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Review

An overview of risk assessment and monitoring of malignant transformation in cirrhotic nodules

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***Abstract*:** Cirrhotic liver nodules can progress to hepatocellular carcinoma (HCC) through a multi-step carcinogenesis model, with dysplastic nodules being particularly high risk. Currently, monitoring the progression of non-HCC cirrhotic nodules is primarily through dynamic observation, but there is a lack of sensitive, efficient, and convenient methods. Dynamic monitoring and risk evaluation of malignant transformation are essential for timely treatment and improved patient survival rates. Routine liver biopsies are impractical for monitoring, and imaging techniques like ultrasound, computed tomography, and magnetic resonance imaging are not suitable for all patients or for accurately assessing subcentimeter nodules. Identifying serum biomarkers with high sensitivity, specificity, and stability, and developing a multi-index evaluation model, may provide a more convenient and efficient approach to monitoring pathological changes in cirrhotic nodules.

Keywords: cirrhotic nodule, hepatocellular carcinoma, liver biopsy, risk factors

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Introduction

Cirrhotic nodules (CNs) are hepatocyte nodules caused by continuous hepatocyte damage and matrix component proliferation in liver cirrhosis and can be classified into regenerative nodules (RNs), dysplastic nodules (DNs), and hepatocellular carcinoma (HCC). Dysplastic nodules are further categorized into low-grade DN (LGDN) and high-grade DN (HGDN) based on cellular atypia. The malignant transformation rate of CN tends to increase progressively through the stages of RN, LGDN, and HGDN. Studies have shown that DN has higher growth and malignant rates compared to RN, with HGDN significantly higher than LGDN.[1,2] Long-term observations have found that some CNs either disappear or remain unchanged. Kobayashi et al.[3] observed 154 patients with small hepatic nodules, finding that 18.8% developed into HCC during the observation period, with a 5-year cumulative incidence of HCC at 12.4% for RN, 36.6% for LGDN, and 80% for HGDN, indicating a higher malignant transformation possibility for HGDN compared to RN and LGDN. Consequently, some CNs can progress to HCC through the RN-LGDN-HGDN multi-step carcinogenesis pathway. Given that the 5-year survival rate of advanced HCC is less than 10%,[4] assessing and monitoring the risk of CN malignant transformation is crucial for early prevention and treatment of HCC.

Unlike other malignant tumors, the imaging diagnosis of HCC is more reliable. Despite variations in the management strategies recommended by The American Association for the Study of Liver Diseases (AASLD), European Association for the Study of the Liver (EASL), and Asian Pacific Association for the Study of the Liver (APASL), all suggest ultrasound examinations every 6 months[5–7] (Table 1). However, contrast-enhanced ultrasound (CEUS), computed tomography (CT), and magnetic resonance imaging (MRI) are particularly significant for diagnosing

Table 1. Differences between AASLD, EASL, and APASL guidelines on the management of hepatocellular carcinoma.

Different contents	AASLD	EASL	APASL
Screening intervals and modality	6 months, US (and/or AFP)	6 months, US	6 months, US and AFP
Radiological diagnosis	CT/MRI	CT/MRI	CT/MRI/Gd-EOB-DTPA-enhanced MRI
CEUS	No recommendation	⩾1 cm Ø	Sensitivity is consistent with CT and MRI
Uncertain nodule	Imaging examination follow-up/contrast enhancement/biopsy	Other imaging examination	Further examination
Liver biopsy	Not recommended for regular applications	Diagnosis of non-HCC cirrhosis	Uncertain nodule (⩾1 cm Ø)

AASLD, The American Association for the Study of Liver Diseases; AFP, alpha-fetoprotein; CEUS, contrast-enhanced ultrasound; Gd-EOB-DTPA, gadolinium ethoxybenzyl diethylenetriamine penta-acetic acid; HCC, hepatocellular carcinoma; US, ultrasound.

DN, especially HGDN patients. Most guidelines recommend a biopsy if imaging fails to provide a definitive diagnosis. While liver biopsy is invaluable for the definitive diagnosis of CN, it is impractical to perform on all CNs due to their size and the complications associated with liver puncture. Additionally, liver biopsy is prone to sampling errors and observer variability,[8] meaning a negative result cannot entirely rule out HCC, necessitating a comprehensive assessment incorporating imaging and serum-related indicators.[9]

Monitoring CN is crucial for the early identification and treatment of HCC, and predicting the risk of malignant transformation is significant for early intervention. Most researchers have invested their funding and efforts into studies on the treatment of HCC, but compared to early intervention, the benefits to patients are clearly limited. Although many studies have evaluated HCC risk factors,[10] there is a paucity of research on the mechanisms underlying CN malignant transformation. While the risk factors for CN malignant transformation may overlap with those of HCC, CN patients require more sensitive and specific monitoring programs for effective early prevention and treatment of HCC. This review aims to clarify how to improve the detection of new nodules and predict the risk of these nodules developing into HCC in cirrhotic patients at risk of HCC.

