

Review

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# Liver carcinogenesis: diagnostic and clinical aspects of preneoplastic nodules

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## Abstract

In multistep hepatocarcinogenesis, sizable lesions can precede the development of hepatocellular carcinoma (HCC). These lesions are currently classified as low grade (LG)- and high grade (HG)-dysplastic nodules. Following international guidelines recommending the surveillance of cirrhotic patients, a growing number of 1-2 cm hepatocellular nodules are recognized including early hepatocellular carcinoma (eHCC) and DN the latter accounting for as many as 70% of nodules < 1 cm. HG-DN are currently considered the most advanced HCC precursors. The histological diagnosis of low-grade dysplastic nodule (LG-DN), high- grade dysplastic nodule (HG-DN) and eHCC in small liver biopsies requires a comprehensive stepwise morphological and immunocytochemical approach. By imaging the differential diagnosis among these lesions is a challenge. According to vascular enhancement at dynamic computed tomography (CT) or magnetic resonance imaging (MRI) these precursors are classified as hypo-vascular/ indeterminate nodules even though distinction between LG-DN and HG-DN is almost impossible. The introduction of gadoexetic acid-enhanced MRI has represented an extremely important advance in this field allowing a better differentiation of dysplastic lesions from eHCC and progressed HCC. Additional MRI features as diffusion-weighted imaging further improved diagnostic accuracy of imaging. According to Liver Imaging Reporting and Data System (LI-RADS), either CT/MRI or Contrast-Enhanced Ultrasound LI-RADS, the dysplastic lesions should be categorized as LR-3 or LR-4. Natural history of these lesions confirmed that HCC can develop from HG-DN but which nodule and when it will undergo malignant transformation is not predictable. The search and validation of radiological and tissue markers able to select lesions more prone to HCC development, is currently underway. Whether and how HG-DN should be ablated or closely followed up is currently debated.

**Keywords:** Low-grade dysplastic nodule, high-grade dysplastic nodule, early hepatocellular carcinoma, progressed hepatocellular carcinoma, dynamic imaging, gadoexetic acid-enhanced resonance imaging, hepatobiliary phase



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## INTRODUCTION

It has long been known that, like in others human carcinogenic models as colo-rectal cancer, in hepatocellular multistep carcinogenesis precancerous lesions both micro- and macroscopic precede the development of hepatocellular carcinoma (HCC)<sup>[1,2]</sup>. Chronic hepatitis and cirrhosis represent the natural background in which precancerous lesions grow through stepwise sequence. These lesions have been described many years ago and include microscopic dysplastic foci (DF) and sizable dysplastic nodules (DN). The latter are further categorized as low-grade (LG-DN) and high-grade dysplastic nodules (HG-DN) with specific and distinctive morphologic characteristics and different carcinogenetic propensity<sup>[3]</sup>. Representation of multistep hepatic carcinogenesis is schematized in [Figure 1]. Given the low attitude to perform liver biopsy in cirrhotic patients, microscopic preneoplastic lesions have been losing interest in clinical practice. Hence, the present review will focus specifically on preneoplastic nodules which can be routinely detected by radiologic techniques and monitored over time for their potential neoplastic transition. Histologic classification, histopathologic features, radiologic diagnostic criteria, natural history and treatment of these lesion will be discussed.

In cirrhosis, application of regular ultrasound surveillance resulted in the discovery of an increasing number of small nodules [ $< 2$  cm according to the definition of small lesion by International Working Party (IWP)]<sup>[4]</sup>. Small nodules usually include definite hepatocellular carcinoma (HCC), either early hepatocellular carcinoma (eHCC) and very-early hepatocellular carcinoma (veHCC) or progressed hepatocellular carcinoma (pHCC) and the large majority of pre-neoplastic nodules<sup>[5-9]</sup>. Other small lesions as metastases or mesenchymal neoplasms are much less frequently detected during surveillance.

Distinction of pre-neoplastic nodules from well-established HCC is mandatory for decision making process, particularly in transplant or surgical setting. To decide whether a given nodule is malignant, premalignant or benign is therefore of paramount importance demanding a great radiologic, morphologic and clinical expertise. The following key points should help clinicians to get oriented in the management of such small nodules:

1. Precancerous nodules have a maximum diameter seldom exceeding 2 cm and are typically detected in cirrhosis/chronic hepatitis.
2. Their prevalence is relevant ranging from 60% to 70% among nodules  $< 1$  cm and from 20% to 30% among nodules  $> 1 < 2$  cm.
3. They can be intercepted as single or multiple lesions and often concomitant with already established HCC.
4. HG-DN account for 30 % of all precancerous nodules and are considered true precancerous lesions whereas LG-DN have a trivial neoplastic risk comparable to that of large regenerative nodules.
5. Transforming risk of HG-DN is largely unpredictable at baseline.

