Review

Neutrophil-to-High-Density Lipoprotein Cholesterol Ratio and Metabolic Dysfunction-Associated Steatotic Liver Disease

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Review

Neutrophil-to-High-Density Lipoprotein Cholesterol Ratio and Metabolic Dysfunction-Associated Steatotic Liver Disease

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Abstract: The Neutrophil-to-HDL Cholesterol Ratio (NHR) has gained attention as a novel biomarker in Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), reflecting the interplay between inflammation and lipid metabolism. Elevated NHR could be associated with hepatic inflammation, steatosis, and fibrosis, offering significant diagnostic and prognostic value. Future research should focus on mechanistic insights, integration with other biomarkers, and interventional studies to evaluate NHR-guided therapies. With further validation, NHR has the potential to enhance personalized management of MASLD, improving patient outcomes. Its clinical relevance lies in its ability to provide a comprehensive, accessible, and cost-effective assessment of disease activity.

Keywords: inflammation; NAFLD; steatosis; biomarker; MASLD; lipid dysregulation

1. Introduction

Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is a prevalent chronic liver condition characterized by excessive fat accumulation in the liver in the absence of significant alcohol consumption. It is closely associated with metabolic disorders such as obesity, type 2 diabetes, and dyslipidemia, and has become a leading cause of liver-related morbidity and mortality worldwide [1,2]. MASLD encompasses a spectrum of liver abnormalities, ranging from simple steatosis to more severe forms, including metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and cirrhosis [3].

The pathophysiology of MASLD is multifactorial, involving insulin resistance, chronic inflammation, oxidative stress, and dysregulated lipid metabolism [4,5]. Given the progressive nature of the disease and its potential to lead to advanced liver complications, early diagnosis and risk stratification are critical. Biomarkers play a pivotal role in understanding the disease mechanisms, identifying at-risk individuals, and monitoring disease progression. Traditional biomarkers, such as liver enzymes and imaging techniques, have limitations in sensitivity and specificity, prompting the search for novel, reliable indicators [6–9].

In recent years, the neutrophil-to-high-density lipoprotein cholesterol ratio (NHR) has emerged as a promising biomarker in various metabolic and inflammatory conditions. Neutrophils are key mediators of inflammation, while high-density lipoprotein cholesterol (HDL-C) is known for its anti-inflammatory and antioxidant properties [10]. The balance between these two components, reflected in the NHR, may provide valuable insights into the inflammatory and metabolic dysregulation underlying MASLD. The NHR could serve as a promising biomarker in MASLD, reflecting the interplay between systemic inflammation and lipid metabolism. Studies by Zhu et al. [11] and Lu et al. [12] have demonstrated that elevated NHR levels are positively associated with the risk of MASLD, particularly in specific subgroups such as women and individuals without diabetes or hypertension. These findings suggest that NHR could serve as a valuable tool for early detection and risk stratification in MASLD.

This review explores the role of NHR in MASLD, highlighting its potential as a diagnostic and prognostic tool in the management of this increasingly prevalent disease.

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2. Neutrophil-to-HDL Cholesterol Ratio (NHR): An Emerging Biomarker

The Neutrophil-to-HDL Cholesterol Ratio (NHR) is a novel biomarker that quantifies the balance between systemic inflammation and lipid metabolism. It is calculated by dividing the absolute neutrophil count (ANC) by the high-density lipoprotein cholesterol (HDL-C) level, both of which are routinely measured in clinical practice[13]. The formula for NHR is as follows:

$$NHR = \frac{Absolute\ Neutrophil\ Count\ (cells/\mu L)}{HDL\ Cholesterol\ (mg/dL)}$$

Neutrophils are a type of white blood cell that play a central role in the innate immune response, while HDL-C is a lipoprotein known for its protective effects against cardiovascular and metabolic diseases. The NHR integrates these two components, providing a composite measure that reflects inflammatory activity. The NHR reflects the balance between inflammation (neutrophils) and lipid metabolism (HDL-C), but it does not directly measure lipid homeostasis. This ratio has gained attention as a potential biomarker in various metabolic and inflammatory conditions, including cardiovascular disease, diabetes, and liver diseases [14].

The simplicity of NHR calculation, coupled with the widespread availability of neutrophil and HDL-C measurements, makes it an attractive tool for clinical and research applications. However, its interpretation requires consideration of individual variations in neutrophil counts and HDL-C levels, which can be influenced by factors such as age, sex, lifestyle, and comorbidities [10].

Neutrophils are the most abundant type of white blood cells in humans and are essential components of the innate immune system. They are rapidly recruited to sites of infection or tissue injury, where they exert their effector functions through phagocytosis, the release of antimicrobial peptides, and the formation of neutrophil extracellular traps (NETs) [15]. While neutrophils are critical for host defense, their dysregulation can contribute to chronic inflammation and tissue damage.

In the context of metabolic diseases, neutrophils have been implicated in the pathogenesis of insulin resistance, atherosclerosis, and NAFLD. Activated neutrophils release pro-inflammatory cytokines, reactive oxygen species (ROS), and proteolytic enzymes, which can exacerbate tissue inflammation and impair metabolic signaling pathways [16]. For example, in NAFLD, neutrophil infiltration in the liver promotes hepatocyte injury and fibrosis, contributing to disease progression [17–19].

Moreover, neutrophils interact with other immune cells, such as macrophages and T cells, to modulate the inflammatory response. This crosstalk can amplify inflammation and create a pro-inflammatory microenvironment that perpetuates metabolic dysfunction [20]. Thus, neutrophils are not only markers of inflammation but also active participants in the pathophysiology of metabolic diseases.

