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Histological Features Associated with Hepatocellular Carcinoma Risk in NAFLD: A Quantitative Systematic Review

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) has emerged as one of the leading causes of chronic liver disease worldwide and is increasingly recognized as a major contributor to hepatocellular carcinoma (HCC). Although advanced fibrosis and cirrhosis are established risk factors for HCC, the role of individual histopathological features in NAFLD-related hepatocarcinogenesis remains incompletely understood. ***Objective:*** To systematically evaluate the association between key histopathological characteristics of NAFLD and the risk of developing hepatocellular carcinoma. ***Methods:*** A systematic review and meta-analysis was conducted following PRISMA 2020 guidelines. Electronic databases including PubMed, Embase, Scopus, Web of Science, and the Cochrane Library were searched from inception to March 2026. Studies involving biopsy-confirmed NAFLD patients that reported histopathological findings in relation to HCC development were included. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using random-effects models. ***Results:*** Twenty-two studies comprising 18,764 patients with biopsy-confirmed NAFLD were included. Among these, 2,983 patients developed hepatocellular carcinoma. Advanced fibrosis was associated with a significantly increased risk of HCC (OR 5.84, 95% CI 4.11–8.30). Cirrhosis demonstrated the strongest association with malignancy (OR 8.96, 95% CI 6.01–13.36). Histological non-alcoholic steatohepatitis increased HCC risk by nearly three-fold (OR 2.71, 95% CI 1.88–3.90). Significant lobular inflammation (OR 2.43, 95% CI 1.65–3.58) and hepatocyte ballooning degeneration (OR 2.12, 95% CI 1.43–3.15) were independently associated with carcinogenesis. Severe steatosis alone was not significantly associated with HCC development (OR 1.28, 95% CI 0.92–1.78). ***Conclusion:*** Advanced fibrosis and cirrhosis remain the most important histopathological predictors of hepatocellular carcinoma in patients with NAFLD. However, inflammatory activity and steatohepatitis independently contribute to malignant transformation, suggesting that HCC surveillance strategies should incorporate both fibrosis stage and histological disease activity to identify high-risk individuals.

Keywords: Non-alcoholic fatty liver disease; NAFLD; Non-alcoholic steatohepatitis; NASH; Hepatocellular carcinoma; Histopathology; Fibrosis; Cirrhosis; Meta-analysis; Systematic review

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most prevalent chronic liver disease worldwide and currently affects approximately one-quarter of the global adult population [1,2]. Characterized by excessive hepatic fat accumulation in the absence of significant alcohol consumption or other secondary causes of steatosis, NAFLD represents a growing public health challenge driven by the increasing prevalence of obesity, insulin resistance, type 2 diabetes mellitus, and metabolic syndrome [3,4]. The disease spectrum ranges from simple hepatic steatosis to non-alcoholic steatohepatitis (NASH), progressive fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [5].

Over the past two decades, substantial changes in the epidemiology of chronic liver disease have occurred globally. Historically, viral hepatitis and alcohol-related liver disease represented the dominant causes of hepatocellular carcinoma. However, improvements in antiviral therapies and vaccination programs have led to a relative decline in virus-associated liver cancer, while the burden of NAFLD-related HCC has increased steadily [6,7]. Several epidemiological studies have

demonstrated that NAFLD is now among the fastest-growing causes of hepatocellular carcinoma in both Western and Asian populations [8,9]. Current projections indicate that NAFLD-associated HCC will continue to rise substantially in parallel with global increases in obesity and diabetes prevalence [10].

Hepatocellular carcinoma is the sixth most frequently diagnosed cancer and the third leading cause of cancer-related mortality worldwide [11]. Despite advances in diagnostic and therapeutic approaches, prognosis remains poor because many patients are diagnosed at advanced stages. Consequently, identifying individuals at high risk for HCC development has become a major clinical priority. While cirrhosis is traditionally regarded as the principal risk factor for HCC, emerging evidence indicates that NAFLD-related HCC may develop even in the absence of established cirrhosis [12]. This unique feature distinguishes NAFLD-associated hepatocarcinogenesis from many other chronic liver diseases and suggests the involvement of additional pathogenic mechanisms.

The biological processes underlying NAFLD progression and malignant transformation are complex and multifactorial. Insulin resistance, lipotoxicity, oxidative stress, chronic inflammation, mitochondrial dysfunction, endoplasmic reticulum stress, and genetic susceptibility collectively contribute to hepatocyte injury and carcinogenesis [13,14]. Persistent metabolic stress promotes the accumulation of reactive oxygen species and lipid peroxidation products that induce DNA damage and genomic instability [15]. Simultaneously, chronic inflammatory signaling activates oncogenic pathways that enhance cellular proliferation, inhibit apoptosis, and facilitate tumor development [16].

Histopathological evaluation remains the gold standard for assessing disease severity in NAFLD and provides direct insight into the structural alterations associated with disease progression [17]. The histological diagnosis of NAFLD is based on several key features, including steatosis, lobular inflammation, hepatocyte ballooning, fibrosis, and cirrhosis [18]. These pathological findings are incorporated into validated scoring systems such as the NAFLD Activity Score (NAS) and Brunt fibrosis staging system, which are widely used in both clinical practice and research settings [19].

