Thrombospondin-2 as a Biomarker of Advanced Liver Fibrosis in Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD)

Catarina Budyono, Fatikha Rudia Ahda***

*Division of Gastroenterohepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Mataram/ General National Hospital, NTB
**Faculty of Medicine, Universitas Mataram/ General National Hospital, NTB

Corresponding author:
Catarina Budyono, Division of Gastroenterohepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Mataram/ General National Hospital, NTB. Email: catarina.budyono@gmail.com; Phone +6282123700051.

ABSTRACT

Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) is a highly prevalent liver disorder associated with obesity and metabolic syndrome, replacing the term Non-Alcoholic Fatty Liver Disease (NAFLD). The diagnosis of MAFLD is established based on the presence of hepatic steatosis accompanied by metabolic abnormalities such as overweight/obesity, type 2 diabetes mellitus, or metabolic dysregulation. Although liver biopsy remains the gold standard for diagnosis, its invasive nature underscores the need for practical, non-invasive biomarkers. This review aims to summarize the current evidence regarding thrombospondin-2 (TSP-2) as a potential biomarker of hepatic fibrosis in MAFLD. Recent data indicate that serum TSP-2 levels correlate with the severity of steatosis, inflammation, and fibrosis. TSP-2 also shows promise in distinguishing simple steatosis from steatohepatitis and in identifying patients at risk of advanced fibrosis. While these findings highlight the potential role of TSP-2 in diagnosis and disease monitoring, further clinical validation is required before its routine implementation in clinical practice.

Keywords: *Hepatic fibrosis, MAFLD, Trombospondin-2 (TSP-2)*

ABSTRAK

Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) merupakan penyakit hati dengan prevalensi tinggi yang berhubungan dengan obesitas dan sindrom metabolik, serta menggantikan istilah Non-Alcoholic Fatty Liver Disease (NAFLD). Diagnosis MAFLD ditegakkan berdasarkan adanya steatosis hepatik yang disertai kelainan metabolik, seperti kelebihan berat badan atau obesitas, diabetes melitus tipe 2, maupun disfungsi metabolik. Meskipun biopsi hati masih merupakan baku emas dalam penegakan diagnosis, sifatnya yang invasif menegaskan perlunya biomarker non-invasif yang lebih praktis. Artikel tinjauan ini bertujuan untuk merangkum bukti terkini mengenai peran thrombospondin-2 (TSP-2) sebagai biomarker potensial fibrosis hati pada MAFLD. Data terbaru menunjukkan bahwa kadar serum TSP-2 berkorelasi dengan tingkat keparahan steatosis, inflamasi, dan fibrosis. Selain itu, TSP-2 berpotensi membedakan steatosis sederhana dari steatohepatitis serta mengidentifikasi pasien dengan risiko fibrosis lanjut. Meskipun temuan ini menyoroti potensi peran TSP-2 dalam diagnosis dan pemantauan penyakit, diperlukan validasi klinis lebih lanjut sebelum dapat diterapkan secara rutin dalam praktik klinis.

Kata Kunci*: Fibrosis hati, MAFLD, Trombospondin-2 (TSP-2)*

INTRODUCTION

Metabolic dysfunction–associated fatty liver disease (MAFLD) represents the hepatic manifestation of metabolic syndrome in the context of obesity and other metabolic risk factors. A recent meta-analysis estimated the global prevalence of MAFLD in adults to be approximately 38.8%.[1] The redefinition to MAFLD emphasizes metabolic dysfunction, rather than the exclusion of alcohol consumption, as the disease's defining characteristic.[2] MAFLD progresses from simple steatosis to metabolic dysfunction–associated steatohepatitis (MASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), with the stage of fibrosis rather than steatosis or inflammation serving as the major determinant of prognosis.[3]

The gold standard for diagnosing MAFLD and differentiating steatohepatitis from simple steatosis remains liver biopsy. However, this procedure has notable limitations due to its invasive nature, including sampling errors from small biopsy specimens, potential complications, high cost, and inter-observer variability.[4] To address these limitations, several non-invasive fibrosis assessment tools have been developed, including the Fibrosis-4 Index (FIB-4), the NAFLD Fibrosis Score (NFS), and the Enhanced Liver Fibrosis (ELF) test.[5] Nevertheless, these conventional biomarkers demonstrate limited accuracy in intermediate fibrosis stages and may be confounded by factors such as age, comorbidities, and non-liver-specific changes. Consequently, there is a pressing need for biomarkers that directly reflect fibrogenic activity and provide robust diagnostic performance in MAFLD.