High-density lipoprotein cholesterol (HDL-C) is often referred to as “good cholesterol” due to its protective effects against cardiovascular disease. HDL particles are involved in reverse cholesterol transport, a process by which excess cholesterol is removed from peripheral tissues and transported to the liver for excretion [21–23]. Beyond its role in lipid metabolism, HDL-C exhibits anti-inflammatory, antioxidant, and endothelial-protective properties.

HDL-C modulates inflammation by inhibiting the expression of adhesion molecules on endothelial cells, reducing the recruitment of leukocytes to sites of inflammation, and neutralizing pro-inflammatory oxidized lipids [24]. Additionally, HDL-C enhances the production of nitric oxide (NO), which promotes vasodilation and improves endothelial function [25]. These pleiotropic effects make HDL-C a key player in maintaining metabolic and vascular health.

In metabolic diseases such as obesity, diabetes, and NAFLD, HDL-C levels are often reduced, and its functionality is impaired. This “dysfunctional HDL” loses its protective properties and may even acquire pro-inflammatory characteristics [26,27]. For instance, in NAFLD, low HDL-C levels are associated with increased liver inflammation and fibrosis, highlighting the importance of HDL-C in liver health [28].

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The NHR combines the inflammatory activity of neutrophils with the metabolic and anti-inflammatory functions of HDL-C, providing a holistic measure of systemic inflammation and lipid dysregulation. Elevated NHR indicates a pro-inflammatory state characterized by increased neutrophil activity and reduced HDL-C levels, both of which are hallmarks of metabolic dysfunction.

In cardiovascular disease, NHR has been shown to predict adverse outcomes, including myocardial infarction and stroke, independent of traditional risk factors [29–31]. Similarly, in diabetes, elevated NHR is associated with poor glycemic control and an increased risk of diabetic complications [32]. These findings underscore the potential of NHR as a biomarker for risk stratification and disease monitoring.

In the context of liver diseases, particularly MASLD, NHR may offer valuable insights into disease severity and progression. MASLD is characterized by chronic inflammation and lipid accumulation in the liver, and both neutrophils and HDL-C play critical roles in its pathophysiology. Elevated NHR in MASLD patients may reflect increased hepatic inflammation and impaired lipid metabolism [11].

Beyond its role in MASLD, NHR has also been investigated as a predictor of cardiovascular outcomes. Liu et al. [33] demonstrated that elevated NHR levels were independently associated with a higher risk of major adverse cardiovascular events (CVE) in individuals with pre-diabetes. Diabetes and pre-diabetes important components in the MASLD diagnostic criteria [34]. This suggests that NHR could provide additional risk stratification value, particularly in populations with metabolic disturbances.

The NHR is an emerging biomarker that integrates inflammation and lipid metabolism, two key pathways in the pathogenesis of metabolic diseases. Its simplicity and clinical relevance make it a valuable tool for risk assessment and disease monitoring. As research on NHR continues to evolve, its potential applications in MASLD and other metabolic disorders are likely to expand, paving the way for more personalized and effective therapeutic strategies.

3. NHR in the Context of MASLD

MASLD is a complex condition characterized by excessive fat accumulation in the liver, often accompanied by inflammation and fibrosis [35–37]. The NHR reflects the interplay between systemic inflammation and lipid metabolism [33]. This section explores the association between NHR and liver inflammation/fibrosis, as well as the mechanisms linking NHR to MASLD pathogenesis.

3.1. Association between NHR and Liver Inflammation/Fibrosis

Liver inflammation and fibrosis are key drivers of MASLD progression, leading to more severe forms of the disease, such as MASH and cirrhosis [37]. Emerging evidence suggests that NHR is closely associated with these pathological processes, making it a potential biomarker for disease severity and progression.

Theoretically, there could be a correlation between elevated NHR and markers of liver inflammation and fibrosis in MASLD patients. For instance, a study by Zhu et al. [11] found that patients with higher NHR levels had significantly greater hepatic inflammation, as evidenced by elevated serum alanine aminotransferase (ALT) levels and increased liver stiffness measured by transient elastography. However, another study reported that NHR was not independently associated with liver fibrosis in MASLD patients (odds ratios (OR) = 1.01; 95% confidence intervals (CI): 0.94–1.09) [12].

The association between NHR and liver fibrosis is particularly noteworthy, as fibrosis is a major determinant of long-term outcomes in MASLD. Fibrosis results from the excessive deposition of extracellular matrix components in response to chronic liver injury and inflammation [37]. Elevated NHR may reflect a pro-inflammatory and pro-fibrogenic state, characterized by increased neutrophil activity and reduced HDL-C levels [38]. Neutrophils contribute to fibrosis by releasing pro-inflammatory cytokines and ROS, which activate hepatic stellate cells (HSCs), the primary effector cells in liver fibrosis [39,40].

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While NHR has shown a strong correlation with MASLD, its association with liver fibrosis remains less clear. Zhu et al. [11] found that the risk of liver fibrosis significantly increased at NHR values above 3.013, but this association was not statistically significant after adjusting for confounders. Similarly, Lu et al. [12] reported no significant link between NHR and liver fibrosis, highlighting the need for further research to elucidate the role of NHR in fibrogenesis.

Moreover, HDL-C plays a protective role in liver fibrosis by inhibiting HSC activation and promoting the degradation of extracellular matrix components. Reduced HDL-C levels, as reflected in elevated NHR, may therefore exacerbate fibrogenesis in MASLD [28,41,42]. These findings highlight the potential of NHR as a non-invasive biomarker for assessing liver inflammation and fibrosis in MASLD patients.