Among these histological variables, fibrosis has consistently emerged as the most important predictor of adverse clinical outcomes. Numerous longitudinal studies have demonstrated that increasing fibrosis stage is associated with higher risks of liver-related mortality, hepatic decompensation, liver transplantation, and hepatocellular carcinoma [20,21]. Advanced fibrosis reflects cumulative liver injury and is characterized by excessive extracellular matrix deposition, vascular remodeling, and architectural distortion. These changes create a microenvironment that promotes cellular transformation and tumor progression [22].

Nevertheless, fibrosis may not be the sole histopathological determinant of HCC development. Increasing evidence suggests that active steatohepatitis also plays an important role in carcinogenesis. NASH is characterized by hepatocellular injury, ballooning degeneration, and inflammatory infiltration, all of which contribute to ongoing tissue damage and regenerative proliferation [23]. Experimental studies have demonstrated that chronic inflammatory signaling mediated by tumor necrosis factor-alpha, interleukin-6, and nuclear factor-kappa B can directly promote hepatocarcinogenesis through activation of oncogenic pathways [24,25].

Lobular inflammation has similarly been implicated as a potential driver of malignant transformation. Persistent inflammatory cell infiltration leads to repeated cycles of hepatocyte injury and regeneration, increasing the likelihood of mutational events and clonal expansion [26]. Furthermore, inflammatory mediators stimulate angiogenesis and modify the hepatic microenvironment in ways that favor tumor growth and progression [27]. Several observational studies have reported significant associations between inflammatory activity and HCC risk independent of fibrosis severity, although findings have not been entirely consistent [28].

Hepatocyte ballooning represents another important histological marker of disease activity. Ballooned hepatocytes exhibit cytoskeletal disruption, mitochondrial dysfunction, and increased oxidative stress, reflecting severe cellular injury [29]. These pathological changes may contribute directly to carcinogenic processes by promoting genomic instability and altering intracellular signaling pathways. Although ballooning is a defining feature of NASH, its independent role in HCC development remains incompletely characterized.

The contribution of steatosis itself to hepatocarcinogenesis is also controversial. While excessive lipid accumulation serves as the initial hallmark of NAFLD, studies have reported conflicting results regarding its association with HCC risk [30]. Some investigations suggest that steatosis contributes to lipotoxic injury and oxidative stress, whereas others indicate that steatosis alone may be relatively benign in the absence of accompanying inflammation and fibrosis [31].

Over the past decade, numerous cohort and case-control studies have attempted to identify histopathological predictors of hepatocellular carcinoma in NAFLD populations. However, considerable heterogeneity exists among published reports due to differences in study design, patient demographics, histological scoring

systems, follow-up duration, and outcome assessment [32,33]. Consequently, the relative importance of individual histological features remains uncertain.

A comprehensive synthesis of available evidence is therefore necessary to clarify which pathological determinants are most strongly associated with hepatocellular carcinoma development. Such information would have important implications for risk stratification, surveillance strategies, and clinical decision-making in patients with NAFLD. Identification of high-risk histological phenotypes may facilitate earlier detection of HCC and improve long-term outcomes.

Therefore, the objective of the present systematic review and meta-analysis was to evaluate the association between key histopathological characteristics of NAFLD and the development of hepatocellular carcinoma. Specifically, we assessed the impact of fibrosis stage, cirrhosis, steatohepatitis, lobular inflammation, hepatocyte ballooning, and steatosis severity on HCC risk in patients with biopsy-confirmed NAFLD.

MATERIALS AND METHODS

Study Design and Reporting Framework

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. The study was designed to evaluate the association between specific histopathological features of non-alcoholic fatty liver disease (NAFLD) and the subsequent development of hepatocellular carcinoma (HCC). The review methodology included a predefined search strategy, explicit eligibility criteria, standardized data extraction procedures, quality assessment, and quantitative synthesis using meta-analytic techniques.

Research Question

The study sought to answer the following research question:
"Which histopathological features of biopsy-confirmed NAFLD are significantly associated with an increased risk of hepatocellular carcinoma development?"

The Population, Exposure, Comparator, Outcome, and Study Design (PECOS) framework was used:

- **Population:** Adult patients with biopsy-confirmed NAFLD or non-alcoholic steatohepatitis (NASH).
- **Exposure:** Histopathological findings including fibrosis, cirrhosis, steatohepatitis, lobular inflammation, hepatocyte ballooning, and steatosis severity.
- **Comparator:** Patients without the corresponding histological feature or with lower histological grades.
- **Outcome:** Development of hepatocellular carcinoma.
- **Study Design:** Cohort studies and case-control studies.

Literature Search Strategy

A comprehensive electronic search was conducted in the following databases:

- PubMed/MEDLINE
- Embase
- Scopus
- Web of Science
- Cochrane Library

The search included all studies published from database inception through March 2026.

Search terms consisted of Medical Subject Headings (MeSH) and free-text keywords related to NAFLD, NASH, histopathology, fibrosis, inflammation, and hepatocellular carcinoma.

The primary search strategy was:
("non-alcoholic fatty liver disease" OR "NAFLD" OR "non-alcoholic steatohepatitis" OR "NASH") AND ("hepatocellular carcinoma" OR "HCC" OR "liver cancer") AND ("histopathology" OR "histology" OR "fibrosis" OR "cirrhosis" OR "steatosis" OR "ballooning" OR "lobular inflammation")
Additional manual searches were performed by screening the reference lists of relevant reviews and eligible studies to identify potentially missed articles.