Methods

By setting keywords such as "cirrhotic nodule," "liver nodule," "hepatocellular carcinoma," "liver cancer," "precancerous lesion," and "dysplastic nodule," a search was conducted for relevant literature in the "PubMed," "Web of Science," and "Google Scholar" databases. In addition, further secondary searches were performed through citations. Finally, the relevance of the literature was assessed by reviewing abstracts, and the selected studies were summarized and compiled.

Liver biopsy

Histopathology

Pathological examination can classify CN based on hepatocyte morphology and angiogenesis. Therefore, direct surgery or liver biopsy is the most accurate method to characterize and evaluate CN. However, distinguishing early hepatocellular carcinoma (eHCC) from DN, especially HGDN, can be challenging[11] (Table 2). Combining histological and disease characteristics may provide valuable references for monitoring CN malignancy. Since changes in fat content within nodules are histopathological features of HGDN and HCC, fat infiltration in lesions may be a significant risk factor for the vascularization of hypovascular nodules.[12] Thus, the accumulation of fat in CN could indicate a shift toward dysplasia. Furthermore, the malignant

Table 2. Differences in histological characteristics of DN and eHCC.

Histological characteristics	LGDN	HGDN	eHCC
Small cell dysplasia	–	+	+
Large cell dysplasia	+/–	+/–	–
Degree of cell atypia	Mild	Moderate to severe	Severe
Nuclear/cytoplasmic ratio	+/–	+	+
Hyperchromasia/nuclear atypia	–	+	+
Increased cell density compared with surroundings	<1.3 times	1.3 to 2 times	>2 times
Pseudoglands	–	+/–	+
Portal tract	+	+/–	–
Reticular scaffold	+	+	+/–
Unpaired arteries	+/–	+/–	+
Interstitial infiltration	–	–	+/–
Nodule-in-nodule	–	–	+/–

eHCC, early hepatocellular carcinoma; DN, dysplastic nodule; HGDN, high-grade DN; LGDN, low-grade DN.

transformation of CN is influenced by the surrounding microenvironment. A study by Borzio et al.[1] demonstrated that when histopathology indicates the presence of both HGDN and extranodular large cell changes, the risk of HCC development is significantly higher compared to when these conditions exist separately. Therefore, changes in the microenvironment within and around CN may be crucial in promoting CN malignancy. Immunohistochemical detection aids in distinguishing different cell types and functions, providing a clearer diagnosis for the qualitative assessment and prognosis of CN. Consequently, detecting markers of abnormal proliferation and metabolism in different CNs can help identify abnormal cell populations and determine the likelihood of HCC.

Abnormal proliferation markers

One important characteristic of tumor cells is their abnormally increased proliferation. Factors reflecting cell proliferation activity are significant for understanding the malignant transformation of CN. Ki-67 is a nuclear antigen related to proliferating cells, and DNA topoisomerase II-α (Topo II-α) is an important enzyme in DNA metabolism. The expression of Ki-67 and Topo II-α in hepatocyte nuclei within nodules increases significantly with the transition from CN to HCC, reflecting abnormal changes in hepatocyte proliferation and serving as markers for the pathological detection of CN malignancy.[13–15] Proteins regulating cell proliferation, such as glypican-3 (GPC3) and squamous cell carcinoma antigen (SCCA), also promote HCC through various signaling pathways. GPC3 regulates tissue and organ growth by influencing multiple molecular pathways,[16] with its expression significantly higher in HGDN than in LGDN.[17] SCCA-1 inhibits tumor infiltration of natural killer cells, suppresses tumor cell apoptosis, and promotes proliferation,[18] with its expression increasing in chronic liver disease, DN, and HCC, but not in normal hepatocytes.[19] These findings suggest that proteins involved in cell proliferation are closely related to CN malignancy and can predict CN malignancy, especially in HGDN patients with hepatitis B or C virus (HBV or HCV).