## HISTOPATOLOGICAL CLASSIFICATION AND FEATURES OF DYSPLASTIC NODULES

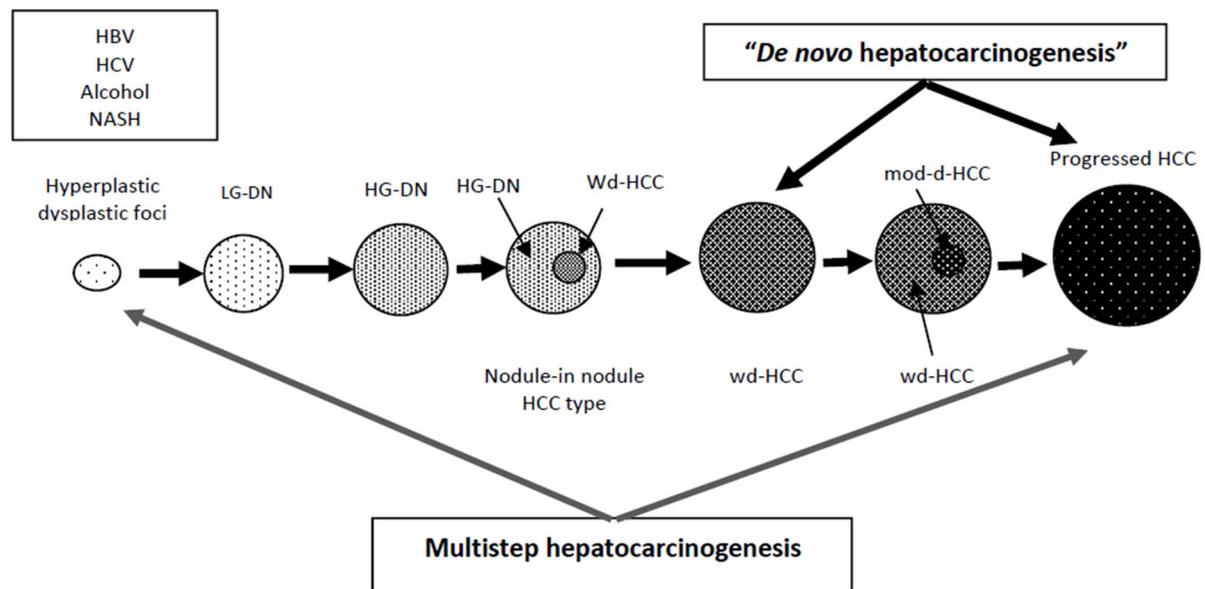
### Nomenclature

Definition and nomenclature of precancerous nodules was firstly proposed by the International Working Party in 1995<sup>[4]</sup> and further updated in an east-western consensus of pathologists in 2008<sup>[10]</sup>.

Non-malignant hepatocellular nodules include large regenerative nodules (LRN) and DN. The latter are subclassified as LG-DN and HG-DN. However, LG-DN share a number of features to non-neoplastic LRN and therefore, the distinction between these two lesions is particularly difficult and unpractical. Hence, the panel of experts recommended not to separate LG-DN from LRN and classify as LG-DN any nodule that cannot be classified as HG-DN<sup>[4]</sup>. Their size ranges from few millimetres to 2 cm rarely exceeding 3 cm.

### Histologic differential diagnosis

Histologic differentiation of LG-DN, HG-DN and eHCC is challenging, particularly when pathologists have to deal with small samples obtained by fine needle biopsy. It has been repeatedly stressed that histology is



**Figure 1.** Schematic illustration of lesions occurring in hepatocarcinogenesis. Two models of human hepatocarcinogenesis. HCC: hepatocellular carcinoma; DN: dysplastic nodules; HG-DN: high-grade dysplastic nodules; LG-DN: low-grade dysplastic nodules

mandatory, and that comparison between intra nodular and extra nodular tissue is of great importance. Cytology should be avoided since it does not allow to appreciate architectural changes useful for differential diagnosis. First diagnostic step is to evaluate whether or not the sample is adequate. Schematically, adequacy can be checked by evaluating the overall nodularity at low magnification [Figure 2] by comparing intra- and extranodular samples. In brief, when both intra- and extra-lesional samples show overlapping cirrhosis-like features, without a major nodule in the background, the sample should not be considered adequate and biopsy should be repeated shortly after. This is another reason why cytology is inadequate to approach this issue.

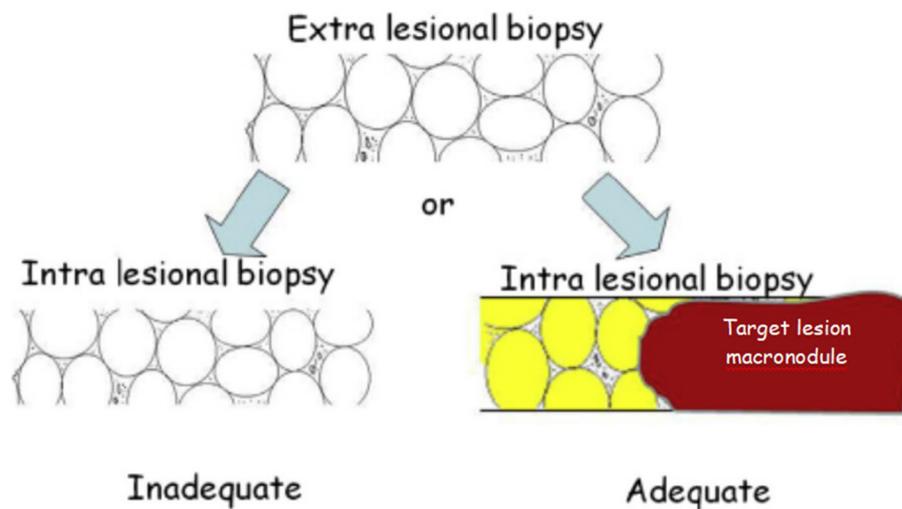
*Small liver cell dysplasia* (SLCD) is a key feature of HG-DN. It refers to areas of increased cell density (more than twice that of the surrounding tissue) and plate thickening, simulating a well-differentiated HCC. Formation of pseudoglands/acini along with SLCD are features helping to distinguish HG-DN from LG-DN. These features are also present in eHCC even though much more pronounced.

*Unpaired arteries*, so called because they are not coupled with bile ducts, is another distinctive feature of liver carcinogenesis and represent neoangiogenesis which is the morphologic counterpart of arterial contrast enhancement on imaging. Similarly, sinusoidal capillarization which can be highlighted by CD34 immunostaining is minimal in cirrhosis and increases from LG- to HG-DN with the highest levels in HCC.

*Reticulin framework* is another feature useful to distinguish benign from malignant nodules since it is usually well preserved in both regenerative and dysplastic nodules whereas it is decreased or lost in advanced HCC; however, in early well-differentiated HCC, the reticulin framework may be retained or only slightly decreased.

*Portal tracts* are retained in HG-DN and can be retained in eHCC as well and their presence does not help differential diagnosis.

*Stromal invasion* is the key feature of definitive malignant transition separating eHCC from HG-DN. Stromal invasion has to be looked for in portal tracts but, unfortunately, it is not always detectable, especially on samples obtained by fine needle biopsy. For iconography please refer to recent review by Roncalli et al.<sup>[11]</sup>.