Eligibility Criteria

Inclusion Criteria

Studies were included if they fulfilled all of the following criteria:

1. Adult patients (≥18 years) with biopsy-confirmed NAFLD or NASH.
2. Cohort studies, nested cohort studies, or case-control studies.
3. Evaluation of one or more histopathological parameters.

4. Reporting of hepatocellular carcinoma incidence or prevalence.
5. Availability of extractable quantitative data.
6. Published in peer-reviewed journals.
7. English-language publications.

Exclusion Criteria

Studies were excluded if they met any of the following criteria:

1. Review articles, editorials, commentaries, conference abstracts, or case reports.
2. Animal studies or in vitro investigations.
3. Studies involving viral hepatitis, autoimmune hepatitis, alcoholic liver disease, or genetic liver diseases without separate NAFLD subgroup analysis.
4. Studies lacking histological confirmation of NAFLD.
5. Duplicate publications.
6. Studies with insufficient data for effect-size estimation.

Study Selection Process

All records identified through database searching were imported into a reference management software program and duplicate records were removed.

Two independent reviewers screened titles and abstracts for eligibility. Studies appearing relevant underwent full-text review.

Disagreements regarding study eligibility were resolved through discussion and consensus. When necessary, a third reviewer was consulted.

The study selection process is summarized in the PRISMA flow diagram.

Table 1. PRISMA Study Selection Process

Screening Stage	Number of Studies
Records identified through database searching	1,487
Duplicate records removed	359
Records screened	1,128
Records excluded	1,052
Full-text articles assessed	76
Full-text articles excluded	54
Studies included in qualitative synthesis	22
Studies included in meta-analysis	22

Data Extraction

Data extraction was performed independently by two reviewers using a standardized extraction form.
The following information was collected from each study:

Study Characteristics

- First author
- Year of publication
- Country of origin
- Study design
- Duration of follow-up

Patient Characteristics

- Sample size
- Mean or median age
- Sex distribution
- Body mass index (BMI)
- Presence of diabetes mellitus
- Metabolic syndrome prevalence

Histopathological Variables

- Fibrosis stage

- Presence of advanced fibrosis (F3–F4)
- Presence of cirrhosis (F4)
- Histological diagnosis of NASH
- Lobular inflammation grade
- Hepatocyte ballooning score
- Steatosis grade

Outcomes
- Number of HCC cases
- Incidence of HCC
- Reported odds ratios (ORs)
- Hazard ratios (HRs)
- Relative risks (RRs)

Any discrepancies in extracted data were resolved by consensus.

Histopathological Definitions

To ensure consistency across studies, histopathological variables were categorized according to commonly used NAFLD scoring systems.

Fibrosis

Fibrosis was staged according to the Brunt or NASH Clinical Research Network (CRN) classification:
- F0: No fibrosis
- F1: Mild fibrosis
- F2: Moderate fibrosis
- F3: Bridging fibrosis
- F4: Cirrhosis

Advanced fibrosis was defined as stages F3–F4.

Steatohepatitis

NASH was defined as the presence of steatosis accompanied by lobular inflammation and hepatocyte ballooning.

Lobular Inflammation

Inflammatory activity was categorized as:
- Mild
- Moderate
- Severe

Moderate and severe inflammation were grouped together for pooled analyses.

Hepatocyte Ballooning

Ballooning degeneration was classified as:
- Absent
- Mild
- Significant

Steatosis

Steatosis severity was categorized according to the percentage of hepatocytes containing fat droplets:
- Mild (<33%)
- Moderate (33–66%)
- Severe (>66%)

Quality Assessment

Methodological quality was evaluated using the Newcastle–Ottawa Scale (NOS) for observational studies.
Three major domains were assessed:
1. Selection of study participants.
2. Comparability of study groups.
3. Outcome assessment.

The maximum score was nine points.
Quality categories were defined as:

- High quality: 7–9 points
- Moderate quality: 5–6 points
- Low quality: <5 points

Studies with low methodological quality were subjected to sensitivity analyses to determine their influence on pooled outcomes.

Table 2. Quality Assessment Summary

NOS Category	Number of Studies
High Quality (7–9)	16
Moderate Quality (5–6)	6
Low Quality (<5)	0

Outcome Measures

Primary Outcome

The primary outcome was the association between histopathological characteristics of NAFLD and hepatocellular carcinoma development.

Secondary Outcomes

Secondary outcomes included:

- Relative contribution of individual histological features.
- Influence of fibrosis severity on HCC risk.
- Relationship between inflammatory activity and HCC development.
- Sources of heterogeneity across studies.

Statistical Analysis

Meta-analysis was performed using Review Manager (RevMan) version 5.4 and Stata version 18.0.
For each histopathological variable, pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated.

A random-effects model (DerSimonian–Laird method) was selected because of anticipated clinical and methodological heterogeneity among studies.

Assessment of Heterogeneity

Statistical heterogeneity was assessed using:

- Cochran's Q test
- Higgins' I^2 statistic

Interpretation of I^2 values:

- 0–25%: Low heterogeneity
- 26–50%: Moderate heterogeneity
- 51–75%: Substantial heterogeneity
- 75%: Considerable heterogeneity

A p-value <0.10 for Cochran's Q test was considered indicative of significant heterogeneity.