Among the emerging candidates, thrombospondin-2 (TSP-2) has attracted considerable attention as an extracellular matrix glycoprotein involved in tissue remodeling, angiogenesis, and wound healing.[6–7] Recent studies have shown that circulating TSP-2 levels are elevated in patients with obesity and type 2 diabetes mellitus and correlate with the degree of steatosis, inflammation, and liver fibrosis.[8–9] These findings suggest that TSP-2 may serve as a promising biomarker for fibrosis assessment in MAFLD, potentially complementing or even outperforming existing non-invasive indices and biomarker panels.

DEFINITION OF MAFLD

MAFLD is diagnosed based on evidence of ≥5% hepatic steatosis (on imaging or histology) in conjunction with one of the following: overweight/obesity, type 2 diabetes mellitus, or at least two metabolic risk factors such as hypertension, dyslipidemia, insulin resistance, or elevated C-reactive protein (CRP).[2] Unlike NAFLD, the diagnosis of MAFLD does not require the exclusion of significant alcohol intake or other liver diseases, acknowledging that MAFLD may coexist with conditions such as viral hepatitis or alcohol-related liver disease. This inclusive diagnostic framework better reflects real-world metabolic liver disease and facilitates more accurate risk stratification.

PATHOPHYSIOLOGY OF MAFLD

The pathogenesis of MAFLD follows a multiple-hit model. Insulin resistance increases the flux of free fatty acids to hepatocytes, promoting triglyceride accumulation, impaired β-oxidation, and enhanced de novo lipogenesis, ultimately resulting in hepatic steatosis.[10] Lipotoxicity induces oxidative stress, mitochondrial dysfunction, and the release of damage-associated molecular patterns (DAMPs), which activate Kupffer cells and inflammasomes—particularly NLRP3 leading to the secretion of interleukin-1β (IL-1β) and other pro-inflammatory cytokines.[11]

Adipose tissue dysfunction contributes further through decreased adiponectin production and increased secretion of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).[12] Gut microbiota dysbiosis exacerbates hepatic injury by increasing intestinal permeability and promoting lipopolysaccharide (LPS)-mediated activation of Toll-like receptor 4 (TLR4) on Kupffer cells.[13]

Chronic hepatocellular injury triggers the activation of hepatic stellate cells (HSCs), which represents the central event in hepatic fibrosis. Under the influence of transforming growth factor-beta 1 (TGF-β1), platelet-derived growth factor (PDGF), and endothelin-1, activated HSCs transdifferentiate into myofibroblasts, producing type I and III collagen as well as other extracellular matrix (ECM) proteins.[14] Excessive ECM accumulation increases hepatic stiffness, disrupts normal architecture, and promotes the progression from fibrosis to cirrhosis.

THROMBOSPONDIN-2

Thrombospondin-2 (TSP-2) is a matricellular glycoprotein encoded by the *THBS2* gene, which regulates extracellular matrix (ECM) assembly, angiogenesis, and wound repair.[6] Unlike structural ECM proteins, TSP-2 modulates collagen fibrillogenesis, metalloproteinase activity, and cell–matrix interactions.[7]

In fibrotic liver tissue, TSP-2 expression is markedly upregulated. Transcriptomic analyses of patients with NAFLD/NASH have identified *THBS2* as one of the top genes associated with fibrosis severity.[9] Studies in TSP-2–deficient mice have revealed abnormal collagen fibril formation and excessive angiogenesis, underscoring its essential role in ECM stabilization.[15]

TSP-2 actively contributes to liver fibrogenesis. Activated hepatic stellate cells (HSCs) are the primary source of TSP-2, and its secretion reinforces HSC activation through multiple mechanisms **(Figure 1):**