The transforming growth factor β (TGF-β) protein family, which encodes cell proliferation,

differentiation, and growth, remains controversial in HCC formation.[20] TGF-β1 can promote tumor cell apoptosis through downstream Smad proteins in the early stages of HCC formation[21] and enhance tumor cell progression and metastasis by inducing immune escape, promoting epithelial-mesenchymal transition, and angiogenesis in the late stages of HCC.[22] The mechanism of TGF-β1 transformation is unclear and may depend on its source and dominant effect. Consequently, the prognostic value of TGF-β1 for CN malignant transformation is still uncertain.

Ductular reaction markers

The ductular reaction (DR) is reactive bile duct hyperplasia induced by liver injury, involving bile duct cells, hepatocytes, and hepatic progenitor cells. Previous studies have confirmed that DR disappears in the invasive areas of early HCC but is abundantly expressed in most noninvasive areas. It is a sign of hepatocyte regeneration in patients with chronic liver disease, particularly in advanced nonalcoholic fatty liver disease (NAFLD).[23] Research by Clerbaux et al.[24] suggests that DR promotes the formation of hepatocyte nodules during liver injury, which may explain the increased expression of DR in CN. Therefore, monitoring the expression of DR-related indicators in pathological tissues such as cytokeratin 7 (CK7), and CK19 may have significant implications for the malignant transformation of CN. In addition, epithelial cell adhesion molecule (EpCAM) is one of the markers of hepatic progenitor cells. Zhang et al.[25] found that EpCAM is abundantly expressed in noninvasive tissues and RNs and DN, but not in HCC tissues. The study by Pei-Pei Hao et al. also demonstrated that EpCAM(+)/CD133(−) hepatic progenitor cells do not have the ability for spontaneous malignant transformation.[26] However, most studies suggest that EpCAM is associated with a poor prognosis in HCC.[27,28] In these studies, EpCAM is considered a marker of liver cancer stem cells, differing from the cell source of DR. Liver cancer stem cells express various markers, such as EpCAM, CD133, and CD44, which differ from the markers found in cells derived from DR. Current research has confirmed that HCC primarily originates from fully differentiated hepatocytes, while benign lesions, such as RN, originate from hepatic progenitor cells.[29] However, hepatic progenitor cells can also transform into tumor cells under certain conditions, which may depend on the type of liver injury and carcinogenic patterns.[30] The different origins of tumor cells under different liver microenvironments may explain the conflicting results regarding EpCAM in HCC research. HCC derived from hepatocyte transdifferentiation may show a lack of EpCAM expression, while HCC originating from hepatic progenitor cells may show increased EpCAM expression. Therefore, EpCAM, combined with other markers such as CD133, CD44, CK7, or CK19, may effectively improve the identification of the malignancy potential of nodules. Further research is needed to clarify the primary origins of tumor cells in different types of liver injury and liver cancer.

Others

Hepatitis virus can cause DNA damage in hepatocytes, which is closely related to the occurrence of HCC. Phosphorylated histone H2AX (γ-H2AX) is a sensitive marker for DNA double-strand breaks and recruits various molecules involved in DNA repair.[31] Matsuda et al.[32] detected the level of γ-H2AX in the liver tissue of patients with chronic hepatitis, cirrhosis, DN, and HCC. Their results showed a significant increase in γ-H2AX expression from normal liver tissue to hepatitis and then to cirrhosis, with DN exhibiting significantly higher levels than cirrhosis and HCC. Additionally, γ-H2AX expression was negatively correlated with the histological grade of HCC, and its expression in adjacent tissues was significantly higher than in cirrhotic tissues. This suggests that γ-H2AX may play a crucial role in the early stages of carcinogenesis and is an important factor in CN malignancy. Furthermore, the crosstalk between liver sinusoidal endothelial cells and other perisinusoidal cell populations is vital in liver regeneration and angiogenesis associated with HCC development.[33] Therefore, indicators related to the dedifferentiation of sinusoidal endothelial cells in CN, such as von Willebrand Factor (vWF),[34] may provide valuable references for assessing CN malignancy. Other HCC-related molecular markers include heat-shock protein 70 (HSP70), enhancer of zeste homolog 2 (EZH2), and glutamine synthetase (GS).[35] While these molecular markers have some significance in suggesting the malignant transformation of CN or HCC formation, their relevance in CN monitoring needs further confirmation. Additionally, due to tissue differences in early

HCC, the combination of multiple molecular markers may prove more valuable, such as HSP70, GPC3, and GS.[36]