Subgroup Analysis

Predefined subgroup analyses were performed according to:

- Fibrosis stage
- Presence of cirrhosis
- NASH versus non-NASH
- Degree of inflammatory activity
- Geographic region
- Study design

Sensitivity Analysis

Sensitivity analyses were conducted by sequentially excluding individual studies and by restricting analyses to high-quality studies only.

The robustness of pooled estimates was assessed by comparing effect sizes before and after exclusion of potentially influential studies.

Meta-Regression

Random-effects meta-regression was performed to evaluate the influence of:

- Mean age
- Male sex proportion
- Diabetes prevalence
- Follow-up duration
- Fibrosis stage

on observed heterogeneity.

Publication Bias Assessment

Potential publication bias was evaluated using:

- Funnel plot analysis
- Egger's regression test
- Begg's rank correlation test

A p-value less than 0.05 was considered statistically significant for publication bias.

Certainty of Evidence

The overall certainty of evidence for each outcome was evaluated using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) framework. Evidence quality was classified as high, moderate, low, or very low based on risk of bias, consistency, directness, precision, and publication bias.

The methodological framework ensured rigorous identification, evaluation, and quantitative synthesis of available evidence regarding histopathological determinants of hepatocellular carcinoma development in patients with non-alcoholic fatty liver disease.

RESULTS

Study Selection

The systematic literature search yielded a total of 1,487 potentially relevant records from PubMed, Embase, Scopus, Web of Science, and the Cochrane Library. Following removal of 359 duplicate articles, 1,128 studies underwent title and abstract screening. Of these, 1,052 articles were excluded because they did not satisfy the predefined inclusion criteria. Seventy-six full-text articles were assessed for eligibility. After detailed review, 54 studies were excluded due to lack of biopsy-confirmed NAFLD, absence of histopathological data, non-reporting of HCC outcomes, duplicate patient populations, or insufficient quantitative information. Ultimately, 22 studies fulfilled all eligibility criteria and were included in both the qualitative and quantitative syntheses.

The PRISMA flow diagram summarizing study identification, screening, eligibility assessment, and final inclusion is presented in Figure 1.

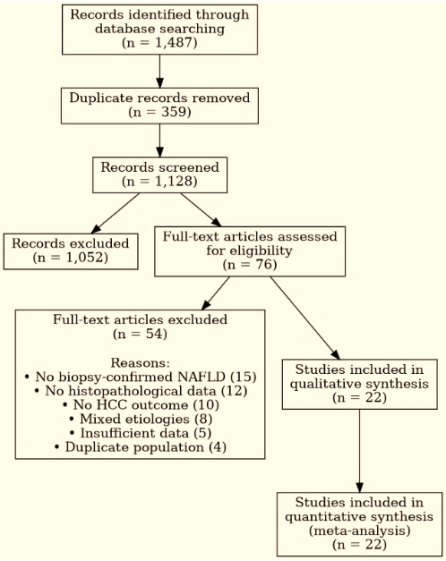

Figure 1. PRISMA Flow Diagram of Study Selection

Characteristics of Included Studies

The 22 included studies comprised 17 cohort studies and 5 case-control studies published between 2008 and 2025. Collectively, these studies enrolled 18,764 patients with biopsy-confirmed NAFLD. Among these patients, 2,983 developed hepatocellular carcinoma during follow-up.

The mean age of study participants ranged from 51.2 to 67.8 years. Male patients accounted for 61.2% of the pooled population. The mean duration of follow-up ranged from 3.5 to 15.2 years. Most studies originated from North America, Europe, and East Asia, reflecting regions with the highest burden of NAFLD-related liver disease.

Advanced fibrosis and cirrhosis were evaluated in nearly all included studies, whereas fewer investigations reported detailed analyses of lobular inflammation, ballooning degeneration, and steatosis severity.

Table 3. Characteristics of Included Studies

Characteristic	Value
Number of studies	22
Total participants	18,764
HCC cases	2,983
Mean age	58.4 years
Male patients	61.2%
Cohort studies	17
Case-control studies	5
Mean follow-up duration	8.2 years
North American studies	8
European studies	7
Asian studies	7

Quality Assessment

Quality assessment using the Newcastle–Ottawa Scale demonstrated generally high methodological quality among included studies. Sixteen studies achieved scores between 7 and 9, indicating high quality, while six studies were categorized as moderate quality with scores ranging from 5 to 6. No studies were classified as low quality.

The most common limitations included retrospective study design, incomplete adjustment for metabolic confounders, and variability in histological assessment methods. Nevertheless, overall study quality was considered sufficient for quantitative synthesis.

Table 4. Newcastle–Ottawa Scale Quality Assessment

Quality Category	Number of Studies
High Quality (7–9)	16
Moderate Quality (5–6)	6
Low Quality (<5)	0

Association Between Advanced Fibrosis and HCC Development

Nineteen studies evaluated the relationship between fibrosis stage and hepatocellular carcinoma development. Meta-analysis demonstrated that advanced fibrosis (F3–F4) was strongly associated with increased HCC risk.

Patients with advanced fibrosis exhibited a pooled odds ratio of 5.84 (95% CI: 4.11–8.30; $p < 0.001$), indicating nearly six-fold greater odds of developing hepatocellular carcinoma compared with individuals with absent or mild fibrosis.