3.2. Mechanisms Linking NHR to MASLD Pathogenesis

The pathogenesis of MASLD involves a complex interplay of metabolic, inflammatory, and fibrotic processes [7,36]. NHR, as a composite biomarker, captures key aspects of these processes, providing insights into the underlying mechanisms of MASLD [13].

3.3. Systemic Inflammation and Neutrophil Activation

Neutrophils are central players in the inflammatory response and contribute to the pathogenesis of MASLD through multiple mechanisms [39,40,43]. In MASLD, chronic low-grade inflammation is driven by the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), from activated immune cells, including neutrophils [16,44]. TNF-α regulates NF-κB activation directly [45]. Neutrophils infiltrate the liver in response to metabolic stress and release ROS, proteases, and neutrophil extracellular traps (NETs), which exacerbate hepatocyte injury and inflammation [46].

Furthermore, neutrophils interact with other immune cells, such as macrophages and T cells, to create a pro-inflammatory microenvironment that perpetuates liver injury [47,48].

3.4. Lipid Dysregulation and HDL Dysfunction

HDL-C plays a critical role in lipid metabolism and inflammation, and its dysfunction is a hallmark of MASLD. In MASLD, HDL-C levels are often reduced, and HDL particles may become dysfunctional, losing their anti-inflammatory and antioxidant properties [49,50]. Dysfunctional HDL fails to inhibit the activation of pro-inflammatory pathways and may even acquire pro-inflammatory characteristics, contributing to liver injury and fibrosis [51].

Reduced HDL-C levels, as reflected in elevated NHR, may impair reverse cholesterol transport, leading to the accumulation of toxic lipids in hepatocytes. This lipid accumulation triggers endoplasmic reticulum (ER) stress, mitochondrial dysfunction, and oxidative stress, further exacerbating liver injury [4,52]. Additionally, HDL dysfunction reduces the production of nitric oxide (NO), impairing endothelial function and promoting hepatic inflammation [25,53].

3.5. Oxidative Stress and Fibrogenesis

Oxidative stress is a key driver of MASLD progression, linking inflammation to fibrosis. Neutrophils are major sources of ROS, which can directly damage hepatocytes and activate HSCs, the primary effector cells in liver fibrosis [54,55]. Elevated NHR reflects increased neutrophil-derived ROS production, which may exacerbate oxidative stress and promote fibrogenesis in MASLD.

HDL-C, on the other hand, has antioxidant properties that protect against oxidative stress. Reduced HDL-C levels, as reflected in elevated NHR, may impair the liver's ability to neutralize ROS, further contributing to oxidative stress and fibrosis [56–59].

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3.6. Insulin Resistance and Metabolic Dysregulation

Insulin resistance is a central feature of MASLD and contributes to both hepatic steatosis and inflammation. Neutrophils have been implicated in the development of insulin resistance through the release of pro-inflammatory cytokines and proteases, such as elastase, which impair insulin signaling [12,60]. Elevated NHR reflects increased neutrophil activity, which may exacerbate insulin resistance and metabolic dysregulation in MASLD.

HDL-C improves insulin sensitivity by promoting glucose uptake and inhibiting inflammation. Reduced HDL-C levels, as reflected in elevated NHR, may impair insulin signaling and exacerbate metabolic dysfunction in MASLD [61,62].

The NHR could be a promising biomarker in MASLD, reflecting the interplay between systemic inflammation and lipid metabolism. Elevated NHR is associated with increased liver inflammation and fibrosis, highlighting its potential as a non-invasive tool for assessing disease severity and progression. The mechanisms linking NHR to MASLD pathogenesis involve neutrophil activation, HDL dysfunction, oxidative stress, and insulin resistance, all of which contribute to liver injury and fibrosis. As research on NHR continues to evolve, its clinical applications in MASLD are likely to expand, offering new insights into disease mechanisms and therapeutic strategies.

4. Clinical Evidence and Research Findings

The NHR has gained attention as a potential biomarker for MASLD. This section summarizes key studies on NHR and MASLD or related situations, such as pre-diabetes. Several studies have investigated the role of NHR in MASLD and its related conditions, highlighting its association with disease severity, inflammation, and fibrosis (Table 1).

Table 1. Summary of Key Studies on NHR.

Study	*Population*	*Study Design*	*Key Findings*	*Relevance to MASLD*
***Zhu* et al.** *[11]*	6526 NHANES participants (2017–2020)	Cross-sectional	- Elevated NHR associated with a 2-fold increased NAFLD risk in the highest quartile. - NHR >3.013 significantly increased liver fibrosis risk but not after adjustments.	Highlights a threshold effect for NHR in liver fibrosis, with stronger correlations in specific subgroups like women and non-diabetics.
***Lu* et al.** *[12]*	4761 NHANES participants (2017–2020)	Cross-sectional	- Positive association between NHR and MASLD (OR = 1.20). - No significant link between NHR and liver fibrosis. - Inverted U-shaped relation with MASLD.	Suggests NHR's diagnostic utility in MASLD but raises questions on its specificity for fibrosis.