**Figure 2.** Schematic illustration of adequacy judgement of liver biopsy for small nodules in cirrhosis. Intra- and extra-lesion samples are mandatory. Schematically, adequacy can be checked by evaluating the overall nodularity at low magnification by comparing intra- and extranodular biopsy samples. When both intra- and extra-lesional samples show overlapping cirrhosis-like features, without a major nodule in the background, the sample should not be considered adequate and biopsy should be repeated shortly after

The immunostaining of tissue biomarkers is a promising tool in the armamentarium of pathologists for improving the accuracy of the differential diagnosis between benign and premalignant/malignant nodules [Table 1]. In the last two decades, translation of data from gene expression profiles to clinical practice has been focused on the search of serum and tissue markers involved in hepatocarcinogenesis and useful for diagnostic purposes. Most of these markers can be tested on paraffin embedded, hematoxylin-eosin stained tissue samples making this diagnostic technique suitable for employment in clinical practice even on fine needle liver biopsies. Among them alpha-fetoprotein and des- $\gamma$ -carboxy prothrombin are serum markers of HCC but are not sensitive enough for eHCC<sup>[12]</sup>. Other tissue markers as heat shock protein 70 (HSP70), glutamine synthetase (GS), glypican 3 (GPC3), CD34, p53, proliferating cell nuclear antigen (PCNA) and Ki67 have been tested in lesions at different neoplastic evolution in the multistep process leading to mature HCC<sup>[12]</sup>. A panel of three biomarkers: HSP70, GS and GPC3 has been applied to differentiate non-malignant nodules (either LG or HG-DN) from HCC and a positivity for any two or three markers detected malignancy with a sensitivity of 72% and specificity of 100%. Sensitivity dropped to 50% when the panel was applied to tissue obtained by fine needle biopsy samples whereas specificity remained absolute<sup>[13]</sup>. These findings were validated by other authors<sup>[14]</sup>. More recently it has been demonstrated that the addition of clathrin heavy chain (CHC) to the above-mentioned panel of biomarkers increased the diagnostic accuracy for small HCCs from 76.9% to 84.3%, with an important gain in sensitivity (from 46.8% to 63.8%)<sup>[15]</sup>. Currently, the use of three biomarkers panel with CD34 is endorsed by AASLD-EASL guidelines as a diagnostic instrument for diagnosis of HCC<sup>[16,17]</sup>. An algorithmic workflow based on sample adequacy and histopathological features including biomarkers immunostaining useful for characterization of small nodules in cirrhosis has been recently proposed by Roncalli *et al.*<sup>[11]</sup>.

### The role of biopsy

International guidelines recommend performing a biopsy whenever a nodule results atypical or indeterminate at conventional dynamic imaging (see below)<sup>[16-20]</sup>. If a biopsy should be done, a 18-20 gauge cutting needle should be employed and intra- and extra-lesion sample should be obtained. In the setting of small nodules in cirrhosis however, the role of biopsy is hampered by the risk of false negative results due to sampling error. Smaller the nodule higher the risk of sampling error. The rate of false negative sample is quite high and a successful result achievable in no more than two-third of patients<sup>[8]</sup>. In the remaining one-third of cases a second biopsy within a short interval is recommended. It is important to note that, contrarily to

**Table 1. Morphologic features and diagnostic tools for differential diagnosis between dysplastic nodules and early HCC**

	LG-DN	HG-DN	eHCC	Diagnostic value between HG-DN and eHCC
Elementary morphologic feature				
Parenchymal changes, cytologic alterations				
SCC	-	+	+	Low
LCC	±	±	-	Low
Clone like	±	+	+	Low
Architectural changes				
Cell density	±	+	+	Low
Pseudoglands/acini	-	±	+	Medium
Non-parenchymal changes				
Portal tracts	+	+	±	Low
Reticulin framework	+	+	±	Medium*
Unpaired arteries or sinusoidal capillarization (CD34)	±	±	+	Low
Diagnostic tools				
Stromal invasion/loss of ductular reaction (K7/19)	-	-	±	High
HCC biomarkers expression (at least 2 markers among HSP70, GPC3, GS, CHC)	-	-	++	High
Nodule-in-nodule	-	-	±	High

\*If frankly decreased or lost, the discriminatory value of reticulin framework is high. -: absent; ±: may be present but not necessarily detectable in biopsy; +: present and usually detectable in biopsy. CHC: clathrin heavy chain; GPC3: glypican 3; HSP70: heat shock protein 70; LCC: large cell change; SSC: small cell change; LG-DN: low-grade dysplastic nodule; HG-DN: high-grade dysplastic nodule; eHCC: early hepatocellular carcinoma

what can be expected, a second biopsy has the same probability of success as the first. Thus, repeated biopsy would finally lead to a successful diagnostic yield in more than 90% of cases<sup>[8,21]</sup>. However, this invasive strategy is seldom adopted in clinical practice mainly for the awareness that *a priori* probability that such indeterminate 1-2 cm nodules are malignant is low<sup>[8,9,22,23]</sup> and that 2 cm is the size threshold to achieve the best result by percutaneous ablation. Based on these assumptions, a *wait and see* strategy with the adoption of a strict monitoring of the nodule by dynamic imaging is often preferred (see below). Nodule location and coagulative disorders are additional features making biopsy difficult or impossible. Lastly, the potential and theoretical risk of tumour seeding, should be considered even though, this risk, ranging between 1% and 2.7%, seems to balance favourably with the risk of inappropriate or delayed treatment<sup>[24-26]</sup>.

### RADIOLOGIC CRITERIA FOR DIAGNOSIS AND CHARACTERIZATION OF PRENEOPLASTIC NODULES IN CIRRHOSIS DYNAMIC IMAGING CT AND MRI

To date, the differential diagnosis between eHCC and dysplastic nodules still remains a radiologic challenge. Premalignant nodules are usually detected by standard US as hypo- or, less frequently, as hyper-echoic nodules completely indistinguishable from well-established HCC. Thus, standard US is inadequate for differential diagnosis and other imaging tools are needed. The physiopathologic basis for the diagnosis and characterization of small hepatic nodules by imaging rests on characteristics of their vascular supply. It is well established that during cirrhosis-related oncogenesis, progressive loss of normal portal vascular supply in favour of increase arterial one, the so called neoangiogenesis, occurs. This nodular arterialization becomes progressively more evident by transition from regenerative to dysplastic and neoplastic nodules reaching the full expression in high-differentiated HCCs. Dynamic CT, magnetic resonance imaging (MRI) and Contrast-Enhanced Ultrasound (CEUS) are the contrast-enhanced imaging to investigate the vascular pattern of nodules detected under surveillance in cirrhosis. A recent meta-analysis including several comparative studies confirmed that MRI is more accurate than dynamic CT for detection and characterization of small lesions and therefore, it should be preferred as a first line panoramic imaging<sup>[27]</sup>. In addition, a comparative 13-years meta-analysis<sup>[28]</sup> showed that CEUS had a sensitivity and PPV close to that of MRI with gadoexetic contrast media.