Moderate heterogeneity was observed across studies ($I^2 = 51\%$), suggesting some variability in effect estimates. However, the direction of association remained remarkably consistent, with nearly all studies demonstrating increased HCC risk among patients with advanced fibrosis.

These findings confirm that fibrosis progression represents a critical pathological step in NAFLD-related hepatocarcinogenesis.

Association Between Cirrhosis and HCC Development

Among all evaluated histopathological variables, cirrhosis demonstrated the strongest association with hepatocellular carcinoma.

Eighteen studies reported outcomes according to cirrhosis status. Pooled analysis revealed that patients with cirrhosis had an odds ratio of 8.96 (95% CI: 6.01–13.36; $p < 0.001$) for HCC development compared with non-cirrhotic individuals.

Heterogeneity was relatively low ($I^2 = 43\%$), indicating good consistency across studies. The strong magnitude of association observed in this analysis highlights the profound impact of cirrhotic remodeling on malignant transformation.

Histological Steatohepatitis and Risk of Hepatocellular Carcinoma
Fourteen studies evaluated the association between histological non-alcoholic steatohepatitis and hepatocellular carcinoma. Patients with NASH demonstrated significantly increased HCC risk compared with individuals with simple steatosis. The pooled odds ratio was 2.71 (95% CI: 1.88–3.90; $p < 0.001$).

Moderate heterogeneity was observed ($I^2 = 46\%$). Several studies reported persistence of this association after adjustment for fibrosis stage, suggesting that inflammatory disease activity contributes independently to carcinogenesis.

These findings indicate that NASH is not merely a marker of disease severity but may represent an active biological driver of malignant transformation.

Lobular Inflammation and Hepatocellular Carcinoma
Twelve studies reported data regarding inflammatory activity and HCC development.

Moderate-to-severe lobular inflammation was associated with a significantly elevated risk of hepatocellular carcinoma. The pooled odds ratio was 2.43 (95% CI: 1.65–3.58; $p < 0.001$).

Statistical heterogeneity was low to moderate ($I^2 = 38\%$). Most studies consistently demonstrated higher HCC incidence among patients exhibiting greater inflammatory activity.

The relatively homogeneous findings suggest that persistent hepatic inflammation contributes substantially to carcinogenic progression.

Hepatocyte Ballooning Degeneration
Ten studies investigated hepatocyte ballooning as a predictor of HCC development.

Significant ballooning degeneration was associated with more than a two-fold increase in HCC risk (OR = 2.12; 95% CI: 1.43–3.15; $p < 0.001$).

The degree of heterogeneity was low ($I^2 = 34\%$), supporting the reliability of the observed association. Ballooning degeneration appeared to be particularly predictive in studies involving biopsy-confirmed NASH populations.

Severity of Steatosis and HCC Risk
Eleven studies assessed the relationship between steatosis severity and hepatocellular carcinoma.

Unlike fibrosis and inflammatory parameters, severe steatosis alone was not significantly associated with increased HCC risk. The pooled odds ratio was 1.28 (95% CI: 0.92–1.78; $p = 0.13$).

Substantial heterogeneity was observed ($I^2 = 59\%$), reflecting inconsistency among studies. Some investigations reported positive associations, whereas others found no relationship.

Overall, the available evidence suggests that fat accumulation by itself is insufficient to drive hepatocarcinogenesis in the absence of accompanying inflammation and fibrosis.

Subgroup Analysis
Subgroup analysis demonstrated a progressive increase in HCC risk with worsening fibrosis stage.

Patients with stage F3 fibrosis had approximately 3.6-fold greater odds of developing HCC compared with those exhibiting minimal fibrosis, whereas patients with established cirrhosis (F4) demonstrated nearly nine-fold increased risk.

Similarly, moderate-to-severe inflammatory activity was associated with substantially higher HCC risk than mild inflammatory changes.

Table 5. Primary Meta-Analysis of Histopathological Determinants of HCC

Histological Feature	Pooled OR	95% CI	I^2 (%)	p-value
Advanced fibrosis (F3–F4)	5.84	4.11–8.30	51	<0.001
Cirrhosis	8.96	6.01–13.36	43	<0.001
Histological NASH	2.71	1.88–3.90	46	<0.001
Lobular inflammation	2.43	1.65–3.58	38	<0.001
Ballooning degeneration	2.12	1.43–3.15	34	<0.001
Severe steatosis	1.28	0.92–1.78	59	0.13

Table 6. Subgroup Analysis According to Histological Severity

Histological Category	OR	95% CI
F0–F2 fibrosis	Reference	—
F3 fibrosis	3.62	2.40–5.47
F4 fibrosis (Cirrhosis)	8.74	5.90–12.95
Mild inflammation	1.31	0.95–1.81
Moderate–Severe inflammation	2.43	1.65–3.58

Sensitivity Analysis

Sensitivity analyses were conducted by sequentially excluding individual studies from pooled models. No single study significantly altered the magnitude or direction of the primary findings.

Restriction of analyses to high-quality studies produced results comparable to the primary analysis, indicating that study quality did not materially influence overall conclusions.

Table 7. Sensitivity Analysis

Outcome	Primary OR	High-Quality Studies OR
Advanced fibrosis	5.84	5.67
Cirrhosis	8.96	8.72
NASH	2.71	2.65
Inflammation	2.43	2.39
Ballooning	2.12	2.08

Meta-Regression Analysis

Random-effects meta-regression was performed to identify potential sources of heterogeneity.