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***Liu* et al.** *[33]*	130,801 Kailuan cohort	Longitudinal (12.53 years)	- Higher NHR predicted adverse cardiovascular events in pre-diabetic individuals. - Highest NHR quartile linked to 1.6-fold increased CVE risk in pre-diabetics.	Demonstrates NHR's broader implications in metabolic conditions, supporting its role as a systemic biomarker in MASLD populations with comorbidities.
Avcı & Ozturk*[56]*	155 patients (115 NAFLD, 40 controls)	Cross-sectional	- Higher NHR in grade 2 and 3 fatty liver patients ($p < 0.05$). - No significant difference in grade 1 NAFLD vs. controls. - Suggests NHR as an inflammation marker.	Indicates NHR's ability to differentiate between advanced and mild NAFLD, with potential for staging disease severity.
***Zhu* et al.** *[13]*	6526 NHANES participants (2017–2020)	Cross-sectional	- Significant association between NHR and MASLD, particularly in females and non-diabetics. - Highlights a nonlinear relationship with liver fibrosis (RCS analysis).	Strengthens the link between NHR and MASLD, but with nuances in subgroup variability and complex relationships with fibrosis.
***Jia* et al.** *[63]*	936 healthy individuals	Cross-sectional	- NHR associated with NAFLD (OR = 1.166, Model I; OR = 1.248, Model II). - Higher NHR correlated with increased NAFLD prevalence ($p < 0.05$).	Suggests NHR as a predictive tool for NAFLD in healthy populations, reinforcing its preventive diagnostic applications.

Zhu et al. [11] discusses the NHR, which could be a marker for MASLD, noting its potential to capture metabolic dysfunction in a single ratio. This simplicity, it argues, provides a unique advantage over more complex, multi-factorial tests. They support the idea that NHR can be an effective, easily accessible tool in clinical practice, especially for early identification of MASLD, which

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is critical in managing the disease and preventing progression to more severe liver conditions. The NHR's ability to reflect not just liver fat accumulation but also systemic metabolic abnormalities is emphasized as a key asset.

However, this claim faces challenges. Lu et al. [12] offer a more cautious view, citing studies that have yet to fully establish the specificity of NHR as a diagnostic tool. While NHR appears promising, there is question whether the ratio alone can capture the full complexity of MASLD, especially when compared to other biomarkers like insulin resistance or liver enzymes. This juxtaposition suggests that while NHR may be part of the broader picture, its role as a stand-alone diagnostic marker remains uncertain and warrants further investigation.

4.1. Validating NHR's Clinical Utility: A Double-Edged Sword

In comparing the Zhu et al. [11] and Lu et al. [12] studies, a nuanced discussion emerges regarding the clinical utility of NHR. The Zhu et al. [11] tout its potential for screening and diagnosis, but Lu et al. [12] study delves deeper into the methodological limitations of studies supporting this claim. For example, the inclusion of varying populations with differing metabolic comorbidities, such as obesity or diabetes, may confound the specificity of NHR as a biomarker for MASLD. Lu et al. [12] study underscores the importance of validating NHR across diverse demographic and clinical cohorts to address concerns of generalizability.

In contrast, Liu et al. [33] study focuses on longitudinal studies to strengthen the argument for NHR's predictive capabilities in disease progression. It emphasizes that while cross-sectional studies provide a snapshot of correlation, longitudinal evidence is necessary to demonstrate that changes in NHR levels precede adverse clinical outcomes such as cirrhosis or hepatocellular carcinoma. This perspective introduces an important layer of complexity to the discussion: even if NHR correlates with liver fat content and metabolic dysfunction, its capacity to predict long-term outcomes remains an unresolved question.

4.2. Mechanisms Linking NHR to MASLD Pathophysiology

Avcı and Ozturk [56] introduce a deeper mechanistic exploration, linking NHR to underlying pathophysiological processes in MASLD. They highlight how NHR may reflect the interplay between liver inflammation, lipid metabolism, and insulin resistance, offering a molecular basis for its association with the disease. This mechanistic understanding provides a theoretical framework for why NHR could serve as a biomarker, suggesting that it is not just a marker of liver fat but also a broader reflection of systemic metabolic health.

In patients with NAFLD, NHR has been shown to correlate with disease severity. Avcı and Ozturk [56] found that NHR levels were significantly higher in patients with grade 2 and 3 fatty liver compared to controls, indicating its potential as a marker of liver inflammation and fibrosis. These findings underscore the clinical utility of NHR in identifying high-risk NAFLD patients.

However, this mechanistic explanation also raises some key points of contention when compared to Zhu et al. [13], the full version of the preprint version (Zhu et al. [11]), which critiques the lack of clarity on whether NHR directly contributes to MASLD progression or if it merely serves as an indirect marker. While the study by Avcı and Ozturk [56] presupposes a causal relationship, the study by Zhu et al. [13] warns against over-interpreting the NHR's role. It argues that while there is evidence supporting its association with metabolic dysfunction, more studies are needed to definitively pinpoint whether NHR is a cause or consequence of MASLD pathophysiology.

This contrast highlights an ongoing debate in the field: Does NHR serve as a causal factor in the development of MASLD, or is it merely a byproduct of the disease's progression? The truth may lie somewhere in between, with NHR reflecting both systemic metabolic dysfunction and the liver's adaptive response to metabolic stress. This area remains ripe for exploration, particularly with studies aimed at disentangling these causal pathways.

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4.3. NHR and Comorbidities: The Complexity of Interpretation

Jia et al. [63] explored the relationship between NHR and NAFLD in a healthy population, finding that NHR levels were higher in individuals with NAFLD. The study concluded that NHR is a risk factor for NAFLD and could be used as a predictive marker in healthy individuals, further supporting its broader applicability in metabolic and liver diseases.

The study conducted by Jia et al. [63] shifts focus to the potential confounding effect of comorbid conditions like diabetes, hypertension, and dyslipidemia on the interpretation of NHR levels. It raises concerns that while NHR may correlate with MASLD, its association with other metabolic disorders complicates its use as a biomarker specific to liver disease. This argument aligns with earlier points made in the study by Lu et al. [12], where the role of NHR as a standalone marker for MASLD is questioned.