Genetic testing

The excessive proliferation of hepatocytes induced by various factors can promote mutations in genes related to cell proliferation and death, leading to the clonal expansion of mutant cells and their malignant transformation.[37] Genetic mutations accumulate gradually in the early stages of CN and accelerate during the progression from CN to HCC.[38] Screening for specific tumor suppressor genes provides critical information for the early detection and treatment of HCC. Multiple chromosomal regions, such as 1p, 4q, 6q, 8p, 9p, 10q, 11p, 13q, 14q, 16p, and 17p, can undergo chromosomal fragment acquisition, loss, translocation, and loss of heterozygosity (LOH) in HCC.[37] These regions often involve well-known tumor suppressor gene loci. Previous studies have identified that the increased copy number of chromosomal regions such as 1q21-23[39] and 7q21.3[40] may be early genomic events in HCC. Simultaneously, the LOH in multiple chromosomal regions, such as 1p, 4q, and 8p, increases progressively from cirrhosis to HGDN, including loci D1S2843 and D1S513 in the 1p36-p32 region.[41,42] This trend supports the transition from RN to DN and ultimately to HCC, suggesting that the LOH of loci such as D1S2843 and D1S513 is a significant risk factor for the malignant transformation of CN.

With the advancement of HCC sequencing research, an increasing number of mutant genes have been identified. Telomerase reverse transcriptase (TERT), tumor protein p53 (TP53), and catenin beta 1 (CTNNB1) are among the most common genetic variants.[43–45] The expression of these genes in HCC and HGDN with malignant potential is consistent, while there is no significant change in HGDN without malignant potential,[46,47] suggesting they may be risk factors for the conversion of HGDN to HCC. Among these, mutations in the TERT promoter may represent the earliest recurrent somatic clonal genetic alterations, leading to telomerase reactivation and promoting the malignant transformation of nodules.[48] Additionally, certain miRNAs and DNA methylation patterns also show significant changes associated with CN malignancy. Studies have shown that miR-145 and miR-199b are gradually downregulated from LGDN to early HCC, while miR-224 and DNA methyltransferases (DNMTs) such as DNMT1, DNMT3a, and DNMT3b are upregulated.[49–51]

Although various genes are correlated with CN malignancy, only a few drive the expansion and invasion of cancer cells. The regulatory genes involved in HCC formation are not yet fully understood, and their prognostic value for CN requires cautious interpretation. Additionally, micronuclei (MN), which are manifestations of chromosomal aberrations in interphase cells, contain damaged chromosome fragments and/or complete chromosomes.[52] Current studies indicate that the number of MNs gradually increases during the transition from RN to DN and HCC, with a significantly higher incidence in DN compared to RN.[53,54] This suggests the potential value of MN in assessing the risk of CN malignancy, though further clinical evidence is needed.

Imaging examinations

The American Association for the Study of Liver Diseases (AASLD) recommends ultrasound (US) and/or alpha-fetoprotein (AFP) examinations every 6 months for patients at high risk of HCC. Considering the economic cost and potential harm, CT and MRI are not recommended as first-line screening tools.[55] However, the US cannot distinguish between RN and DN, and CEUS cannot differentiate between intrahepatic cholangiocarcinoma (ICC) and HCC.[56] When CN is detected by the US, advanced imaging methods with contrast agents, including CT, MRI, and CEUS, are necessary to evaluate its properties.

Although the typical enhancement and washout patterns seen in CT and MRI are highly specific for HCC, their sensitivity is low. Approximately 30% of HCC cases do not exhibit these typical features, which can lead to missed diagnoses.[57] This may be because the vascular system in early HCC is not fully developed, a scenario that is also likely in the malignant transformation of HGDN. Since metabolic dysfunction in hepatocytes may precede angiogenesis in early HCC formation,[58] detecting early abnormal liver cells could indicate the onset of carcinogenesis. Compounds that target hepatocytes, such as gadolinium ethoxybenzyl diethylenetriamine penta-acetic acid (Gd-EOB-DTPA), can enhance the sensitivity of HCC