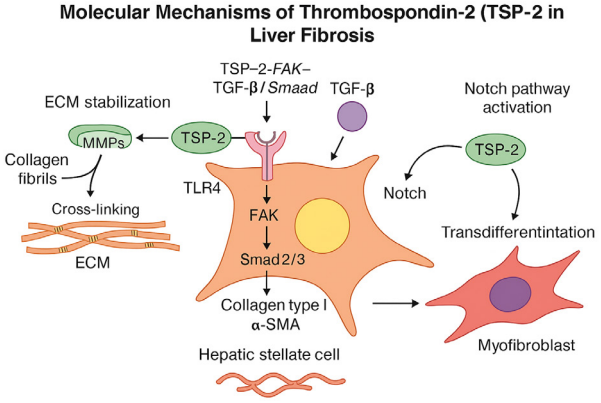

Figure 1. Molecular Mechanisms of TSP-2 in Liver Fibrosis

1. **The TLR4–FAK–TGF-β/Smad Pathway:** TSP-2 binds to Toll-like receptor 4 (TLR4) on HSC membranes, activating focal adhesion kinase (FAK) in the cytoplasm, which amplifies downstream TGF-β signaling. TGF-β receptors phosphorylate Smad2/3 proteins that form complexes with Smad4, translocate into the nucleus, and initiate transcription of *COL1A1* (collagen type I) and α-smooth muscle actin (α-SMA), thereby promoting fibrogenesis.[16]
2. **Activation of the Notch Pathway:** TSP-2 stimulates Notch receptors, promoting HSC transdifferentiation into myofibroblasts. These myofibroblasts secrete large quantities of ECM components, accelerating scar formation.[17]
3. **Extracellular Matrix Stabilization:** TSP-2 enhances collagen cross-linking and ECM stiffness while inhibiting matrix metalloproteinases (MMPs)—the enzymes responsible for ECM degradation—resulting in persistent scar accumulation and progression to cirrhosis.[15]

SERUM TSP-2 AS BIOMARKER FOR ADVANCED FIBROSIS IN MAFLD

Accurate, non-invasive identification of advanced fibrosis (≥F3) is critical in the management of MAFLD, as fibrosis stage is the strongest predictor of liver-related outcomes and overall mortality.[3] While liver biopsy remains the diagnostic gold standard, its invasiveness and associated limitations necessitate the development of reliable non-invasive biomarkers.[4–5]

Conventional non-invasive fibrosis scores—such as FIB-4, the NAFLD Fibrosis Score (NFS), and the Enhanced Liver Fibrosis (ELF) test—rely on indirect markers of hepatic injury, inflammation, or systemic metabolic abnormalities. Their diagnostic accuracy is often reduced in intermediate fibrosis stages and influenced by confounding factors including age, diabetes, obesity, and systemic inflammation.[5] Similarly, direct fibrosis biomarkers such as Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA$^+$-M2BP) and PRO-C3 (N-terminal type III collagen propeptide) have shown clinical utility but remain limited by accessibility and cost in routine practice.[18–20]

By contrast, serum TSP-2 directly reflects fibrogenic activity, as it is secreted primarily by activated HSCs during ECM remodeling.[6–7,15] This biological specificity confers an advantage over indirect markers that are affected by systemic metabolic conditions.

Recent clinical studies have underscored the diagnostic value of circulating TSP-2. In patients with NAFLD and type 2 diabetes mellitus, elevated serum TSP-2 levels correlated strongly with histological fibrosis severity, independent of metabolic confounders.[8] Transcriptomic analyses further identified *THBS2* expression as one of the top genes associated with disease progression to advanced fibrosis and cirrhosis.[9] In obesity-associated MAFLD, serum TSP-2 levels increased progressively with steatosis, inflammation, and fibrosis burden, effectively discriminating high-risk steatohepatitis.[1] In a large biopsy-confirmed cohort, TSP-2 demonstrated an area under the receiver operating characteristic curve (AUROC) of 0.80–0.87 for detecting advanced fibrosis, outperforming conventional indices such as FIB-4 and NFS and showing comparable or superior accuracy to ELF and PRO-C3 panels.[8,16,20]

Moreover, longitudinal studies indicate that serum TSP-2 levels decline in parallel with reductions in liver stiffness following lifestyle modification or metabolic therapy, suggesting its potential utility as a dynamic biomarker for fibrosis regression.[5] Collectively, these findings support serum TSP-2 as a direct and

responsive biomarker that complements existing non-invasive scores and refines decision-making regarding referral, imaging, and biopsy, as summarized in **Table 1.**