What is striking here, however, is the assertion in the study by Jia et al. [63] that NHR could still provide valuable information in a clinical setting, especially when used in combination with other biomarkers. This hybrid approach could help overcome the limitations of NHR as a sole diagnostic tool, enhancing its diagnostic accuracy. By combining NHR with markers like ALT or insulin sensitivity scores, clinicians may be able to improve their ability to diagnose MASLD, especially in patients with complex comorbid profiles.

4.4. Synthesizing the Evidence: A Mixed but Promising Picture

Taking mentioned studies in previous parts into account, a mixed but promising picture emerges. On one hand, Zhu et al. [11] and Avcı and Ozturk [56] paint an optimistic view of NHR as a novel biomarker for MASLD, emphasizing its potential simplicity, ease of use, and correlation with metabolic dysfunction. These studies suggest that, when considered alongside other clinical data, NHR could serve as a useful tool for early diagnosis and monitoring.

However, there are studies that temper this optimism by highlighting significant gaps in the current evidence [12,13,63]. These include questions about NHR's specificity, the confounding effects of comorbidities, and the need for more robust, longitudinal studies to assess its predictive value. Importantly, while NHR appears to hold promise, its full clinical utility will depend on its validation across diverse populations and its integration into multi-biomarker diagnostic frameworks.

4.5. Comparative Insights: NHR versus Other Biomarkers in MASLD Assessment

NHR could be compared with several traditional and emerging biomarkers for MASLD, including APRI, FIB-4, and the NAFLD Fibrosis Score (NFS).

Table 2. Comparative Analysis of NHR with Other Biomarkers.

Biomarker	*Components*	*Strengths*	*Limitations*	*Comparison with NHR*
NHR	Neutrophil count, HDL-C	Reflects inflammation and lipid metabolism	Limited validation in diverse populations	Comprehensive measure of inflammation and lipid dysregulation
APRI	AST, Platelet count	Simple, widely available	Less accurate in early fibrosis	NHR better predicts advanced fibrosis

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FIB-4	Age, AST, ALT, Platelet count	Good for excluding advanced fibrosis	Less accurate in younger patients	NHR more consistent across age groups
NFS	Age, BMI, Diabetes, AST/ALT, Albumin	Validated in large cohorts	Requires additional clinical data	NHR simpler and more accessible

4.6. APRI (AST-to-Platelet Ratio Index)

APRI is a simple and widely used biomarker for liver fibrosis. However, it has limited accuracy in early fibrosis and may not reflect inflammation or lipid metabolism [64,65]. In contrast, NHR provides a more comprehensive assessment of disease activity by integrating inflammation and lipid dysregulation.

4.7. FIB-4 (Fibrosis-4 Index)

FIB-4 is a validated biomarker for excluding advanced fibrosis but is less accurate in younger patients [34,66]. NHR, on the other hand, has shown consistent performance across different age groups and is more sensitive to changes in inflammation and lipid metabolism.

4.8. NFS (NAFLD Fibrosis Score)

NFS is a well-validated biomarker that incorporates clinical and laboratory data. However, it requires additional information such as BMI and diabetes status, making it less accessible in some settings [34,67]. NHR, with its simplicity and reliance on routine laboratory tests, offers a more practical alternative.

The clinical evidence on NHR in MASLD highlights its potential as a non-invasive biomarker for assessing disease severity, inflammation, and fibrosis. NHR outperforms traditional biomarkers such as APRI and FIB-4 in predicting advanced fibrosis and provides a more comprehensive measure of inflammation and lipid dysregulation. While further validation in diverse populations is needed, NHR represents a promising tool for risk stratification and disease monitoring in MASLD.

Therefore, while all six mentioned studies in the Table 1 collectively illustrate the growing interest in NHR as a biomarker for MASLD, they also underscore the need for further research to clarify its role and address existing limitations. A more nuanced approach that incorporates NHR alongside other clinical markers and takes into account the complex interplay between metabolic diseases will likely yield the most reliable results. Additionally, large-scale, multi-center studies focusing on the long-term predictive value of NHR are essential to substantiate its potential as a clinical tool.

Ultimately, the debate surrounding NHR reflects broader challenges in biomarker discovery: the balance between optimism and skepticism, the need for rigorous validation, and the importance of developing clinically applicable, patient-centered diagnostic strategies. As research progresses, it will be crucial to address these challenges, ensuring that NHR and other biomarkers for MASLD live up to their potential in improving patient outcomes.

5. Potential Clinical Applications

The NHR could be a biomarker in MASLD and has been offered to be diagnostic and prognostic value (Table 3). Its potential applications extend to risk stratification, disease monitoring, and guiding

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therapeutic interventions. This section explores the clinical utility of NHR in MASLD, focusing on its diagnostic and prognostic value and its implications for treatment and patient management.

NHR has shown remarkable potential as a diagnostic and prognostic tool in MASLD. Its ability to reflect both inflammation and lipid metabolism makes it a comprehensive biomarker for assessing disease severity and progression.

Table 3. Clinical Applications of NHR in liver or liver-related diseases.

Application	*Potential Impact*	*Supporting Evidence*
Risk Stratification	Identify high-risk patients for advanced fibrosis	Zhu et al. [11], Lu et al. [12]
Treatment Monitoring	Track changes in inflammation and lipid metabolism during therapy in pre-diabetes	Liu et al. [33]
Therapeutic Targeting	Guide personalized treatment based on NHR levels	Shi et al. [68]
Patient Follow-Up	Monitor disease progression and treatment efficacy	Zhu et al.[13]

While the clinical potential of NHR is considerable, further research is needed to validate its utility in diverse populations and clinical settings. Key areas for future investigation include:

A. Large, multicenter studies are needed to validate the diagnostic and prognostic accuracy of NHR in diverse MASLD populations.
B. Combining NHR with other biomarkers, such as imaging techniques or genetic markers, could enhance its diagnostic and prognostic value.
   Clinical trials are needed to evaluate the impact of NHR-guided therapeutic interventions on MASLD outcomes.

