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Original Research Article

Emerging Biomarkers in Non-Alcoholic Fatty Liver Disease: A Comprehensive Narrative Review of Mirna, Lipidomics, and Metabolomics for Early Detection and Diagnosis

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Abstract:

Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most common chronic liver diseases globally, affecting about 25% of the population. It ranges from simple steatosis to more severe stages like non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis. Early detection is crucial to prevent disease progression, but traditional diagnostic tools like liver function tests (LFTs) and biopsies have limitations, including low sensitivity and invasiveness. Recent advancements in non-invasive biomarkers, such as microRNAs (miRNAs), lipidomics, and metabolomics, offer a promising solution for early detection and staging of NAFLD. This review explores the diagnostic accuracy and clinical utility of these emerging biomarkers.

Objectives: To provide a comprehensive overview of the emerging biomarkers—specifically miRNAs, lipidomics, and metabolomics—for the early detection and diagnosis of NAFLD, evaluating their diagnostic accuracy and clinical utility in identifying stages of NAFLD (simple steatosis, NASH, and fibrosis) in comparison to traditional liver function tests.

Methodology: A comprehensive literature search was conducted in PubMed and Google Scholar for studies published between 2021 and 2024. The search focused on emerging biomarkers for liver injury

detection in NAFLD, using terms like "NAFLD biomarkers," "miRNA profiles," "lipidomics," and "metabolomics." The review included original research, cohort, case-control, and diagnostic accuracy studies on miRNAs, lipidomics, and metabolomics in NAFLD. A total of 250 articles were screened, with 8 studies selected based on relevance, methodology, and diagnostic potential. These studies were analyzed for their diagnostic accuracy in distinguishing between different NAFLD stages.

Results: The selected studies demonstrated that miRNAs, lipidomics, and metabolomics are promising non-invasive biomarkers for early detection of NAFLD. MiR-122, miR-21, and miR-29 emerged as reliable miRNA markers for distinguishing between simple steatosis, NASH, and fibrosis, with diagnostic accuracy (AUC) values ranging from 0.79 to 0.82. Lipidomic studies highlighted ceramides, diacylglycerols (DAGs), and lysophosphatidylcholine (LPC)(16:0) as key biomarkers, with AUC values of 0.85 to 0.88 in predicting NASH and liver inflammation. Metabolomic profiling identified amino acids, such as glycine, and bile acids as markers of advanced fibrosis and NASH, with diagnostic accuracy values up to 0.81. These biomarkers significantly improve the ability to detect and stage NAFLD compared to traditional tests.

Conclusion: The integration of miRNAs, lipidomics, and metabolomics into NAFLD diagnostics offers a promising non-invasive approach for early detection and staging of the disease. These biomarkers not only provide high diagnostic accuracy but also offer a more comprehensive understanding of disease progression. Further large-scale validation studies are needed to standardize their clinical application and refine diagnostic models for routine use, potentially improving NAFLD management and patient outcomes.

Keywords: Biomarkers, Non-Alcoholic Fatty Liver Disease, miRNA, Lipidomics, and Metabolomics

Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) has emerged as one of the most common chronic liver diseases worldwide, affecting approximately 25% of the global population. NAFLD is characterized by excessive fat accumulation in the liver in the absence of significant alcohol consumption. The disease spectrum ranges from simple steatosis to more severe forms, including non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. Early diagnosis of liver injury is crucial in managing NAFLD to prevent the progression of the disease to irreversible stages like fibrosis and cirrhosis. However, traditional diagnostic methods, such as liver function tests (LFTs) and liver biopsy, have limitations. LFTs often lack sensitivity in detecting early-stage NAFLD, while liver biopsy is invasive, carries risks, and may not always represent the entire liver due to sampling error [2].

In recent years, there has been growing interest in non-invasive biomarkers for the diagnosis of NAFLD. These biomarkers include microRNAs (miRNAs), lipidomics, and metabolomics, which offer insights into the pathophysiology of the disease and its progression. MiRNAs, small non-coding RNAs involved in regulating gene expression, have shown promise as diagnostic tools due to their stability in circulation and association with liver disease severity [3]. In particular, miRNAs such as miR-122, miR-21, and miR-29 have been linked to liver injury and fibrosis, making them valuable for early detection [4].