At imaging using the so-called extracellular contrast media, mature HCC shows a typical vascular pattern characterized by homogeneous and intense contrast uptake in the arterial phase known as “wash in” followed by a progressive washout of contrast in venous or late phases. Opposite, regenerative nodules, being a simple hypertrophic inflammatory growth of normal hepatocytes, maintain a normal vascular supply and share the same contrast uptake as the surrounding cirrhotic parenchyma during arterial and venous phases. Dysplastic nodules, either LG-DN or HG-DN lack a well-developed neoangiogenesis and maintain normal or partially normal portal inflow appearing as non-enhancing or partially enhancing nodules. Conventional dynamic imaging “per se” cannot differentiate LG-DN from HG-DN. Diagnostic difficulties are also represented by those incipient eHCCs with still poorly developed neoangiogenesis which usually appear as non-enhancing lesions (the so-called hypovascular HCC) and, therefore, hardly distinguishable from HG-DNs.

According to all the international guidelines<sup>[16-20]</sup> arterial wash-in and late washout, when present, are sufficient for the diagnosis of HCC and histologic confirmation is not necessary even for small lesions. This paradigm served to design the non-invasive diagnostic algorithm for nodules detected in cirrhotic patients under surveillance proposed by AASL-EASL-ERTO guidelines<sup>[16-17]</sup>. According to this algorithm, new lesions < 1 cm should be strictly monitored without biopsy. Nodules > 1 cm showing definite hallmarks of malignancy by a single enhancing imaging do not require confirmation by a second one and diagnosis of HCC is accepted. In case of equivocal/inconclusive results by the first imaging, a second one should be sequentially performed. If uncertainty still persists after two dynamic techniques, lesion should be categorized as indeterminate or atypical and biopsy is recommended<sup>[16,29]</sup>.

This sequential strategy demonstrated high specificity and almost absolute PPV for diagnosis of HCC and, in clinical practice, proved to be cost-effective and useful for minimizing the number of futile biopsies<sup>[9,22]</sup>.

Although specificity and positive predictive value of radiologic hallmarks of malignancy (wash in and late washout) are close to 100%, their sensitivity in small lesions is suboptimal (71%)<sup>[30,31]</sup>. According to vascular-based imaging modalities one-third of small nodules discovered under surveillance remain indeed indeterminate or atypical even by dynamic MRI. Indeterminate/atypical lesions include approximately 30% of hypovascular eHCCs<sup>[7,22,32]</sup> and the large majority of pre-neoplastic lesions (the one-third rule: one-third of small lesions are indeterminate and one-third of them are HCCs).

Premalignant nodules are usually detected by standard US as hypo- or, less frequently, as hyper-echoic small nodules. Hence, standard US cannot be used for differential diagnosis being dysplastic nodules completely indistinguishable from well-established HCC. On dynamic imaging these nodules appear as non- or weakly enhancing lesions on arterial phase and hypo/iso-enhancing on venous/delayed phases (hypovascular nodules). Therefore, they fall into the group of indeterminate/atypical lesions and, as recommended by AASLD/EASL guidelines, they should require histologic diagnosis. In this setting however, the role of biopsy is still debated (see above). The differentiation between DN and eHCC is challenging on conventional dynamic imaging. The recent introduction of MRI hepato-specific post-vascular contrast media has represented a relevant diagnostic advance in the characterization of small nodules in cirrhosis<sup>[33,34]</sup>. Gadolinium-chelate agents as gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd EOB DTPA, Primovist<sup>®</sup>) or gadobenate dimeglumine (Gd-BOPTA, MultiHance<sup>®</sup>) enter the hepatocyte by the organic anion transporter polypeptide 1 (OATP1) and are excreted in the bile canaliculi by MRP2,3,4 protein transporters. These agents make possible to achieve information not only on the vascular profile of a given lesion (dynamic phase) but also on specific hepatocyte functions in the so-called hepatobiliary phase (HE phase). During carcinogenetic process, proteins responsible of uptake and excretion of gadolinium-chelates are progressively lost and this derangement may precede changes on vascular profile<sup>[35]</sup>. Most eHCCs and vECCs, are in fact no longer able to incorporate gadolinium-chelates and appear hypo-intense on HE phase. Opposite, LG-DN and most HG-DN maintain still preserved uptake function and consequently

appear as iso- or even hyper-intense nodules. These contrast agents are therefore potentially able to distinguish differently progressed lesions<sup>[35]</sup>. Although the full potential of liver-specific-contrast media needs to be fully assessed in prospective studies, the use of these agents is now considered mandatory in this setting since they provide better diagnostic accuracy than the vascular contrast media<sup>[36]</sup>. Recent data on histologically well characterized small atypical nodules in cirrhosis, confirmed the high diagnostic accuracy of Gd EOB DTPA MRI to correctly distinguish eHCC from premalignant nodules. Ninety-seven percent of iso/hypovascular nodules were correctly classified as premalignant or malignant in HE-phase with a specificity of 100% for malignancy when hypointensity in HP phase was coupled with hypervascularity in arterial phase<sup>[32]</sup>. These data are well in keeping with those of Choi *et al.*<sup>[37]</sup>. In a recent prospective study<sup>[38]</sup> carried out on 111 small nodules defined as atypical at CEUS or dynamic CT and histologically classified as eHCCs or dysplastic, either LG-DN and HG-DN, emerged that HE-phase hypointensity by itself using Gd-EOB-DTPA was the strongest predictor of malignancy superior to vascular-based hallmarks and T2w behaviour. All hypointense nodules at HE phase were malignant/premalignant (overt HCC or eHCC/HG-DN) and hypointensity captured 45/51 malignant nodules either hyper- or hypovascular, with a sensitivity of 88% and specificity of 97% reaching 100% when associated with arterial enhancement. In this study, all benign nodules (large regenerative or LG-DN) displayed iso-hyperintensity at HE-phase. However, it could be outlined that a very low rate (roughly 3%) of well-differentiated hepatocellular carcinomas (wdHCCs), are hyper vascularized at arterial phase but are iso-hyperintense on HE-phase mainly because they retain still functioning hepatocytes<sup>[39-43]</sup>. It is wise to consider nodules with arterial enhancement but hyperintense in HE as highly suspicious of incipient malignant transition.