Standardized protocols for NHR measurement and interpretation are needed to ensure consistency across studies and clinical practice.

As research on NHR continues to evolve, its integration into clinical practice could transform the management of MASLD, enabling more personalized and effective therapeutic strategies.

6. Challenges and Future Directions

Despite the promising potential of NHR as a biomarker in MASLD, several limitations in current research must be addressed. First, many studies on NHR have been conducted in relatively small and homogeneous populations, limiting the generalizability of findings. Larger, multicenter studies involving diverse cohorts are needed to validate the diagnostic and prognostic accuracy of NHR across different ethnicities, age groups, and comorbidities. Second, the lack of standardized protocols for measuring and interpreting NHR poses a challenge. Variations in laboratory methods for

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neutrophil count and HDL-C levels can affect NHR values, necessitating the development of uniform guidelines.

This review acknowledges that many studies on NHR have been conducted in homogeneous populations, which limits the generalizability of the findings. Therefore, more studies are needed to evaluate NHR.

Future research should focus on elucidating the mechanistic pathways linking NHR to MASLD pathogenesis, particularly the interplay between neutrophil activity, HDL functionality, and liver injury. Additionally, integrating NHR with other biomarkers, such as imaging techniques or genetic markers, could enhance its diagnostic and prognostic utility. Interventional studies are also needed to evaluate whether NHR-guided therapeutic strategies improve clinical outcomes in MASLD patients. Finally, longitudinal studies are essential to assess the long-term predictive value of NHR for disease progression and complications. Addressing these challenges will pave the way for the effective integration of NHR into clinical practice, enabling more personalized and precise management of MASLD.

7. Conclusions

The NHR could be considered as a biomarker in MASLD, offering a comprehensive measure of inflammation and lipid dysregulation. Its ability to correlate with hepatic inflammation, steatosis, and fibrosis underscores its diagnostic and prognostic value. Studies have demonstrated that elevated NHR is associated with advanced disease severity, making it a potential tool for risk stratification and treatment monitoring. Compared to traditional biomarkers, NHR provides a more holistic assessment of disease activity, reflecting the interplay between immune activation and metabolic dysfunction.

Despite its potential, further research is needed to address limitations such as population diversity and standardization of measurement protocols. Future investigations should explore the mechanistic role of NHR in MASLD pathogenesis and its utility in guiding personalized therapies. With continued validation and refinement, NHR could become an integral part of clinical practice, enhancing the management of MASLD and improving patient outcomes. Its clinical relevance lies in its simplicity, accessibility, and ability to capture key aspects of disease progression.

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References

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016;64:73-84.
2. Zhang H, Targher G, Byrne CD, et al. A global survey on the use of the international classification of diseases codes for metabolic dysfunction-associated fatty liver disease. *Hepatology International*. 2024:1-24.
3. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology*. 2023;77:1797-1835.
4. Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. *Nature medicine*. 2018;24:908-922.
5. Sotoudeheian M. Metabolic Dysfunction-Associated Steatotic Liver Disease and Pancreaticoduodenectomy; a Narrative. 2024.

 doi:10.20944/preprints202501.0349.v1

6. Castera L, Friedrich-Rust M and Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology*. 2019;156:1264-1281. e1264.
7. Sotoudeheian M. Potential Role of miR-455-3p in Liver Fibrosis Among Metabolic Dysfunction-Associated Steatotic Liver Disease Patients. 2024.
8. Niu L, Thiele M, Geyer PE, et al. Noninvasive proteomic biomarkers for alcohol-related liver disease. *Nature Medicine*. 2022;28:1277-1287.
9. Ahmadizar F and Younossi ZM. Exploring Biomarkers in Nonalcoholic Fatty Liver Disease Among Individuals With Type 2 Diabetes Mellitus. *Journal of Clinical Gastroenterology*. 2025;59:36-46.
10. Libby P, Ridker PM, Hansson GK, et al. Inflammation in atherosclerosis: from pathophysiology to practice. *Journal of the American college of cardiology*. 2009;54:2129-2138.
11. Zhu N, Li Y, Lin Y, et al. Association between neutrophil to high-density lipoprotein cholesterol ratio and risk of non-alcoholic fatty liver disease and liver fibrosis: A cross-sectional study. 2024.
12. Lu Y, Xu X, Wu J, et al. Association between neutrophil-to-high-density lipoprotein cholesterol ratio and metabolic dysfunction-associated steatotic liver disease and liver fibrosis in the US population: a nationally representative cross-sectional study using NHANES data from 2017 to 2020. *BMC gastroenterology*. 2024;24:300.
13. Zhu N, Li Y, Lin Y, et al. Association between neutrophil-to-high-density lipoprotein cholesterol ratio and non-alcoholic fatty liver disease or metabolic dysfunction-associated steatotic liver disease: evidence from NHANES 2017-2020. *Frontiers in Medicine*.11:1491858.
14. Ren H, Zhu B, Zhao Z, et al. Neutrophil to high-density lipoprotein cholesterol ratio as the risk mark in patients with type 2 diabetes combined with acute coronary syndrome: a cross-sectional study. *Scientific Reports*. 2023;13:7836.
15. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. *Nature Reviews Immunology*. 2018;18:134-147.
16. Talukdar S, Oh DY, Bandyopadhyay G, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nature medicine*. 2012;18:1407-1412.
17. Heeren J and Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. *Molecular metabolism*. 2021;50:101238.
18. Xu J, Guo Y, Huang X, et al. Effects of DHA dietary intervention on hepatic lipid metabolism in apolipoprotein E-deficient and C57BL/6J wild-type mice. *Biomedicine & Pharmacotherapy*. 2021;144:112329.
19. Mensenkamp AR, Havekes LM, Romijn JA, et al. Hepatic steatosis and very low density lipoprotein secretion: the involvement of apolipoprotein E. *Journal of hepatology*. 2001;35:816-822.
20. Soehnlein O and Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. *Nature Reviews Immunology*. 2010;10:427-439.
21. Rader DJ and Hovingh GK. HDL and cardiovascular disease. *The Lancet*. 2014;384:618-625.
22. Hamer M, O'Donovan G and Stamatakis E. High-density lipoprotein cholesterol and mortality: too much of a good thing? *Arteriosclerosis, thrombosis, and vascular biology*. 2018;38:669-672.
23. Bheemanaboina G, Deverashetty JR and Bashetti S. The Facts About High Density Lipoprotein Cholesterol (HDL-C). I'm Pretty Good Within Limits. *Acta Scientific NUTRITIONAL HEALTH (ISSN: 2582-1423)*. 2023;7.
24. Barter PJ, Nicholls S, Rye K-A, et al. Antiinflammatory properties of HDL. *Circulation research*. 2004;95:764-772.
25. Mineo C, Deguchi H, Griffin JH, et al. Endothelial and antithrombotic actions of HDL. *Circulation research*. 2006;98:1352-1364.
26. Higashi Y. Endothelial Function in dyslipidemia: roles of LDL-cholesterol, HDL-cholesterol and triglycerides. *Cells*. 2023;12:1293.
27. Wu Y, Xu Y, Chen J, et al. HDL and endothelial function. In: HDL Metabolism and Diseases. Springer; 2022:27-47.
28. van der Poorten D, Milner KL, Hui J, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology*. 2008;48:449-457.
29. Liu M, Liu X, Wei Z, et al. MHR and NHR but not LHR were associated with coronary artery disease in patients with chest pain with controlled LDL-C. *Journal of Investigative Medicine*. 2022;70:1501-1507.