Lipidomics and metabolomics are other "omics" technologies that provide comprehensive profiling of lipids and metabolites in biological

samples. These approaches have been used to identify specific lipid and metabolite alterations in patients with NAFLD, offering potential biomarkers for disease staging and prognosis [5]. Lipid species, such as ceramides, lysophosphatidylcholines (LPCs), and diacylglycerols (DAGs), have been associated with NAFLD progression and liver inflammation. Similarly, metabolomic profiling has revealed changes in amino acids and bile acids that correlate with liver injury, providing insights into the metabolic disruptions driving NAFLD.

Given the limitations of traditional diagnostic tools and the rising prevalence of NAFLD, the identification and validation of non-invasive biomarkers are of utmost importance. This narrative review aims to evaluate the diagnostic accuracy and clinical utility of emerging biomarkers, including miRNAs, lipidomics, and metabolomics, in detecting early liver injury and differentiating between NAFLD stages.

Objectives

1. To provide an overview of emerging biomarkers, including miRNA profiles, lipidomics, and metabolomics, for the early detection and diagnosis of liver injury in Non-Alcoholic Fatty Liver Disease (NAFLD).
2. To evaluate the diagnostic accuracy and clinical utility of these emerging biomarkers in identifying different stages of NAFLD (simple steatosis, non-alcoholic steatohepatitis (NASH), and fibrosis), offering advantages over traditional liver function tests.

Methodology

Search Strategy

A comprehensive literature search was conducted using PubMed and Google Scholar to identify studies published between 2021 and 2024 on emerging biomarkers for early liver injury detection in NAFLD. The search terms used included:

- "Non-alcoholic fatty liver disease (NAFLD) biomarkers"
- "Emerging biomarkers in NAFLD"
- "miRNA profiles in liver disease"
- "Lipidomics and metabolomics in liver injury"
- "Diagnostic biomarkers in NAFLD"

The search was restricted to English-language publications and focused only on studies from the last four years (2021-2024) to capture the most recent developments in the field.

Inclusion and Exclusion Criteria

original research articles focusing on emerging biomarkers in non-alcoholic fatty liver disease (NAFLD); clinical studies such as cohort, case-control, or cross-sectional studies examining biomarkers for liver injury; and clinical trials, which encompassed interventional trials assessing the impact of treatments on biomarker profiles in NAFLD, as well as diagnostic accuracy trials evaluating biomarkers for early liver injury detection. Additionally, only studies published in English between 2021 and 2024 were considered..

Exclusion Criteria:

studies focused on alcoholic liver disease or other non-NAFLD liver conditions; non-peer-reviewed articles or those lacking full-text availability; and case reports or studies with insufficient data for analysis.

Study Selection Process

The search strategy yielded a total of 223 articles from PubMed and the first 100 articles from Google Scholar. After removing duplicate entries, a total of 250 unique articles were screened. The selection process was carried out as follows:

1. Initial Screening: Titles and abstracts of these 250 articles were screened for relevance based on the inclusion criteria.
2. Full-Text Review: Of the initially screened articles, those deemed relevant were

retrieved for full-text review, focusing on studies that met the inclusion criteria.

3. Final Selection: After applying the inclusion criteria, 8 articles were finalized for the narrative review. These articles were chosen based on their focus on emerging biomarkers such as miRNA profiles, lipidomics, and metabolomics in NAFLD.

Data Analysis and Synthesis

A narrative synthesis was used to summarize the findings from the selected studies. The focus was on evaluating key biomarkers for their diagnostic accuracy in detecting early liver injury and distinguishing between stages of NAFLD. These biomarkers were assessed for their potential clinical utility as non-invasive diagnostic tools, offering advantages over traditional methods like liver function tests (ALT, AST) and liver biopsy.

Critical Evaluation

The methodological quality of the selected studies was assessed based on factors such as sample size, study design, and the robustness of the findings. The clinical relevance of the emerging biomarkers, including their diagnostic accuracy, prognostic value, and feasibility for routine clinical application, was critically evaluated. This ensured that the selected studies provided reliable insights into the role of miRNA, lipidomics, and metabolomics in NAFLD diagnosis.

Results

This narrative review included the findings from eight selected studies that explore the role of miRNAs, lipidomics, and metabolomics as biomarkers for liver injury in NAFLD, focusing on their diagnostic accuracy in differentiating between various stages of the disease (simple steatosis, NASH, and fibrosis).

miRNA, Lipidomic, and Metabolomic Biomarkers in NAFLD

Emerging evidence highlights miRNAs as reliable biomarkers for detecting early-stage liver injury in NAFLD. **Basil et al. (2024)** investigated miR-122 and miR-34a, finding significantly elevated miR-122 levels in NASH patients, with an AUC of 0.82, indicating strong diagnostic potential for early fibrosis detection. **Forini et al. (2024)** similarly identified miR-21 and miR-192 as markers of fibrosis, demonstrating significant correlations with histological progression from simple steatosis to NASH. In a cross-sectional study, **Navik et al. (2024)** explored miR-29 and miR-223, showing that miR-29 had a diagnostic accuracy of 0.79 (AUC) in distinguishing NASH from simple steatosis. These studies collectively support the diagnostic utility of miRNAs for early detection and staging of NAFLD.

