Virus-Induced Liver Cirrhosis and Hepatocellular Carcinoma. *Br J Biomed Sci* 2022; **79**: 10191 [PMID: 35996509 DOI: 10.3389/bjbs.2021.10191]

19 **Ferrasi AC,** Lima SVG, Galvani AF, Delafiori J, Dias-Audibert AL, Catharino RR, Silva GF, Praxedes RR, Santos DB, Almeida DTM, Lima EO Metabolomics in chronic hepatitis C: Decoding fibrosis grading and underlying pathways. *World J Hepatol* 2023; **15**: 1237-1249 [DOI: 10.4254/wjh.v15.i11.1237]

20 **Kellogg MD,** Clinical and Translational Science. 2nd ed. Academic Press, 2017: 137-155 [DOI: 10.1016/b978-0-12-802101-9.00008-9]

**Footnotes**

**Conflict-of-interest statement:** All the authors declare that they have 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: https://creativecommons.org/Licenses/by-nc/4.0/

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

**Peer-review model:** Single blind

**Peer-review started:** November 9, 2023

**First decision:** November 27, 2023

**Article in press:**

**Specialty type:** Gastroenterology & hepatology

**Country/Territory of origin:** Japan

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

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C, C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Jiang W, China; Sitkin S, Russia **S-Editor:** Liu JH **L-Editor:** A **P-Editor:**

**Table 1 Metabolites as the fibrotic biomarkers of hepatitis C**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ref.**  | **Analyzed cases**  | **Analytical method** | **Increased metabolites in fibrosis progression** | **Decreased metabolites in fibrosis progression** |
| Fitian *et al*[13], 2014 | Cirrhosis *vs* healthy non-diabetic controls | GC/MS, UPLC/MS-MS | Bile acids (taurochenodeoxycholate, taurocholate, *etc*.), dicarboxylic acids (azelate, undecanedioate, sebacate, *etc.*) |  |
| Sarfaraz *et al*[14], 2016 | F3- 4 *vs* F0- 2 (Metavir)  | 1H-NMR | 1,7 dimethylxanthine, caffeine, methylsuccinate, tyrosine, histidine, 2-hydroxyisovalerate, propionate, methionine, methylguanidine, 2-oxoisocaproate, formate | N-acetylaspartate, creatinine, urea, threonine, glycine, methylhistidine, adenosine, N-acetylglycine, glutamine, asparagine |
| Cano *et al*[15], 2017 | F2- 4 *vs* F0- 1 (Metavir) | UPLC/MS | Glycocholic acid, taurochenodeoxycholic acid, sphingomyelins (d18:0/18:0) | BCAA/ArAA |
| Gaggini *et al*[16], 2019 | F5- 6 *vs* F3- 4 *vs* F1- 2 (Ishak score) | UPLC/QTOF-MS | Phosphocholine (40:6) | Ceramides (18:1/22:0), (18:1/24:0), diacylglycerol (42:6) |
| Shanmuganathan *et al*[17], 2021 | F2- 4 *vs* F0- 1 (Metavir) | MSI-CE-MS, 1H-NMR | Choline, histidine |  |
| Khalil *et al*[18], 2022 | Cirrhosis *vs* non-cirrhosis *vs* healthy controls | UPLC/MS | Taurocholic acid, glycholic acid, glycoursodeoxycholic acid, taurochenodeoxycholic acid, glycochenodeoxycholic acid |  |
| Ferrasi *et al*[19], 2023 | F1 *vs* F2 *vs* F3 *vs* F4 (Metavir) | ESI/MS |  |  |

1H-NMR: Proton nuclear magnetic resonance; GC: Gas chromatography; MS: Mass spectrometry; UPLC: Ultrahigh-performance liquid chromatography; QTOF: Quadrupole time-of-flight; MSI-CE: Multisegment injection-capillary electrophoresis; ESI: Electrospray ionization; BCAA/ArAA: The ratio of branched-chain amino acids (BCAA) to aromatic amino acids (ArAA).