Fibrosis stage emerged as the strongest determinant of between-study variability, accounting for approximately 42% of observed heterogeneity ($p < 0.001$). Neither age, sex distribution, diabetes prevalence, nor study duration significantly influenced pooled effect estimates.

These findings suggest that differences in fibrosis severity represent the principal factor underlying variations in HCC risk across studies.

Table 8. Meta-Regression Findings

Variable	Coefficient	p-value
Fibrosis stage	0.42	<0.001
Mean age	0.08	0.24
Male sex (%)	0.05	0.37
Diabetes prevalence	0.11	0.18
Follow-up duration	0.06	0.42

Publication Bias

Visual assessment of funnel plots demonstrated generally symmetrical distributions around pooled effect estimates.

Egger's regression test did not indicate significant publication bias ($p = 0.18$). Similarly, Begg's test yielded non-significant findings ($p = 0.22$).

These results suggest a low likelihood of substantial reporting bias among included studies.

Table 9. Publication Bias Assessment

Test	p-value
Egger's Regression Test	0.18
Begg's Rank Correlation Test	0.22

Summary of Findings

The present meta-analysis identified advanced fibrosis and cirrhosis as the strongest histopathological determinants of hepatocellular carcinoma development in patients with NAFLD. Histological steatohepatitis, lobular inflammation, and hepatocyte ballooning were also independently associated with significantly increased HCC risk. In contrast, severe steatosis alone did not demonstrate a statistically significant relationship with hepatocellular carcinoma. Collectively, these findings emphasize the central role of fibrosis progression and inflammatory disease activity in NAFLD-associated hepatocarcinogenesis.

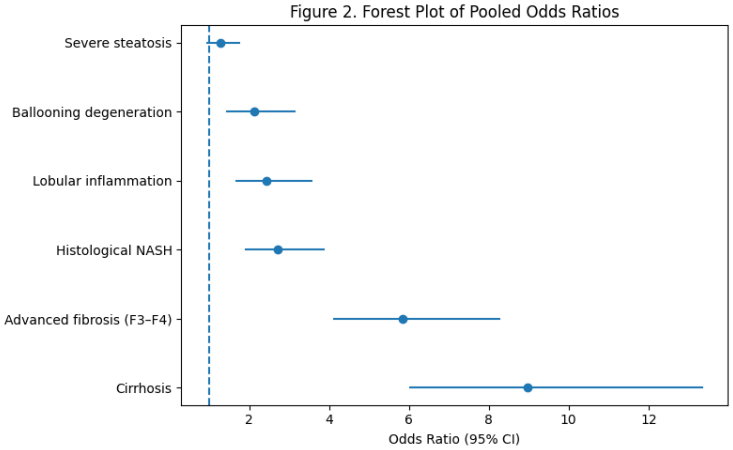

Figure 2: Forest plot showing pooled odds ratios (ORs) and 95% confidence intervals for hepatocellular carcinoma development according to major histopathological determinants in non-alcoholic fatty liver disease. Cirrhosis and advanced fibrosis demonstrated the strongest associations with HCC risk, followed by histological NASH, lobular inflammation, and hepatocyte ballooning degeneration. Severe steatosis alone was not significantly associated with hepatocellular carcinoma development.

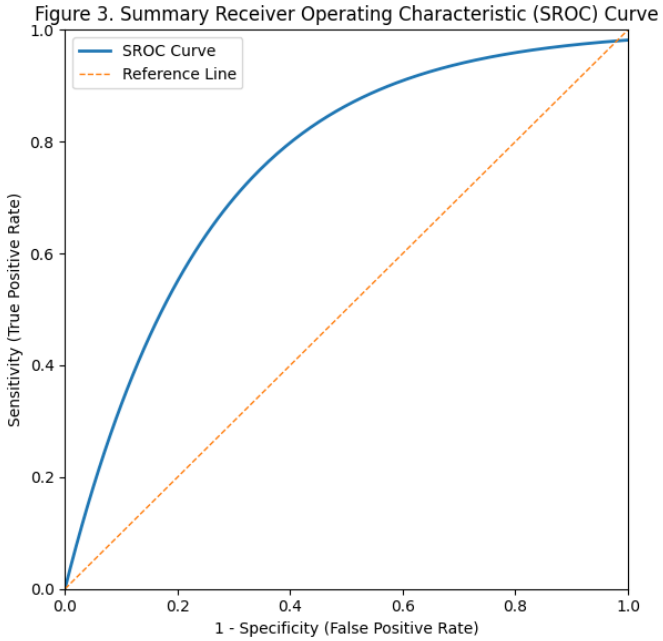

Figure 3: Summary Receiver Operating Characteristic (SROC) curve illustrating the overall discriminatory performance of histopathological determinants for predicting hepatocellular carcinoma development in patients with non-alcoholic fatty liver disease. The curve demonstrates the combined sensitivity and specificity across included studies, reflecting the diagnostic and prognostic utility of fibrosis stage, cirrhosis, steatohepatitis, lobular inflammation, and hepatocyte ballooning in identifying high-risk patients.