Table 1. Diagnostic Performance of Serum TSP-2 Versus Established Fibrosis Biomarkers for Detecting Advanced Fibrosis (≥F3) in MAFLD

Biomarker (class)	Biology (Direct/ Indirect)	AUROC for ≥F3	Sensitivity	Specificity	strengths	limitations	Refs
TSP-2	ECM matricellular protein from activated HSCs (Direct)	0.80–0.87	75–85%	70–85%	Direct readout of fibrogenic activity; tracks treatment response	Assay availability/ cut-off standardization; potential influence of extrahepatic fibrosis	5,8,9
ELF (HA+PIIINP+TIMP-1)	ECM turnover panel (Direct)	0.83–0.88	70–85%	75–90%	Strong evidence base; regulatory acceptance in some regions	Cost; limited access in some settings	9,18
PRO-C3 / ADAPT	Type III collagen formation (Direct)	0.83–0.88	70–85%	70–85%	Captures active collagen synthesis; good rule-in	Assay standardization and availability	20
WFA+-M2BP	Glycoprotein remodeling marker (Direct)	0.80–0.85	70–80%	70–85%	Useful across etiologies; single-analyte test	Performance varies by ethnicity/ etiology	19
Hyaluronic acid (HA)	ECM degradation product (Direct)	0.70–0.79	60–75%	60–80%	Simple assay; low cost	Lower specificity; inflammation-sensitive	18
FIB-4 (Age, AST, ALT, Plt)	Composite clinical index (Indirect)	0.76–0.82	High at low cut-off; low at high cut-off	Complementary trade-off	Inexpensive; widely adopted; good rule-out at low cut-off	Wide indeterminate zone; age-dependent inflation	5,8
NFS (Age, BMI, glycemia, AST/ALT, albumin, Plt)	Composite clinical index (Indirect)	0.75–0.81	Moderate	Moderate	Validated; easy to compute	Poorer discrimination in T2DM/obesity; large gray zone	5

Abbreviations — TSP-2: thrombospondin-2; ELF: Enhanced Liver Fibrosis test; PRO-C3: N-terminal propeptide of type III collagen; WFA+-M2BP: *Wisteria floribunda* agglutinin-positive Mac-2 binding protein; HA: hyaluronic acid; NFS: NAFLD Fibrosis Score; FIB-4: Fibrosis-4 index. **Note** — Sensitivity/specificity vary by cohort and cut-points; values below summarize representative ranges reported in the cited studies.

CONCLUSION

TSP-2 represents a biologically compelling and clinically promising direct biomarker for advanced fibrosis in MAFLD. It is mechanistically linked to HSC activation and ECM remodeling,[6–7,15–17] demonstrates strong diagnostic performance (AUROC 0.80–0.87) in biopsy-validated cohorts,[8–9,20] and exhibits on-treatment declines corresponding to improvements in liver stiffness.[5] These characteristics underscore its dual role in diagnosis and disease monitoring, complementing indirect scores and imaging modalities.

In clinical pathways, TSP-2 could effectively complement low-cost indirect indices (e.g., FIB-4, NFS) by resolving indeterminate results and refining referrals for elastography or biopsy. Nevertheless, important gaps remain. Generalizability must be established across multiethnic, non-diabetic, and community-based populations; assay harmonization and consensus cut-offs are required to define decision thresholds; and head-to-head, biopsy-anchored comparisons with established ECM-based tests (e.g., ELF, PRO-C3, WFA^{+}-M2BP) are needed to quantify incremental value and cost-effectiveness. Furthermore, potential contributions from extrahepatic fibrosis to circulating TSP-2 warrant investigation.

If these challenges are addressed, integrating serum TSP-2 into MAFLD diagnostic algorithms could reduce dependence on liver biopsy, enhance risk stratification, and provide a sensitive pharmacodynamic readout for emerging antifibrotic therapies.

Conflict of Interest

The authors declare no conflicts of interest related to this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

Catarina Budyono contributed to the conception, design, and drafting of the manuscript. Fatikha Rudia Ahda contributed to data acquisition, literature review, and manuscript revision. Both authors approved the final version of the manuscript.