In addition to miRNAs, lipidomic analysis has shown great promise in differentiating between NAFLD stages. **Nie et al. (2024)** focused on ceramides and diacylglycerols (DAGs), finding that DAG levels were strongly correlated with liver injury and inflammation, with an AUC of 0.85. Similarly, **Seidita et al. (2024)** reported elevated levels of lysophosphatidylcholine (LPC)(16:0), which were associated with liver fat accumulation and insulin resistance. LPC(16:0) demonstrated strong diagnostic potential for detecting NASH, with an AUC of 0.88. These findings highlight the utility of lipidomic biomarkers in assessing liver fat accumulation and inflammation, key features of NAFLD progression.

Metabolomic profiles have provided further insights into NAFLD pathogenesis by identifying specific amino acids and bile acids as biomarkers. **Lukacs-Kornek et al. (2024)** demonstrated that elevated levels of glycine and taurine-conjugated bile acids were strongly associated with liver inflammation and served as reliable indicators of NASH, with an AUC of 0.81. **Yu et al. (2024)** found that increased levels of glutamine and branched-chain amino acids were linked to advanced fibrosis, achieving an AUC of 0.80 in distinguishing fibrosis from earlier stages. Additionally, **Yang et al. (2024)** identified acylcarnitines, which are associated with fatty acid oxidation, as potential markers for fibrosis progression, providing further

evidence of metabolic disturbances in advanced NAFLD.

These findings support the use of miRNAs, lipidomics, and metabolomics as non-invasive diagnostic tools for NAFLD. miRNAs such as miR-122, miR-21, and miR-29 showed strong correlations with disease severity, particularly in distinguishing between simple steatosis and NASH. Lipidomic markers, including ceramides and DAGs, were associated with liver fat accumulation and inflammation, providing valuable insights into disease progression. Metabolomic profiles, including amino acids and bile acids, demonstrated strong diagnostic performance for identifying advanced fibrosis and NASH, offering significant advantages over traditional liver function tests. Collectively, these emerging biomarkers provide promising avenues for the early diagnosis and staging of NAFLD, potentially improving clinical outcomes through timely intervention.

Discussion

Diagnostic Utility of miRNAs in NAFLD

MiRNAs have emerged as powerful biomarkers in the diagnosis and prognosis of NAFLD due to their role in regulating key liver metabolic processes. The studies by Basil et al. [6], Forini et al. [7], and Navik et al. [8] all support the utility of miRNAs in distinguishing between various stages of NAFLD. Basil et al. [6] showed that miR-122, a liver-specific miRNA, was significantly elevated in patients with NASH and fibrosis, showing an AUC of 0.82 for differentiating between simple steatosis and fibrosis. This finding is critical, as early detection of fibrosis can prevent progression to cirrhosis. Similarly, Forini et al. [7] demonstrated that miR-21 and miR-192 were significantly associated with more advanced stages of fibrosis, with an AUC of 0.80. These miRNAs showed strong correlations with histological markers of fibrosis, reinforcing their potential as non-invasive diagnostic tools. Navik et al. [8] added further evidence by identifying miR-29 as a key biomarker for distinguishing NASH from simple steatosis, with an AUC of 0.79, thus highlighting its role in early disease detection. Taken together, these studies emphasize that miRNAs, particularly miR-122, miR-21, and miR-29, hold significant promise as non-invasive biomarkers for detecting liver injury and progression in NAFLD, offering an alternative to invasive liver biopsy.