The differential diagnosis between HG-DN and eHCC still remains difficult even by Gd-EOB-DTPA MRI since HG-DN show variable behaviour on HE-phase being frequently hypointense like eHCC<sup>[38,44-48]</sup>. Indeed, the specificity of hypointensity on HE-phase as a unique hallmark of malignancy, is suboptimal ranging from 33% to 97%<sup>[38,49]</sup>. It has been recently outlined that coupling Gd-EOB-DTPA MRI with diffusion-weighted imaging (DWI) may result in increased accuracy for diagnosis of overt HCC and also for the differential diagnosis of eHCC and HG-DN<sup>[44,45]</sup>. DWI is based on the simple assumption that water diffusion in the extracellular compartment is influenced by cellular density which, in turn, reduces the width of interstitial spaces and water diffusion<sup>[50,51]</sup>. Water diffusion can be quantified by a mathematical index called apparent diffusion coefficient (ADC); low ADC values mean low diffusivity, namely hypercellularity and, as previously stated, hypercellularity progressively increases from HG-DN, to eHCC reaching maximum expression in progressed HCC. Unfortunately, DWI as a unique tool for assessing hepatic lesions is inaccurate due to the considerable overlap between benign and malignant lesions and normal liver tissue<sup>[52,53]</sup>. In addition, DWI images are highly influenced by artefacts of liver motion due to respiration and artefacts in the left lobe derived from the heart beating<sup>[54,55]</sup>. Therefore, DWI should be used in conjunction to the other conventional imaging features. Renzulli *et al.*<sup>[56]</sup> reported data from a prospective study evaluating the imaging criteria of HCC, early HCC and HG-DNs using gadoxetic acid-enhanced liver MRI in 228 patients prospectively enrolled with 480 small nodules detected under surveillance for cirrhosis. Using three MRI findings: HE-phase hypointensity, arterial hyperintensity and diffusion-weighted imaging (DWI) the authors designed an algorithm which yielded an overall sensitivity of 96.6% and a specificity of 92.7% and a very high sensitivity (94.7%) and specificity (99.3%) in classifying HG-DN. Although some technical aspects need to be fully standardized (type of MRI machine, breath old method, *b* value for DWI), gadoxetic acid-enhanced MRI including DWI analysis as part of a diagnostic algorithm, may represent a promising approach to better defining those small lesions still atypical at vascular and HE phases being eHCC hyperintense and HG-DN isointense al DWI. DWI behaviour is currently part of ancillary features for liver nodules characterization in Liver Imaging Reporting and Data System (LI-RADS) v17 system (see above)<sup>[57]</sup>.

These advances on MRI prompted JHS and APASL guidelines to incorporate gadoxetic acid-enhanced MRI for definition of atypical nodules before performing biopsy<sup>[18,19]</sup>. Conversely, the late version of guidelines from the Western world<sup>[58,59]</sup>, still endorse a diagnostic algorithm for HCC that have remained the same for

at least 16 years not giving due relevance to the above discussed imaging innovation. Furthermore, western guidelines erroneously consider CT as equivalent to MRI in diagnostic accuracy even though recent meta-analyses<sup>[60-61]</sup>, one of which conducted by the same authors who contributed to the last version of AASLD guidelines, confirmed, the superiority of the latter. Other hepato-specific contrast media as Sonazoid for CEUS (s-CEUS) or Super paramagnetic iron oxide agents for MRI (SPIO-MRI) showed promising results but they are not available worldwide and the experience with these compounds is limited to Eastern countries<sup>[18,19]</sup>. Sonazoid (GE Healthcare, Waukesha, WI, USA), is a second-generation contrast agent available in Japan, South Korea, and Norway (August 2015). The microbubbles of this agent are captured in the liver parenchyma by reticuloendothelial (Kupffer) cells and, therefore, s-CEUS provides information in both haemodynamic-phase and accumulated-phase images. Because malignant tumours contain few or no Kupffer cells, they appear as perfusion defects in late phase of s-CEUS. Conversely, non-neoplastic nodules with preserved or only minimally reduced reticuloendothelial system lack wash-out in the late-phase. Sonazoid proved to be particularly accurate for characterization of small lesions in cirrhosis. In a literature review covering 10 years experience with Sonazoid<sup>[62]</sup>, s-CEUS emerged as accurate as gadoxetic acid-enhanced MRI in differentiating benign nodules from wdHCC. Furthermore, s-CEUS provides a better characterization of some small lesions at contrast-enhanced CT or MRI. Takahashi *et al.*<sup>[63]</sup> in a study comparing s-CEUS and gadoxetic acid-enhanced MRI on characterization of small < 3 cm lesions in cirrhosis reported that 27% of indeterminate hypo-vascular lesions at MRI turned out to be hyper-enhancing at s-CEUS and were histologically diagnosed as HCC. For these reasons s-CEUS is currently included by Japanese and APASL guidelines in the diagnostic algorithm for indeterminate lesions discovered in cirrhosis during surveillance.

## LI-RADS CRITERIA

In 2011 the American College of Radiology issued a new system of liver imaging reporting (LI-RADS) aimed at providing a precisely defined terminology for interpreting and reporting contrast-enhancing CT and MRI examination of nodules detected in cirrhosis<sup>[64]</sup>. This system also included guidance for the interpretation of data obtained by MRI with hepato-specific agents. According to LI-RADS, lesions detected at dynamic CT or MRI in patients at high risk of having HCC (cirrhotic patients under surveillance), are categorized as definitively benign (LR-1), probably benign (LR-2), intermediate probability of being HCC (LR-3), probably HCC (LR-4) and definitively HCC (LR-5). This system would provide more guidance to clinicians to adopt proper clinical decisions such as accelerated follow-up, biopsy or even treatment of small lesions in cirrhosis. According to criteria proposed by LI-RADS the great majority of dysplastic nodules are categorized as LR-3 and LR-4. LR-3 lesions should be strictly monitored and LR-4 lesions require biopsy<sup>[64]</sup>.