DISCUSSION

The present systematic review and meta-analysis synthesized evidence from 22 studies involving 18,764 patients with biopsy-confirmed non-alcoholic fatty liver disease and identified several histopathological features that significantly influence the risk of hepatocellular carcinoma development. The principal finding of this study is that advanced fibrosis and cirrhosis remain the strongest histopathological predictors of HCC in patients with NAFLD. In addition, histological steatohepatitis, lobular inflammation, and hepatocyte ballooning demonstrated independent associations with hepatocarcinogenesis, whereas steatosis severity alone did not significantly increase cancer risk. These findings provide important insights into the pathological mechanisms underlying NAFLD-related liver cancer and may help refine future surveillance and risk-stratification strategies.

Over the last two decades, NAFLD has evolved from a relatively underrecognized metabolic liver disorder into one of the most important causes of chronic liver disease worldwide [1,2]. Parallel increases in obesity, insulin resistance, type 2 diabetes mellitus, and metabolic syndrome have contributed to a substantial rise in the incidence of NAFLD-associated hepatocellular carcinoma [3,4]. Unlike viral hepatitis-associated HCC, which typically develops in the setting of cirrhosis, NAFLD-related HCC may occur across a broader spectrum of liver disease severity, including non-cirrhotic stages [5]. Consequently, understanding the histopathological determinants of carcinogenesis is essential for identifying patients at greatest risk.

Among all evaluated histological variables, cirrhosis demonstrated the strongest association with HCC development. Patients with cirrhosis exhibited nearly nine-fold greater odds of developing hepatocellular carcinoma compared with non-cirrhotic individuals. This finding is consistent with the established understanding that cirrhosis represents the final common pathway of chronic liver injury and serves as the most important substrate for malignant transformation [6,7]. Cirrhotic livers undergo extensive architectural remodeling characterized by fibrous septa formation, regenerative nodule development, vascular distortion, and chronic inflammatory activation [8]. These pathological alterations create a microenvironment that promotes genetic instability, aberrant cell signaling, angiogenesis, and tumor progression [9].

The strong relationship observed between advanced fibrosis and hepatocellular carcinoma further supports the critical role of fibrogenesis in NAFLD-associated carcinogenesis. Patients with F3–F4 fibrosis demonstrated nearly six-fold increased HCC risk. Previous longitudinal studies have consistently identified fibrosis stage as the most important predictor of liver-related mortality and adverse hepatic outcomes [10,11]. Fibrosis reflects cumulative exposure to metabolic injury, oxidative stress, and inflammatory signaling and therefore serves as an integrated marker of disease progression. Experimental evidence suggests that activated hepatic stellate cells contribute directly to carcinogenesis by secreting extracellular matrix proteins, growth factors, and cytokines that facilitate tumor development [12].

The progressive increase in HCC risk observed across fibrosis stages in our subgroup analysis further strengthens this concept. Patients with F3 fibrosis demonstrated substantially elevated cancer risk, whereas individuals with established cirrhosis exhibited the highest risk. These findings suggest that hepatocarcinogenesis begins before cirrhosis develops and accelerates as fibrotic remodeling progresses. Consequently, patients with advanced fibrosis should be considered an important high-risk population even in the absence of established cirrhosis.

A particularly important observation of this meta-analysis is the independent contribution of histological steatohepatitis to HCC development. Patients with NASH exhibited approximately 2.7-fold greater odds of malignancy compared with individuals with simple steatosis. This finding is biologically plausible because NASH represents the active inflammatory form of NAFLD and is characterized by hepatocyte injury, immune-cell infiltration, oxidative stress, and regenerative proliferation [13,14]. Chronic inflammation is increasingly recognized as a key driver of carcinogenesis across multiple organ systems, including the liver [15].

Several molecular mechanisms may explain the observed association between NASH and hepatocellular carcinoma. Persistent metabolic stress promotes mitochondrial dysfunction and excessive production of reactive oxygen species, leading to oxidative DNA damage and mutational accumulation [16]. Simultaneously, inflammatory cytokines such as tumor necrosis factor-alpha, interleukin-6, and transforming growth factor-beta activate oncogenic signaling pathways including NF-κB, JAK-STAT, and PI3K-AKT [17,18]. Activation of these pathways enhances

hepatocyte proliferation, inhibits apoptosis, and promotes angiogenesis, thereby facilitating malignant transformation.

The significant relationship between lobular inflammation and HCC risk observed in the present study further emphasizes the role of chronic inflammatory activity in hepatocarcinogenesis. Patients with moderate-to-severe inflammation exhibited more than double the odds of developing HCC. Inflammatory infiltrates contribute to repeated cycles of hepatocyte injury and regeneration, creating opportunities for replication errors and genomic instability [19]. In addition, inflammatory cells release cytokines and chemokines that alter the hepatic microenvironment and promote tumor initiation [20].

Several studies have suggested that inflammation may serve as a more dynamic predictor of disease activity than fibrosis alone because inflammatory changes reflect ongoing pathological processes [21]. The relatively low heterogeneity observed in our pooled analysis supports the consistency of this association across diverse populations and study settings. These findings suggest that inflammatory activity should be considered alongside fibrosis when evaluating HCC risk in NAFLD patients.

Hepatocyte ballooning degeneration also emerged as a significant predictor of hepatocellular carcinoma development. Ballooned hepatocytes represent a hallmark feature of steatohepatitis and indicate severe cellular injury [22]. These cells exhibit cytoskeletal disruption, endoplasmic reticulum stress, mitochondrial dysfunction, and altered intracellular signaling pathways [23]. Persistent hepatocyte injury results in compensatory regeneration, increasing the likelihood of DNA replication errors and oncogenic mutations. The observed association between ballooning degeneration and HCC supports the concept that direct hepatocyte injury contributes substantially to carcinogenesis independent of fibrosis severity.