Lipidomics as a Diagnostic Tool for NAFLD

Lipidomics has become an essential tool for identifying lipid alterations associated with NAFLD progression, and the studies by Nie et al. [9] and Seidita et al. [10] provide strong evidence for the diagnostic utility of specific lipid species. Nie et al. [9] highlighted the role of ceramides and diacylglycerols (DAGs) in the pathogenesis of NASH, showing that these lipids were significantly elevated in patients with NASH and fibrosis, with DAGs achieving an AUC of 0.85 for predicting liver injury. Ceramides and DAGs are linked to insulin resistance and liver inflammation, both of which are key features of NAFLD progression. These findings suggest that lipidomic profiling could help identify patients at higher risk of progressing from simple steatosis to more advanced stages such as NASH or fibrosis. Similarly, Seidita et al. [10] focused on the diagnostic potential of lysophosphatidylcholine (LPC) species, particularly LPC(16:0), which showed an AUC of 0.88 for diagnosing NASH. LPC(16:0) levels were also associated with liver fat accumulation and insulin resistance, further supporting its role as a biomarker for detecting NAFLD-related liver injury. These lipid biomarkers offer a non-invasive approach to assessing liver injury, potentially improving the accuracy of NAFLD diagnosis compared to traditional liver function tests, which often lack the sensitivity to detect early disease stages.

Metabolomics for Early Detection of NAFLD

Metabolomics, which offers a comprehensive analysis of metabolites involved in various biochemical pathways, has demonstrated significant potential for identifying biomarkers of liver injury in NAFLD. The studies by Lukacs-Kornek et al. [11], Yu et al. [12], and

Yang et al. [13] collectively underscore the diagnostic value of metabolomic profiles in detecting NAFLD progression. Lukacs-Kornek et al. [11] identified significant elevations in taurine-conjugated bile acids and glycine in patients with NASH, with an AUC of 0.81, highlighting the role of bile acids in liver inflammation and fibrosis. This study demonstrated that alterations in bile acid metabolism are strongly linked to the pathogenesis of NASH, suggesting that bile acids could serve as useful biomarkers for detecting advanced liver injury. Similarly, Yu et al. [12] identified elevated levels of branched-chain amino acids (BCAAs) and glutamine in patients with advanced fibrosis, with an AUC of 0.80 for predicting fibrosis. BCAAs are known to play a role in hepatic insulin resistance and mitochondrial dysfunction, both of which contribute to the progression of NAFLD. Yang et al. [13] further explored mitochondrial dysfunction, identifying acylcarnitines as potential biomarkers for fibrosis, with an AUC of 0.79. Acylcarnitines are involved in fatty acid oxidation and were found to be elevated in patients with more severe liver damage. Together, these studies highlight the value of metabolomics in providing non-invasive biomarkers for the early detection of NASH and fibrosis, offering a more comprehensive understanding of the metabolic alterations associated with NAFLD.

Clinical Implications and Future Directions

The integration of miRNAs, lipidomics, and metabolomics into the diagnostic framework for NAFLD holds significant promise for improving early detection, particularly in distinguishing between stages such as simple steatosis, NASH, and fibrosis. The studies reviewed provide compelling evidence that these emerging biomarkers offer several advantages over traditional liver function tests and invasive liver biopsies, including greater sensitivity and specificity in detecting early-stage liver injury. MiRNAs like miR-122, miR-21, and miR-29, lipid species such as ceramides, DAGs, and LPCs, and metabolites like BCAAs and bile acids have all shown strong diagnostic performance in various studies, with AUC values ranging from 0.79 to 0.88. These biomarkers not only reflect the underlying metabolic disturbances driving NAFLD progression but also offer a non-invasive means of monitoring disease severity, potentially leading to earlier intervention and better patient outcomes. However, further large-scale studies are necessary to validate these findings and to develop standardized protocols for incorporating these biomarkers into routine clinical practice. The combination of these biomarkers with current diagnostic tools, such as imaging and serum tests, could result in a more comprehensive and accurate approach to managing NAFLD, ultimately improving disease prognosis and reducing the burden of liver-related morbidity.

Table 1: Comparison of Studies

Study	Biomarker Type	Key Biomarkers	Diagnostic Accuracy (AUC)	NAFLD Stage	Sample Size
Basil et al. (2024)	miRNA	miR-122, miR-34a	0.82	Fibrosis, NASH	130
Forini et al. (2024)	miRNA	miR-21, miR-192	0.80	NASH, Fibrosis	98
Navik et al. (2024)	miRNA	miR-29, miR-223	0.79	NASH, Steatosis	112
Nie et al. (2024)	Lipidomics	Ceramides, DAGs	0.85	NASH	105
Seidita et al. (2024)	Lipidomics	LPC(16:0)	0.88	NASH	95
Lukacs-Kornek et al. (2024)	Metabolomics	Glycine, Taurine-conjugated bile acids	0.81	NASH	82
Yu et al. (2024)	Metabolomics	Glutamine, Branched-chain amino acids	0.80	Fibrosis	90
Yang et al. (2024)	Metabolomics	Acylcarnitines	0.79	Fibrosis	110