LI-RADS system was strongly encouraged by the US regulatory agency for liver transplantation (UNOS) to avoid or minimize futile transplants for false positive cases. Indeed, LI-RADS criteria proposed by LI-RADS v2014 and by the updated 2017 version (v2017)<sup>[57]</sup> for diagnosis of HCC are more restrictive than those proposed by AASLD-EASL and do not accept the diagnosis of definitive-HCC (LR-5) for lesions sized 1-2 cm even in presence of wash in and washout. In these cases a 50% growth in at least 6 months is needed for definite classification as LR-5. These stringent criteria would limit *de facto* the early diagnosis of HCC in the group of 1-2 cm nodules. These limits were emphasized by a prospective study comparing LI-RADS classification with AASLD criteria for HCC on 133 small (< 2 cm) newly detected nodules in cirrhosis studied by MRI<sup>[65]</sup>. In this study 21 histologically proven eHCCs were erroneously subcategorized as LR-4 according to LI-RADS criteria even though they were hyper-enhancing at arterial phase with late wash-out. In addition, 29 small HCCs fell into LR-3 category making the overall sensitivity of LI-RADS for HCC 43%, relevantly lower than the sensitivity of AASLD criteria (58.6%). Similar results were obtained by Ronot *et al.*<sup>[66]</sup> in a prospective study on 595 nodules < 3 cm in cirrhosis, where specificity of LI-RADS criteria for diagnosis of HCC relevantly increased and became better than AASLD criteria when LR-4 and LR-5 categories were considered in combination. On the other hands and more important, in the study by Darnell *et al.*<sup>[65]</sup>

none of the non-malignant hepatocellular nodules fell into LR5 category making the LI-RADS specificity for HCC diagnosis close to 100%. The high specificity of LI-RADS criteria was further emphasized in a recent systematic review in which data from 2,760 patients were analysed<sup>[67]</sup>. The authors found that only 3% of observations categorized as LR5 were non-malignant giving a 97% overall specificity of LI-RADS for malignancy. To overcome the low sensitivity of LI-RADS, the most recent version (v2018)<sup>[68]</sup> provides updated criteria for small (10-19 mm) LR-5 observations and a simplified definition for threshold growth. In this version, in order to align LI-RADS criteria with AASLD/EASL criteria of HCC and increase simplicity, a 10-19 mm nodule with arterial phase hyperenhancement and non-peripheral “washout” is definitely categorized as LR-5.

According to LI-RADS v2018, hypointensity in the HE-phase and DWI hyperintensity are considered as ancillary features but they should not allow upgrading a suspected malignant lesion (LR-4) to LR-5<sup>[66]</sup>. In order to significantly increase the diagnostic efficacy of the MRI LI-RADS criteria it has been suggested that they should be modified (mLI-RADS) by incorporating hypointensity in the HE-phase and hyperintensity at diffusion restriction among the major MRI features of malignancy<sup>[68]</sup>.

A further progress in the diagnostic accuracy small HCCs comes from the recently released Contrast-Enhanced Ultrasound LI-RADS (CEUS LI-RADS v2017) which provides a refined definition of the typical CEUS pattern of HCC<sup>[69]</sup>. According to CEUS LI-RADS criteria, a nodule showing a rapid arterial enhancement and a delayed wash out (> 60 s) can be classified as definite HCC (LR-5) regardless its size. These criteria were recently validated in a series of 1086 well-defined lesions detected in cirrhosis and studied by CEUS. Applying CEUS LI-RADS criteria 58.5 % of HCCs were correctly classified as LR-5 with a PPV of 98.5% and only 3% of non-malignant nodules were erroneously classified as HCC<sup>[70]</sup>. Thus, LR-5 CEUS is an extremely reliable criteria for HCC, given its excellent PPV, without misdiagnosis for other malignancies. Using CEUS LI-RADS criteria most of LG and HG-DN were classified as LR-3 and LR-4<sup>[70]</sup>. These results are extremely important and support the use of CEUS in clinical practice not only for the definite diagnosis of HCC when other imaging is inconclusive but also for monitoring indeterminate lesions at risk of neoplastic transformation considering that it is cheaper and more accessible than MRI.

## NATURAL HISTORY OF PRENEOPLASTIC LESIONS

Although preneoplastic lesions have been described several years ago, their natural history has been clarified only recently by prospective studies carried out on cirrhotic patients undergoing US surveillance for early detection of HCC. Early small series from Japan<sup>[71-73]</sup> collected in a pre-dynamic imaging era, and including ultrasonically detected non malignant lesions classified according to different histologic criteria, provided preliminary evidence of the preneoplastic role of adenomatous/dysplastic nodules. More robust and convincing data derived from two successive prospective studies<sup>[5,74]</sup> comparing the natural history of different non-malignant lesions detected by ultrasounds in cirrhosis and categorized according to IWP classification. These studies confirmed that HG-DN were the true precursors of HCC with a risk of neoplastic transformation significantly higher as compared to LG-DN and LRN. Similar conclusions emerged from a single centre study on 66 LRNs and 20 DN with a 28-years follow-up by Sato *et al.*<sup>[75]</sup>. However, in these studies, the overall rate of malignant evolution of HG-DN nodules was relatively low ranging from 9% to 31% and neoplastic evolution could be documented in large regenerative/LG-DN as well albeit at a lower rate<sup>[5,74]</sup> [Table 2]. In addition, transforming progression of HG-DNs was hardly predictable in terms of elapsing time from US detection to HCC transformation. In fact, some HG-DNs remained stable over a long time (20% to 50%), often exceeding the follow-up time suggested by AASLD/EASL guidelines (18 months), and some disappeared during follow-up. These data rise concern on the low PPV of morphology as a unique tool for the correct identification of nodules at risk of transformation when applied to small samples supporting a “watchful waiting” policy based on a strict radiologic surveillance. Conversely, Iavarone *et al.*<sup>[76]</sup> in a retrospective-prospective study carried out on 36 non-malignant nodules histologically classified as