Acknowledgement

The authors gratefully acknowledge the Division of Gastroenterohepatology, Department of Internal Medicine, Faculty of Medicine, Universitas Mataram / General National Hospital NTB, Indonesia, for their institutional support during the preparation of this work.

Data Availability

All data supporting the findings of this study are included in this published article.

REFERENCES

1. Zheng M-H, Pan J-W, Lu J-Q, et al. A meta-analysis and systematic review of 10 739 607 individuals: overall prevalence of MAFLD was 38.77% (95% CI 32.94-44.95). J Clin Endocrinol Metab. 2024;107(9):2691-2702.
2. Eslam M, Sarin SK, Wong VWS, et al. Asian Pacific Association for the Study of the Liver Clinical Practice Guidelines for the diagnosis and management of metabolic dysfunction-associated fatty liver disease (MAFLD). *Hepatol Int.* 2020;14(6):889-919.
3. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with NAFLD. *Gastroenterology*. 2015;149(2):389–97.
4. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. *Hepatology*. 2009;49(3):1017–44.
5. Mak JHC, Lui DTW, Fong CHY, Cheung CYY, Wong Y, Lee ACH, et al. Serum thrombospondin-2 level changes with liver stiffness improvement in patients with type 2 diabetes. *Clin Endocrinol (Oxf)*. 2024;100(3):230–7.
6. Bornstein P. Thrombospondins: structure and regulation of expression. *FASEB J*. 1992;6(14):3290–9.
7. Calabro NE, Kristofik NJ, Kyriakides TR. Thrombospondin-2 and extracellular matrix assembly. *Biochim Biophys Acta*. 2014;1840(8):2396–402.
8. Lee CH, Seto WK, Lui DTW, Fong CHY, Wan HY, Cheung CYY, et al. Circulating thrombospondin-2 as a novel fibrosis biomarker of NAFLD in type 2 diabetes. *Diabetes Care*. 2021;44(9):2089–97.
9. Kozumi K, Kodama T, Murai H, Sakane S, Govaere O, Cockell S, et al. Transcriptomics identify thrombospondin-2 as a biomarker for NASH and advanced liver fibrosis. *Hepatology*. 2021;74(5):2452–66.
10. Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology*. 2010;52(5):1836–46.
11. Xu R, Tao A, Zhang S, Deng Y, Chen G. Roles of NLRP3 inflammasome in liver diseases: focusing on nonalcoholic fatty liver disease. *Front Immunol*. 2020;11:609924.
12. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol*. 2006;6(10):772–83.
13. Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of metabolic dysfunction-associated fatty liver disease. *Nat Rev Gastroenterol Hepatol*. 2021;18(6):387–400.
14. 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(7):397–411.
15. Kyriakides TR, Zhu YH, Smith LT, Bain SD, Yang Z, Lin MT, et al. Mice that lack thrombospondin 2 display connective tissue abnormalities associated with disordered collagen fibrillogenesis, increased vascular density, and a bleeding diathesis. *J Cell Biol*. 1998;140(2):419–30.
16. Zhang N, Wu X, Zhang W, Sun Y, Yan X, Xu A, et al. Targeting thrombospondin-2 retards liver fibrosis by inhibiting TLR4-FAK/TGF-β signaling. *JHEP Rep*. 2024;6(3):101014.
17. Chen W, Wu X, Yan X, Xu A, Yang A, You H. Multi-transcriptome analyses reveal prioritized genes specifically associated with liver fibrosis progression. *Am J Physiol Gastrointest Liver Physiol*. 2019;316(6):G744–54.
18. Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, et al. Non-invasive markers of fibrosis in NAFLD: validating the European Liver Fibrosis panel and exploring simple markers. *Hepatology*. 2008;47(2):455–60.
19. Nishikawa H, Enomoto H, Iwata Y, Nishimura T, Kita R, Kimura T, et al. Serum WFA+-M2BP as a diagnostic marker of liver fibrosis in NAFLD. *Hepatol Res*. 2016;46(10):1042–51.
20. Daniels SJ, Leeming DJ, Eslam M, Hashem AM, Nielsen MJ, Krag A, et al. ADAPT: an algorithm incorporating PRO-C3 accurately identifies patients with NAFLD and advanced fibrosis. *Hepatology*. 2019;69(3):1075–86.