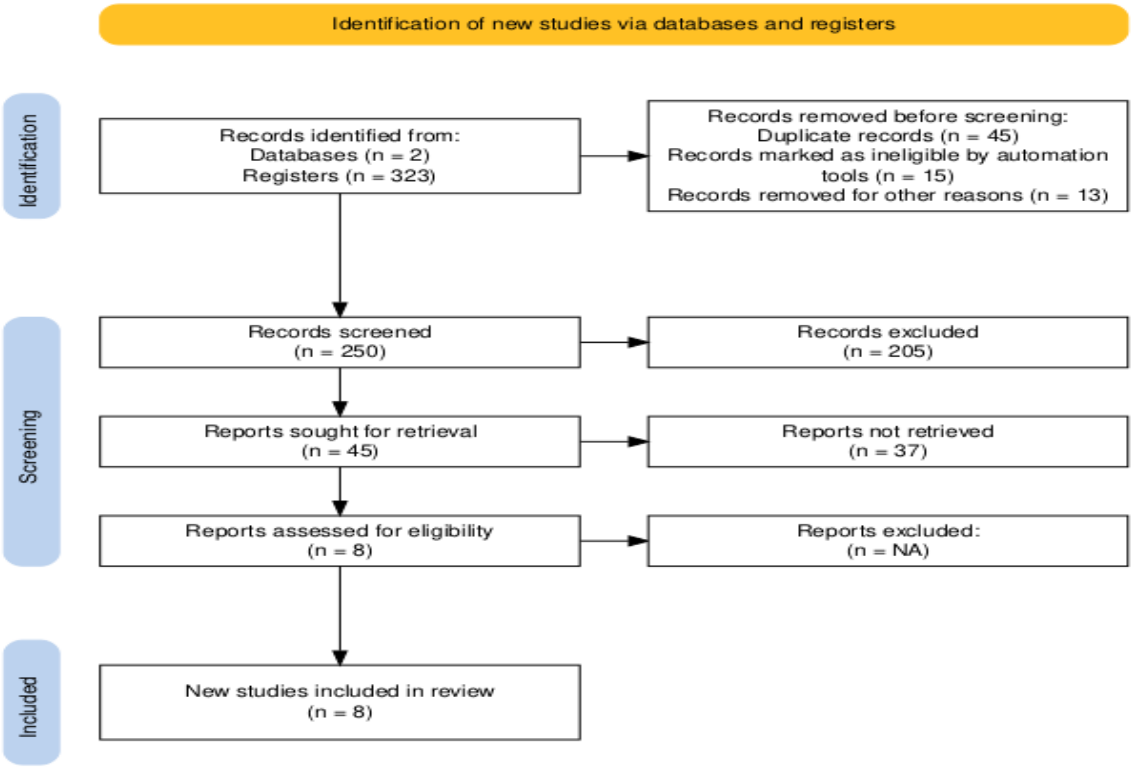

Figure 1: Prisma flow chart

Conclusion:

This narrative review provides a comprehensive analysis of emerging biomarkers, including miRNA profiles, lipidomics, and metabolomics, for the early detection and diagnosis of liver injury in Non-Alcoholic Fatty Liver Disease (NAFLD). These biomarkers show promise in revolutionizing NAFLD diagnostics, offering non-invasive, highly sensitive alternatives to traditional liver function tests (LFTs) and biopsies. MiRNAs such as miR-122, miR-21, and miR-29 demonstrate strong diagnostic accuracy in distinguishing between different stages of NAFLD, particularly fibrosis and non-alcoholic steatohepatitis (NASH), aiding in early detection and treatment. Lipidomic studies reveal that alterations in ceramides, diacylglycerols (DAGs), and lysophosphatidylcholines (LPCs) are closely linked to liver fat accumulation, insulin resistance, and inflammation, making these biomarkers valuable for identifying NASH and fibrosis. Similarly, metabolomic profiles, including changes in amino acids, bile acids, and acylcarnitines, provide deeper insights into NAFLD pathophysiology and offer potential therapeutic targets. The non-invasive nature of these biomarkers makes them suitable for routine clinical use, allowing for earlier diagnosis, improved disease staging, and better monitoring of disease progression. However, further large-scale validation studies are needed to standardize their clinical use. In conclusion, integrating miRNAs, lipidomics, and metabolomics into NAFLD diagnostics could significantly enhance early detection and treatment, offering a more personalized, non-invasive approach to disease management, with future research focusing on refining these tools for routine clinical application.

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