**Table 2. Natural history of regenerative/dysplastic nodules in cirrhosis**

Author (years)	Type	No	Malignant changes	Unchanged	Disappeared	Time (months) to HCC progression Mean (range)
Kondo <i>et al.</i> <sup>[71]</sup> , 2011	LRN	17	-	13 (76%)	4 (24%)	NA
Terasaky <i>et al.</i> <sup>[72]</sup> , 1998	LRN/DN	34	5 (15%)	4 (12%)	25 (73%)	NA
Seki <i>et al.</i> <sup>[73]</sup> , 2000	DN	33	4 (9%)	14 (42%)	15 (49%)	18 (16-21)
Borzio <i>et al.</i> <sup>[5]</sup> , 2003	LRN/DN	90	28 (31%)	44 (49%)	18 (20%)	22 (8-48)
Kobayashi <i>et al.</i> <sup>[74]</sup> , 2006	LRN/DN	154	29 (19%)	81 (53%)	44 (28%)	NS
Iavarone <i>et al.</i> <sup>[76]</sup> , 2013	LRN/DN	36	11 (31%)	21 (53%)	4 (16%)	13 (7-27)
Sato <i>et al.</i> <sup>[75]</sup> , 2015	LRN/DN	92	19 (21%)	30 (32%)	43 (47%)	NS

NA: not available; NS: not specified. DN: dysplastic nodules; LRN: large regenerative nodules

LG- or HG-DNs found that neither size increase nor changing on enhancing pattern emerged as accurate predictors of neoplastic transformation since some DNs transformed into HCC without enlarging and others without acquiring arterial hypervascularity.

In the dynamic imaging era, the natural history of pre-malignant nodules can be extrapolated from mainly retrospective studies including nodules classified as non-enhancing/indeterminate at both CT and MRI to differentiate them from progressed HCC. Studies before 2010 and mainly based on dynamic CT, confirmed that some of these hypo-enhancing nodules carry a risk to acquire true radiologic hallmarks of malignancy over time even though this risk at an individual level still remains unpredictable. From these studies, size at baseline (larger the nodule, higher the risk) and changes in size and/or in the vascular pattern during follow-up (shift from hypo- to hyper-enhancing pattern on arterial phase), were reliable predictors of malignant transformation<sup>[77,78]</sup>. In the retrospective study by Chuo *et al.*<sup>[79]</sup> including a large series of indeterminate (hypo-vascular) small nodules observed during surveillance of HBV-related cirrhosis, emerged that old age, initial size > 1 cm and arterial enhancement were risk factors for neoplastic progression. The authors developed a useful and accurate risk score model for predicting HCC progression of indeterminate nodules. Unfortunately, this risk score model was not further validated in series with different etiologic liver diseases.

From 2010 onwards, most data on natural history of small hypovascular lesions observed in cirrhosis derived from studies carried out by gadoxeti-acid enhanced MRI<sup>[80-86]</sup>. In these studies neoplastic evolution was assumed to occur by acquisition of hypervascularity. Unfortunately, all these studies were retrospective and hypovascular/indeterminate nodules lacked histological classification. Furthermore, in most of these studies, one of the inclusion criteria was hypointensity at HE-phase. As previously stated, most of small nodules hypovascular, hypo-intense at He-phase, are eHCC/HG-DN. Thus, in the strict sense, these studied focused on the natural history of early hypovascular HCCs or progressed HG-DN rather than on precursor lesions as a whole<sup>[80-86]</sup>. This may partially explain why the transition rate to hyper-vascularized pHCC found in these studies was extremely high ranging from 12% to 35% at one year [Table 3].

At the best of our knowledge, only two studies by gadoexetic-acid enhanced MRI provided reliable information on the natural history of premalignant lesions with exclusion of hypovascular HCC.

The first by Kim *et al.*<sup>[85]</sup> focused on hypovascular, HE hypo-intense lesions without T2W hyperintensity. Authors assumed that having excluded T2 hyperintense nodules, the risk of inclusion of eHCC among hypovascular hypointense nodules on HE phase was marginal.

In this study the rate of hypervascular transformation of precursor nodules, such as LG-DN and HG-DN was 23 % at 3 years. In the second study, Sano *et al.*<sup>[86]</sup> in a large series of small hypo-vascular nodules, iso/hyperintense at HE found that acquisition of hypervascularity was extremely rare (0.6%) over 3 years follow-up and no nodules evolved into mature HCC after the fourth year. In this study the only independent risk factor for progression was the initial size of the nodule (> 10 mm). In addition, the nodule growth rate showed 85% PPV in predicting of hypervascularization.

**Table 3. Natural history of small hypovascular nodules at MRI and evaluated by gadoteric acid uptake at hepatic phase**

Author (years)	Type	HE intensity at baseline	Nodules (n)	Acquired hypervascularity	Mean follow-up (months)	Risk factors
Kumada <i>et al.</i> <sup>[81]</sup> , 2011	Retrospective	Hypo-	49	6 (27%)	12	Size ≥ 15 mm
Motosugi <i>et al.</i> <sup>[80]</sup> , 2011	Retrospective	Hypo-	135	16 (12%)		Size ≥ 10 mm Fat content enlargement
Kim <i>et al.</i> <sup>[83]</sup> , 2012	Retrospective	Hypo-	214	75 (35%)	11	Hyperintensity at DWI
Hyodo <i>et al.</i> <sup>[82]</sup> , 2013	Retrospective	Hypo	160	50 (31%)	12	Rapid growth (tumor volume doubling time = 542 days) T2W hyper-intensity
Higaki <i>et al.</i> <sup>[84]</sup> , 2014	Retrospective	Hypo	60	10 (17%)	12	Higher growth rate
Kim <i>et al.</i> <sup>[85]</sup> , 2016	Retrospective	Hypo No T2W hyperintensity	114	26 (23%)	42	T1w hyperintensity Size > 10.5 mm Previous HCC Rapid growth rate
Sano <i>et al.</i> <sup>[86]</sup> , 2017	Retrospective	Iso-hyper	663	6 (0.9)	36	Size > 10 mm

HCC: hepatocellular carcinoma

In summary, results from imaging-based studies provide evidence showing that indeterminate hypovascular nodules may evolve into mature HCC but this transition is hardly predictable based on initial clinical characteristics and MR imaging features. The risk and speed of neoplastic evolution of hypovascular nodules seems to depend mostly on their behaviour at HE phase on gadoexetic-acid enhanced MRI. Hypo-intense nodule at HE phase, have an elevated risk to become hypervascular pHCC in a short interval, whereas nodules iso/hyper-intense at HE phase, showed a minimal oncogenic risk<sup>[80-86]</sup>. Among the numerous risk factors found, the growth rate per se seems to have the highest positive predictive value and should be regarded as the most reliable alarm ring for radiologic evolution to progressed HCC.