diagnosis.[59] Carlo Bartolozzi et al.[60] analyzed 102 nodules using Gd-EOB-DTPA-enhanced MRI, finding that hyperenhancement in the arterial phase and hypointensity in the late phase are specific indicators of HCC. Nodular hypointensity in the hepatobiliary phase was detected in 39 of 40 HCCs and in 21 of 30 HGDNs, but not in LGDN. A study by Tatsuya Shimizu et al.[61] found that hepatobiliary-phase hypointense nodules without arterial phase hyperenhancement on Gd-EOB-DTPA-enhanced MRI were closely related to the subsequent development of hypervascular HCC. This relationship may be influenced by the initial size of the nodule. Utaroh Motosugi et al.[62] studied 135 hypovascular nodules and found that nodules larger than 10 mm and containing fat were high-risk factors for hypervascularization. A systematic review indicated that the critical size for CN malignant transformation is 9 mm.[63] Additionally, the presence of hypointensity in the hepatobiliary phase, along with hyperintensity in the arterial phase and on diffusion-weighted imaging (DWI), highly indicates the malignant transformation of HGDN.[59] Therefore, although most current guidelines do not list hepatobiliary-phase hypointensity as a standard for HCC imaging diagnosis, the monitoring period should be shortened for hypointense nodules in the hepatobiliary phase observed on Gd-EOB-DTPA-enhanced MRI, especially in HGDN.

Similarly, Gd-EOB-DTPA-enhanced MRI also holds significant predictive value for the postoperative recurrence of HCC. Recent studies have shown that the presence of hypointense nodules in the hepatobiliary phase without obvious arterial phase hyperenhancement before surgery is an important predictor of postoperative HCC recurrence.[64–66] A retrospective study by Inoue et al.[67] found that the intrahepatic distant recurrence rates within 5 years following radiofrequency ablation were significantly higher in patients with non-hypervascular hypointense nodules compared to those without (89.1% vs 48.7%). Further, the study by Matsuda et al.[68] demonstrated that 20.7% of late recurrence cases originated from hepatobiliary-phase hypointense nodules without arterial phase hyperenhancement, while the remaining 79.3% of late recurrence cases originated from regions where no nodule was initially detected. These findings suggest that hepatobiliary-phase hypointense nodules without arterial phase hyperenhancement are not only indicative of high malignancy but also serve as independent risk factors for HCC occurrence throughout the liver. Given the multicentric nature of HCC, the emergence of new CN after surgery may indicate a higher risk of malignant transformation.

Additionally, the growth rate of CN is a crucial risk factor for their malignant transformation, especially when characterized by hypointensity in the hepatobiliary phase. Higaki et al.[69] conducted a follow-up study of 33 patients with 60 hepatobiliary-phase hypointense nodules and found that the growth rate of nodules showing hypervascular transformation (6.3 ± 4.5 mm/year) was significantly higher than that of nodules without hypervascular transformation (3.4 ± 7.2 mm/year). This indicates that growth rate is a significant predictor of hypervascular transformation. Another retrospective study demonstrated that an absolute growth rate of 5 mm or greater in 6 months or a relative growth rate of 30% or greater in 6 months is closely related to CN malignant transformation.[70] For subcentimeter CN in patients with a history of HCC, vigilance should be heightened if there is arterial phase enhancement. Song et al.[71] found that 89.9% of subcentimeter hypervascular nodules progressed to HCC within 12 months in patients with a history of HCC, and the progression rate for nodules larger than 5.5 mm was 100%. However, most studies rely on imaging examinations without pathological verification, which may result in false positives. Nevertheless, sufficient attention should be paid to subcentimeter CN in patients with a history of HCC. For CN larger than 1 cm, imaging should be the primary detection method. It is important to note that although imaging results are specific, they cannot absolutely exclude HCC. For CN with nonspecific signs, liver biopsy should still be performed, along with close observation using other indicators.

Gd-EOB-DTPA-enhanced MRI is widely used in the clinical detection of CN. RN retains hepatocyte function and lack angiogenesis, typically not visible on T1 and T2 weighted imaging, but their signal intensity can change due to iron, glycogen, and lipid content.[72] Both RN and LGDN show iso to hyperintensity in the hepatobiliary phase, making them difficult to distinguish, but this has no adverse consequences. HGDN and eHCC show hypointensity in the hepatobiliary phase, distinguishable by hyperintensity in both DWI

and T2. For some difficult-to-distinguish cases of DN and eHCC, research has utilized the pathological characteristic of reduced iron content during nodule malignancy transformation to develop MRI-based quantitative iron analysis methods.[73] These algorithms may help improve the accuracy and sensitivity of MRI diagnosis, though further clinical validation is still needed. Most studies suggest that MRI is more sensitive than CT,[74] although some are based on retrospective data at risk of bias. Current evidence is insufficient to determine which modality is more sensitive, but the contrast media used in both are not suitable for patients with renal failure. The microbubble contrast agent used in CEUS has not shown nephrotoxicity, making it safer for patients with renal failure, and more sensitive for detecting arterial phase hyperenhancement (APHE) than CT and MRI.[75] At the same time, CEUS has also shown good accuracy and sensitivity in distinguishing different types of nodules. For example, the study by Yu Duan et al. suggests that a multivariate regression model constructed using qualitative CEUS characteristics and the contrast arrival time ratio can effectively differentiate between RN, DN, and HCC.[76] However, CEUS is less reliable than CT and MRI in assessing changes in CN size due to variability among examiners. Thus, the method of clinical monitoring of CN should be selected based on the patient's condition.