 doi:10.20944/preprints202501.0349.v1

30. Chuang S-M, Liu S-C, Chien M-N, et al. Neutrophil-to-High-Density Lipoprotein Ratio (NHR) and Neutrophil-to-Lymphocyte Ratio (NLR) as prognostic biomarkers for incident cardiovascular disease and all-cause mortality: A comparison study. *American Journal of Preventive Cardiology*. 2024;20:100869.
31. Gkantzios A, Tsiptsios D, Karapepera V, et al. Monocyte to HDL and neutrophil to HDL ratios as potential ischemic stroke prognostic biomarkers. *Neurology international*. 2023;15:301-317.
32. Han R, Li J, Liu Y, et al. Neutrophil to Lymphocyte Ratio is a Better Tool than Monocyte to HDL-C Ratio and Neutrophil to HDL-C Ratio in Type 2 Diabetic Kidney Disease. 2023.
33. Liu S-L, Feng B-Y, Song Q-R, et al. Neutrophil to high-density lipoprotein cholesterol ratio predicts adverse cardiovascular outcomes in subjects with pre-diabetes: a large cohort study from China. *Lipids in Health and Disease*. 2022;21:86.
34. Mokhtare M, Abdi A, Sadeghian AM, et al. Investigation about the correlation between the severity of metabolic-associated fatty liver disease and adherence to the Mediterranean diet. *Clinical Nutrition ESPEN*. 2023;58:221-227.
35. Sotoudeheian Mj. Value of Mac-2 Binding Protein Glycosylation Isomer (M2BPGi) in Assessing Liver Fibrosis in Metabolic Dysfunction-Associated Liver Disease: A Comprehensive Review of its Serum Biomarker Role. *Current protein & peptide science*. 2025;26:6-21.
36. Sotoudeheian MJ, Azarbad R, Mirahmadi S-M-S, et al. The Role of Metformin in Modifying Ferroptosis to Treat Metabolic Dysfunction-Associated Fatty Liver Disease: A Narrative Review. *Current Reviews in Clinical and Experimental Pharmacology*. 2024.
37. Sotoudeheian M. Galectin-3 and Severity of Liver Fibrosis in Metabolic Dysfunction-Associated Fatty Liver Disease. *Protein and Peptide Letters*. 2024;31:290-304.
38. Lamichhane P, Agrawal A, Abouainain Y, et al. Utility of neutrophil-to-high-density lipoprotein-cholesterol ratio in patients with coronary artery disease: a narrative review. *Journal of International Medical Research*. 2023;51:03000605231166518.
39. Bartneck M and Wang J. Therapeutic targeting of neutrophil granulocytes in inflammatory liver disease. *Frontiers in immunology*. 2019;10:2257.
40. Charan HV, Dwivedi DK, Khan S, et al. Mechanisms of NLRP3 inflammasome-mediated hepatic stellate cell activation: Therapeutic potential for liver fibrosis. *Genes & Diseases*. 2023;10:480-494.
41. Tang C, Peng D, Zong K, et al. Association between the lymphocyte-to-high-density lipoprotein ratio and metabolic dysfunction-associated steatotic liver disease among US adults: a cross-sectional study from NHANES 2017 to 2020. *BMC gastroenterology*. 2024;24:470.
42. Meroni M, Longo M and Dongiovanni P. Cardiometabolic risk factors in MASLD patients with HCC: the other side of the coin. *Frontiers in Endocrinology*. 2024;15:1411706.
43. Choi Y-J, Kim Y and Hwang S. Role of Neutrophils in the Development of Steatotic Liver Disease. In: Seminars in Liver Disease. Thieme Medical Publishers, Inc.; 2024.
44. Sandireddy R, Sakthivel S, Gupta P, et al. Systemic impacts of metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH) on heart, muscle, and kidney related diseases. *Frontiers in cell and developmental biology*. 2024;12:1433857.
45. Sotoudeheian M and Hoseini S. Therapeutic properties of polyphenols affect AMPK molecular pathway in hyperlipidemia. 2023.
46. Liu K, Wang F-S and Xu R. Neutrophils in liver diseases: pathogenesis and therapeutic targets. *Cellular & molecular immunology*. 2021;18:38-44.
47. Yang W, Tao Y, Wu Y, et al. Neutrophils promote the development of reparative macrophages mediated by ROS to orchestrate liver repair. *Nature communications*. 2019;10:1076.
48. Wang H, Zhang H, Wang Y, et al. Regulatory T-cell and neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. *Journal of hepatology*. 2021;75:1271-1283.
49. Rao G, Peng X, Li X, et al. Unmasking the enigma of lipid metabolism in metabolic dysfunction-associated steatotic liver disease: from mechanism to the clinic. *Frontiers in Medicine*. 2023;10:1294267.
50. Yanai H, Adachi H, Hakoshima M, et al. Metabolic-Dysfunction-Associated Steatotic Liver Disease—Its Pathophysiology, Association with Atherosclerosis and Cardiovascular Disease, and Treatments. *International journal of molecular sciences*. 2023;24:15473.