### SURVEILLANCE AND TREATMENT

The proper follow-up of non-malignant lesions is still debated and, theoretically, it should be dictated by the intrinsic risk of neoplastic evolution [Table 4]. Guidelines do not specifically address this issue even though a strict follow up is recommended. Once a nodule is histologically classified as dysplastic, either LG-DN or HG-DN, it should enter an enhanced follow-up the goal of which is to promptly capture its neoplastic transformation. However, indications on such an enhanced surveillance are not uniform among different guidelines. An interval of 3-4 months seems to be reasonable since it would ensure that, in case of malignant transformation, nodule would not grow beyond curability. For nodule diagnosed only by imaging, the interval should be dictated by radiologic characteristics. Hypovascularity coupled with hypo-intensity at HE by gadoexetic-acid MRI call for a strict follow up. Conversely, hypovascular nodules showing iso/hyperintensity at HE-phase should be monitored by standard six months interval and with a follow-up no longer than 3 years. Eighteen months observation period as that recommended by AASLD/EASL guidelines seems to be inadequate since neoplastic transformation of some DN may take longer interval. As to which imaging technique should be employed, ideally it should be able to detect changes either in size and/or in vascular profile. Therefore, a dynamic imaging is preferable to standard ultrasound. CEUS, being cheaper, safer and more accessible than CT or MRI seems to be preferable in clinical practice since it can catch changes either in size or vascularity. Gadoexetate-enhancing MRI with DWI evaluation remains the recall imaging of reference to confirm neoplastic transition of nodules.

Early treatment of dysplastic nodules, which may theoretically improve survival is controversial. Unlike in others human models of gastrointestinal carcinogenesis (colo-rectal and gastroesophageal cancer) where treatment of precancerous lesions is recommended, in hepatocarcinogenetic model data supporting this policy are still lacking and evidences from the natural history of preneoplastic lesions discourage their systematic treatment. Recommendations on this issue by international guidelines are discordant. American

**Table 4. Clinical, radiologic and morphologic features useful to predict neoplastic evolution of dysplastic nodules**

Features	Risk of neoplastic evolution
Size	
< 1 cm	Low
> 1.5 cm	High
Arterial enhancement at CT, MRI, CEUS	
Hypo-vascularity	Low
Hyper-vascularity	High
HE phase at gadoxetate-enhanced MRI	
Iso/hyper-intense	Low
Hypo-intensity	High
DWI	
Hypo-intensity	Low
Hyper-intensity	High
Imaging features at follow-up	
Stable size	Low
Increasing size	High
Stable vascular pattern	Low
Acquired hyper-vascularity	High
Sincronous HCC	
No	Low
Yes	High
Histologic diagnosis	
LG-DN	Low
HG-DN	high

LG-DN: low-grade dysplastic nodule; HG-DN: high-grade dysplastic nodule; HCC: hepatocellular carcinoma

and European guidelines do not recommend systematic treatment of these lesions while Asian and Japanese guidelines are in favour of treatment of HG-DN. These discrepancies can be explained by the confusion in the pathological interpretation of early HCC and DNs among Japanese and Western pathologists. In particular, many of the vaguely nodular well-differentiated HCCs diagnosed by Japanese pathologists tend to be interpreted as high-grade DNs rather than HCC by Western pathologists while, many of the high-grade DNs diagnosed by Western pathologists are interpreted as well-differentiated HCC by Japanese pathologists<sup>[87]</sup>. This grey zone is particularly worrisome considering that the pursued goal is to treat any lesion arising in cirrhosis within an optimal curable stage (within 2 cm as the maximum diameter). However, according to western point of view, concerns are raised on the indiscriminate treatment of DNs that might be regarded as futile due to their longer and unpredictable natural history. In addition, the accurate selection of lesions with true neoplastic potential is still difficult in particular when multiple lesions are encountered. To date, only few studies addressed this issue with questionable conclusions. In 2008, Kim *et al.*<sup>[88]</sup> reported in a retrospective study the results of radiofrequency ablation (RFA) of 21 HG-DNs as compared to 41 small HCCs. Although complete necrosis was successfully obtained in 100 % of DN, this result did not translate into either long-term overall and disease-free survival benefit owing to the occurrence of “*de novo*” HCCs aside the initial DNs (48%) as the natural course of multicentric hepatocarcinogenesis. Owing to the lack and the difficulties to organize and conduct well-designed prospective controlled trials, Korean authors addressed this issue by a simulation model comparing two treatment strategies: RFA versus follow-up and timely resection. This model could not provide any evidence supporting that nodular ablation was superior to follow-up and timely resection for overall survival. Furthermore, in patients with multiple HG-DNs, RFA ablation of all nodule is not clinically feasible, as it can compromise liver function<sup>[89]</sup>. In conclusion, the rationale for systematic treatment of DN in cirrhosis at present is weak and carries the risk of falling into an overtreatment, i.e., treatment of lesions which may not cause significant disease in the patient.

## CONCLUSION

Like in other gastrointestinal oncogenetic models, in multistep cirrhosis-related hepatocarcinogenesis the development of HCC is preceded by sizable dysplastic lesions. The IWP classification distinguishes DN into

LG- and HG-DN, being the latter the true HCC precursors. Although relevant advances have been obtained in the last 2 decades as to morphology, and radiologic behaviour of these precursors, their management still represents a challenge for clinicians, radiologists and pathologists. In particular, major difficulties arise in surgical and transplant setting where experts have to face with these small lesions and make decisions about early treatment or simple observation without impacting negatively on the single patient outcome<sup>[90]</sup>. This remain an open and hot issue and efforts of scientific community are targeted to search and validate radiological and tissue markers helping to select lesions more prone to evolve into well-established HCC and deserving early treatment. Due to the limitations of biopsy and considering the complexity of radiologic work up, strict follow-up remains the most reliable alternative and the nodule growth the most confident and easy-to-use parameter driving decision-making process.

## DECLARATIONS

### Authors' contributions

Planned the review: Borzio M

Performed data research and data extraction: Borzio M, Francica G

Drafted the manuscript and approved the final version of the manuscript: Borzio M, Paladino F, Francica G

Controlled for data extraction: Borzio M, Paladino F, Francica G

### Availability of data and materials

Not applicable.

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None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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