The widely accepted view is that patients with CN should undergo enhanced MRI, enhanced CT, or CEUS at least once every 6 months. However, when monitoring CN via imaging, factors such as the patient's anxiety, financial burden, and the risk of malignancy should be taken into account. Due to the lack of comparative evaluations of proactive interventions, such as interventional procedures or surgical resection, it remains uncertain whether early intervention is the optimal choice for HGDN. However, some researchers suggest shortening the monitoring interval for high-risk nodules. For example, the 2024 Guidelines for Diagnosis and Treatment of Primary Liver Cancer in China recommend that high-risk nodules, which cannot be clinically diagnosed as liver cancer, undergo imaging examinations every 2–3 months, along with serum AFP, des-gamma-carboxy prothrombin (DCP), and a combination of seven microRNAs (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801).[77] *Chinese Journal of Practical Surgery* 2024; 44(4): 361–386. Article in Chinese). Therefore, considering the economic burden and the relatively low risk of malignancy, imaging surveillance every 6 months for RN and LGDN may be sufficient to monitor disease progression. However, for HGDN, the interval may need to be shortened to 3 months.

Serological markers

Serum alpha-fetoprotein (AFP) is a key serological marker for diagnosing HCC. Several observational studies involving patients with CN have shown that AFP is an independent high-risk factor for the malignant transformation of subcentimeter CN and CN with arterial phase hyperenhancement as indicated by contrast-enhanced MRI.[78,79] Smereka et al.[79] demonstrated that an AFP threshold of 10.1 ng/mL had a sensitivity of 52.1% and a specificity of 81.6% for predicting HCC. Similarly, Marrero et al.[80] found that an AFP threshold of 10.9 ng/mL had a sensitivity of 66% and a specificity of 81%. Another retrospective analysis revealed that an AFP threshold of 20 ng/mL had a sensitivity of 70.1% and a specificity of 89.8% for HCC detection.[81] These results suggest that 10 ng/mL may be a critical AFP threshold for monitoring CN malignant transformation, particularly when other high-risk factors are present.

However, it is important to note that serum AFP levels can be influenced by various factors, especially baseline alanine aminotransferase (ALT) levels and may increase in HCV patients.[82] Therefore, AFP is more sensitive to non-HCV-related liver diseases. Additionally, current research indicates that several serological markers, including DCP, Lens culinaris agglutinin-reactive AFP (AFP-L3), Dickkopf-1 (DKK1), glypican-3 (GPC-3), alpha-1-fucosidase (AFU), and squamous cell carcinoma antigen-IgM (SCCA-IgM), exhibit good sensitivity and specificity in the early diagnosis of HCC.[83,84] These markers may be valuable in predicting CN malignant transformation and combining multiple markers may further enhance the sensitivity and specificity of predictions, serving as an effective supplement to imaging examinations.

In recent years, liquid biopsy, which includes circulating free microRNA, circulating tumor cells, cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), free mitochondrial DNA, free viral

DNA, and extracellular vesicles, has demonstrated significant value in the early diagnosis and therapeutic evaluation of HCC.[85–88] Tomohiro Umezu et al.[89] used Streptozotocin to induce a mouse model of HCC and dynamically observed the differences in serum miRNA levels between mice that developed HCC and those that did not during nodule formation. These results were compared with clinical studies involving patients with hepatitis C-related cirrhosis and liver cancer. The study revealed that the expression levels of six miRNAs (let-7f-5p, miR-10b-5p, miR-143-3p, miR-191-5p, miR-21-5p, and miR-26a-5p) were significantly higher in both the mouse model and human clinical samples compared to the non-HCC groups, suggesting their potential as early diagnostic markers for HCC and for dynamic monitoring of DN. Additionally, tumor cells can influence platelet mRNA expression, and detecting specific platelet mRNA expression may help indicate tumor formation. For example, Walifa Waqar et al.[90] found that the expression of platelet mRNAs CTNNB1, SERPIND1, and SPINK1 was significantly higher in HCC patients with cirrhosis than in those with cirrhosis alone. Given the limitations in the sensitivity of imaging techniques, especially for detecting subcentimeter nodules, liquid biopsy may become a powerful supplement or even a replacement for imaging in monitoring nodules, potentially reducing the need for liver biopsy. However, liquid biopsy still faces challenges related to sensitivity, cost, and accessibility, which require large-scale clinical studies and the development of cost-effective and efficient detection methods.