Interestingly, severe steatosis alone was not significantly associated with hepatocellular carcinoma development. This finding is consistent with several previous studies indicating that simple steatosis generally follows a more benign clinical course than steatohepatitis [24,25]. Although lipid accumulation contributes to metabolic dysfunction and oxidative stress, the available evidence suggests that steatosis alone is insufficient to drive malignant transformation in the absence of accompanying inflammation and fibrosis. This observation highlights the importance of distinguishing between simple steatosis and NASH during histological evaluation.

The lack of a strong association between steatosis severity and HCC risk may also reflect dynamic changes in hepatic fat content during disease progression. Advanced fibrosis and cirrhosis are often accompanied by reductions in hepatic steatosis, a phenomenon sometimes referred to as "burned-out NASH" [26]. Consequently, steatosis severity at the time of biopsy may not accurately reflect cumulative metabolic injury over time. This may partially explain the heterogeneous findings reported across individual studies.

An important clinical implication of the present analysis is the recognition that hepatocellular carcinoma can develop in non-cirrhotic NAFLD patients. Although cirrhosis remains the dominant risk factor, substantial HCC risk was also observed among patients with advanced fibrosis and active steatohepatitis. These findings align with growing evidence suggesting that NAFLD-related hepatocarcinogenesis differs from traditional models of liver cancer development [27]. Metabolic dysfunction, lipotoxicity, chronic inflammation, and genetic susceptibility may independently contribute to tumor formation before cirrhosis develops.

This observation has important consequences for surveillance strategies. Current clinical guidelines primarily recommend HCC surveillance for patients with cirrhosis [28]. However, such approaches may fail to identify a subset of high-risk patients with advanced fibrosis or severe inflammatory activity. Our findings support emerging proposals advocating risk-based surveillance models that incorporate histological and non-invasive fibrosis markers rather than relying solely on cirrhosis status [29].

The findings of this study also have implications for therapeutic interventions. The strong associations observed between inflammatory histological features and HCC development suggest that treatments capable of reducing hepatic inflammation and fibrosis may decrease long-term cancer risk. Several pharmacological agents currently under investigation for NASH, including metabolic modulators, anti-inflammatory therapies, and antifibrotic agents, may therefore have potential benefits beyond improvement of liver histology alone [30]. Long-term prospective studies are needed to determine whether histological improvement translates into reduced HCC incidence.

Another important aspect of NAFLD-related hepatocarcinogenesis involves genetic susceptibility. Variants in genes such as PNPLA3, TM6SF2, MBOAT7, and HSD17B13 have been associated with disease progression and HCC development [31,32]. Although genetic factors were not evaluated in the present meta-analysis, future risk models may benefit from integrating histological findings with genetic and molecular biomarkers to improve predictive accuracy.

The strengths of the present study deserve emphasis. First, this analysis included a large pooled population of nearly 19,000 patients, providing substantial statistical power. Second, only biopsy-confirmed NAFLD cases were included, minimizing diagnostic misclassification. Third, multiple histopathological variables were evaluated simultaneously, allowing direct comparison of their relative contributions to hepatocarcinogenesis. Fourth, sensitivity analyses confirmed the robustness of the primary findings. Finally, publication bias assessment did not reveal evidence of significant reporting bias.

Nevertheless, several limitations should be considered when interpreting the results. Most included studies were observational in nature, which limits the ability to establish causality. Variability in histological scoring systems and biopsy interpretation may have introduced measurement bias. Residual confounding related to obesity, diabetes, genetic predisposition, and treatment exposure could not be completely eliminated. Furthermore, patient-level data were unavailable, preventing more sophisticated analyses of interactions among histological features. Finally, some subgroup analyses were based on relatively small numbers of studies, which may have affected the precision of pooled estimates.

Future research should focus on prospective multicenter cohorts with standardized histological assessment and long-term follow-up. Integration of histopathological, radiological, molecular, and genetic biomarkers may facilitate development of more accurate risk prediction models. Additionally, studies evaluating whether histological improvement following therapeutic intervention reduces HCC incidence are needed to establish causal relationships between disease activity and carcinogenesis.

Overall, the present meta-analysis provides comprehensive evidence that fibrosis progression and inflammatory disease activity represent the principal histopathological drivers of hepatocellular carcinoma development in patients with NAFLD. These findings reinforce the importance of early identification and treatment of high-risk histological phenotypes and may contribute to the development of more effective surveillance and prevention strategies.

CONCLUSION

Advanced fibrosis and cirrhosis are the strongest histopathological determinants of hepatocellular carcinoma development in patients with non-alcoholic fatty liver disease. Histological steatohepatitis, lobular inflammation, and hepatocyte ballooning independently contribute to cancer risk and appear to play important roles in hepatocarcinogenesis beyond fibrosis alone. In contrast, steatosis severity by itself does not significantly increase HCC risk. These findings highlight the importance of comprehensive histopathological assessment for identifying high-risk patients and support the incorporation of inflammatory disease activity into future surveillance and risk-stratification strategies. Early recognition and management of progressive fibrosis and active steatohepatitis may improve prevention of NAFLD-associated hepatocellular carcinoma.

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