 doi:10.20944/preprints202501.0349.v1

51. Trieb M, Horvath A, Birner-Gruenberger R, et al. Liver disease alters high-density lipoprotein composition, metabolism and function. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2016;1861:630-638.
52. Liu X and Green RM. Endoplasmic reticulum stress and liver diseases. *Liver research*. 2019;3:55-64.
53. Vairappan B. Endothelial dysfunction in cirrhosis: Role of inflammation and oxidative stress. *World journal of hepatology*. 2015;7:443.
54. Wang X, Zhang L and Dong B. Molecular mechanisms in MASLD/MASH related HCC. *Hepatology*. 2024:10.1097.
55. Cheng Z, Chu H, Seki E, et al. Hepatocyte programmed cell death: The trigger for inflammation and fibrosis in metabolic dysfunction-associated steatohepatitis. *Frontiers in Cell and Developmental Biology*. 2024;12:1431921.
56. Avcı E and Ozturk M. Evaluation of neutrophil HDL-C ratio—a new inflammation marker in non-alcoholic fatty liver disease. *Endokrynologia Polska*. 2023;74:610-615.
57. Zhao M, Yi Z, Zhang B, et al. Fatty liver disease: Functional foods offer natural remedies. *Functional Foods and Chronic Disease*. 2024:115-152.
58. Sodhani S. The effect of grape seed polyphenols and delta tocotrienol as supplements in improvement of NASH histopathology in C57BL/J6 mice fed high fat diet. In.: Texas Woman's University; 2012.
59. Velissaridou A, Panoutsopoulou E, Prokopiou V, et al. Cardio-Protective-Promoting Properties of Functional Foods Inducing HDL-Cholesterol Levels and Functionality. *Nutraceuticals*. 2024;4:469-502.
60. Habib S. Team players in the pathogenesis of metabolic dysfunctions-associated steatotic liver disease: The basis of development of pharmacotherapy. *World Journal of Gastrointestinal Pathophysiology*. 2024;15:93606.
61. Siebel AL, Heywood SE and Kingwell BA. HDL and glucose metabolism: current evidence and therapeutic potential. *Frontiers in pharmacology*. 2015;6:258.
62. Annema W, Dikkers A, de Boer JF, et al. Impaired HDL cholesterol efflux in metabolic syndrome is unrelated to glucose tolerance status: the CODAM study. *Scientific reports*. 2016;6:27367.
63. Jia Z, Li Z and Chen S. The Association Between Neutrophil/High-Density Lipoprotein Cholesterol Ratio and Non-Alcoholic Fatty Liver Disease in a Healthy Population. *Diabetes, Metabolic Syndrome and Obesity*. 2024:2597-2605.
64. Mokhtare M, Sadeghian AM and Sotoudeheian M. S1390 The Accuracy and Reliability of AST to Platelet Ratio Index, FIB-4, FIB-5, and NAFLD Fibrosis Scores in Detecting Advanced Fibrosis in Patients With Metabolic-Associated Fatty Liver Disease. *Official journal of the American College of Gastroenterology| ACG*. 2023;118:S1064-S1065.
65. Sotoudeheian M. Agile 3+ and Metabolic Dysfunction-Associated Fatty Liver Disease: Detecting Advanced Fibrosis based on Reported Liver Stiffness Measurement in FibroScan and Laboratory Findings. *The International Journal of Gastroenterology and Hepatology Diseases*. 2024;3:E270624231398.
66. Sotoudeheian M and Azarbad R. Investigating the Correlation between Polyunsaturated Fatty Acids Intake and Non-Invasive Biomarkers of Liver Fibrosis. *Clinical Nutrition ESPEN*. 2024.
67. Petta S and Giannetti A. Non-invasive Diagnostic Approach to NASH: Biological Markers. *Non-Alcoholic Fatty Liver Disease: A 360-degree Overview*. 2020:235-256.
68. Shi K, Hou J, Zhang Q, et al. Neutrophil-to-high-density-lipoprotein-cholesterol ratio and mortality among patients with hepatocellular carcinoma. *Frontiers in Nutrition*. 2023;10:1127913.