Others

Patients with HBV and HCV-related cirrhosis have a higher incidence of HCC,[91] and the combination of viral infection and alcohol use may synergistically accelerate HCC formation.[92] These factors significantly influence the malignant transformation of CN, and etiological treatment may reduce the incidence of HCC. However, the impact of direct-acting antiviral (DAA) treatment for eradicating HCV on DN remains controversial. Some studies suggest that DAA drugs, such as sofosbuvir, may promote the malignant transformation of existing DN nodules,[93] while others argue that DAA treatment can reduce the risk of malignancy in hepatobiliary-phase low-signal nodules without arterial phase enhancement, as indicated by Gd-EOB-DTPA-enhanced MRI in HCV-infected livers.[94] Therefore, further evaluation is necessary to assess the impact of DAA therapy on patients with HCV-related CN. For patients with NAFLD-related cirrhosis, the risk of CN malignant transformation is also significantly increased compared to non-cirrhotic patients.[95] Given that a lower proportion of NAFLD patients receive HCC monitoring compared to those with viral hepatitis-related cirrhosis, research on NAFLD-related CN is limited, making it difficult to fully evaluate the impact of related factors on CN deterioration.

Additionally, gender, age, and a previous history of HCC are important risk factors for CN malignant transformation.[79,96,97] The incidence of HCC is higher in men than in women, possibly because estrogen inhibits HCC formation in the early stages by suppressing inflammatory factors and their downstream signaling pathways.[98] The immune microenvironment may play a role in estrogen's inhibitory effect on HCC formation, and the weakening of immune regulation with age may partly explain the age-related increase in HCC formation. Given the complexity, sensitivity, and specificity of HCC pathogenesis, constructing a predictive model based on multiple risk factors may be an effective way to monitor CN. Cho et al.[99] conducted a large-scale, single-center retrospective study to stratify the risk of CNs in patients with HBV cirrhosis and developed a risk score model that included age, arterial enhancement, nodule size, serum albumin level, serum AFP level, prior HCC history, and HBeAg status. This risk score model showed good performance, with an area under the curve of 0.886 at 3 years and 0.920 at 5 years in leave-one-out cross-validation. These results indicate that the model can effectively assess the risk of HCC progression in CN patients with HBV cirrhosis. The traditional Child-Pugh score is also significant in predicting the malignant transformation of CN. A study by Gazelakis et al.[100] demonstrated that the Child-Pugh classification is the only independent predictor of the transformation of nonmalignant hypervascular nodules in cirrhosis into HCC. Compared to patients with Child-Pugh Class A, the risk of HCC transformation increases by 10.1 times in Child-Pugh Class B patients and 32.6 times in Child-Pugh Class C patients. Currently, there is a lack of comprehensive prediction models for CN malignant transformation, and more extensive and in-depth clinical research is needed to develop better prediction models and

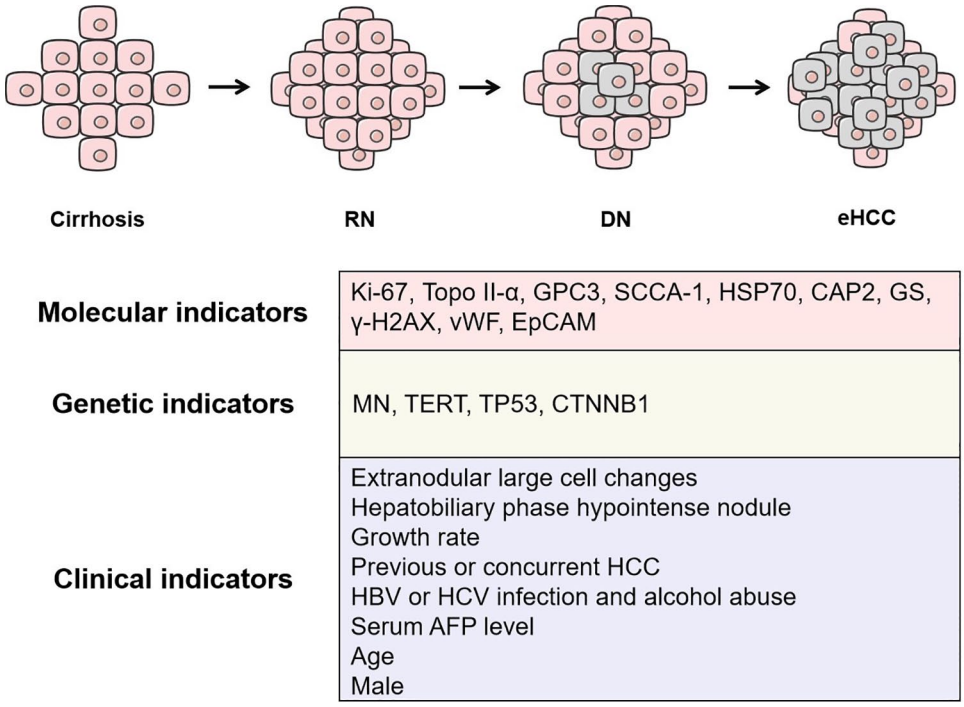

Figure 1. Risk factors for malignant transformation of cirrhotic nodules.

evaluation methods for different types of CN. Predictive models incorporating imaging, serology, and disease characteristics can serve as supplements or replacements for pathological diagnosis and as effective strategies for dynamic CN monitoring.

Conclusion

CN is crucial for the early prevention and intervention of HCC. Recent studies have explored the mechanisms and risk factors associated with CN malignant transformation. However, there are currently no high-sensitivity detection indicators and protocols available for predicting and evaluating the malignant transformation of CN. Histopathology remains the most accurate detection method, providing detailed differentiation between parenchymal and non-parenchymal cells and precise localization. Additionally, immunohistochemistry offers valuable information on angiogenesis and cell proliferation.

While genetic analysis of liver biopsy tissue presents certain insights, research on the molecular mechanisms of tumorigenesis is not yet comprehensive, and the detection of trace tissue samples can be challenging due to insufficient sample sizes. Despite the high diagnostic value and suggestive significance of liver biopsies for CN classification and prognosis, there are notable limitations. Most current research is based on surgical samples from HCC and nonmalignant CN samples obtained during liver transplantation, which may not accurately represent CN development in patients with liver cirrhosis. More liver biopsy samples are needed to validate relevant findings. Additionally, the invasive nature of liver biopsies limits their use as routine monitoring tools, especially in patients with decompensated cirrhosis and coagulation dysfunction.

Imaging and serological detection methods offer more convenience but may lack detailed understanding of HCC biological characteristics, potentially impacting targeted therapy applications. Active risk assessment and prediction are essential for timely intervention and improved prognosis for CN patients. Current studies indicate that hepatobiliary-phase hypointense nodules, growth rate, serum AFP levels, HBV or HCV infection, prior HCC history, and male gender are high-risk factors for CN malignant transformation (Figure 1). Although some studies are retrospective and may introduce bias, they provide useful references for future research.

To enhance the specificity and sensitivity of risk prediction, especially for subcentimeter CN, more accurate CN classification, stratification schemes, and sensitive indicators are needed. Liver biopsy can provide molecular biological indicators, but only noninvasive or minimally invasive biomarkers are practical for routine evaluation and prediction. Blood biomarkers such as miRNA and DNA methylation have shown promise in early HCC detection, but their role in CN malignant evolution remains unclear. Combining blood biomarkers with imaging and other indicators is expected to improve CN diagnosis accuracy, particularly for identifying HGDN and early HCC, and to offer risk prediction and assessment for CN malignant trends. The development of liquid biopsy technology has provided more possibilities for improving the accuracy of noninvasive HCC diagnosis. However, there is still a lack of research on the dynamic progression of CN, which clearly requires more attention and investment in research and development. Imaging studies remain the primary method for monitoring CN progression, but in clinical practice, a more reasonable monitoring strategy is necessary, considering both cost-effectiveness and diagnostic accuracy. Developing a multi-index evaluation model could be an effective approach for monitoring CN progression, requiring validation through larger-scale clinical samples. Unfortunately, research in this area is still limited. In addition, the frequent use of contrast agents may increase the likelihood of adverse reactions in patients. Therefore, monitoring strategies based on different categories of CN may require tailored time interval protocols to balance the risks and benefits effectively. Therefore, it is essential to actively explore specific, sensitive, and stable blood biomarkers during CN progression and to construct an efficient multi-index risk assessment model.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions
Zhun Xiao: Conceptualization; Resources; Writing – original draft.

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The authors declare that there is no conflict of interest.

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