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Editorial

Modern diagnosis of hepatocellular carcinoma: Utilization of liver biopsy and genomic markers ☆

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The incidence of Hepatocellular Carcinoma (HCC) has been rising in both Europe and the United States, largely due to the growing prevalence of hepatitis C (HCV) cirrhosis [1]. Despite advances in technology and available treatments, there has been little improvement in the overall survival likely due to the fact that most patients are diagnosed at advanced stages [2,3]. Cirrhosis is the most important risk factor for the development of HCC [4]. HCV and hepatitis B (HBV) are the major etiological agents that lead to HCC [5,6], though all etiologies that lead to cirrhosis also increase the risk of developing HCC. The rationale for surveillance is the use of a relatively simple and inexpensive test in a large number of individuals at risk for cancer with the goals of reducing morbidity and mortality [7]. Because patients with cirrhosis are at a high risk for developing HCC, guidelines recommend surveillance of these patients [8].

An algorithm for the diagnosis of HCC has been established for surveillance tests that result abnormal [8]. These guidelines indicate that nodules developed in cirrhotic patients >2 cm in diameter with typical findings for HCC (enhancement in arterial phase followed by washout in portal venous phase) on cross-sectional imaging or contrast-enhanced ultrasound do not require biopsy confirmation. If lesions reveal atypical features on imaging, then biopsy is required to establish the diagnosis of HCC. Importantly, for nodules in cirrhosis between 1 and 2 cm in diameter, the guidelines are more stringent and required two imaging tests (cross-sectional imaging, contrast-enhanced ultrasound and/or arteriogram) for the diagnosis of HCC if they have typical features (enhancement in arterial phase followed by washout in portal venous phase). Otherwise, a biopsy of the lesion is required for the diagnostic confirmation of HCC. A recent study showed that 84% of nodules >2 cm in diameter met the typical criteria of HCC and did not require biopsy confirmation, but only 44% of the nodules between 1 and 2 cm met this criteria [9]. A study that validated the AASLD guidelines showed that the sensitivity of these non-invasive criteria was 33% and biopsy was required for the diagnosis of HCC of nodules in patients with cirrhosis [10]. The goal of surveillance in patients with cirrhosis would be the detection of early HCC (single lesion between 2 and 5 cm or <3 lesions each <3 cm) or very early HCC (single lesion <2 cm) in which curative therapy with either radiofrequency ablation or surgical resection can be applied [8]. Very early HCC can be determined by size (<2 cm) but also by the presence of tumor cell invasion into the intratumoral portal tracts (stromal invasion) in the pathological examination [11]. Therefore, for the diagnosis of HCC in nodules <2 cm in patients with cirrhosis, liver biopsy will be a critical part of the diagnostic armamentarium.

There are concerns with performing biopsy in patients with cirrhosis. One is bleeding. The rate of bleeding has been established to be 0–1.4% [12]. Therefore the use of biopsy for the diagnosis of HCC should be restricted to patients with cirrhosis in a surveillance

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program with compensated liver disease and an acceptable risk of bleeding. The second concern is the potential for seeding along the needle track. We have shown that using a coaxial cutting needle technique with a 17-gauge introducer and 18-gauge biopsy needle reduces seeding significantly [13], and it is considered safe from this aspect. The third most important concern with biopsy is the lack of agreement even among expert pathologists regarding the histological diagnosis of HCC [14]. There are now international guidelines with consensus as to pathological criteria for the diagnosis of HCC and more importantly the difference between high-grade dysplastic nodules and very well-differentiated HCC. Hence histological evaluation, using liver biopsy, of nodules seen during surveillance of cirrhotic patients is necessary and will most likely be employed more widely for this purpose in the future.

In a short time genomic studies have rapidly evolved from the theory to elegant translational studies that have the potential to impact clinical medicine. The genomic profile of HCC has been identified [15,16]. One of the most important studies that translated genomic studies into clinical practice evaluated 55 genes to determine a signature for the diagnosis of early HCC [17]. The authors found that a 3-gene set had a discriminative accuracy of 94%, but, however, glypican-3 (GPC3) provided most of the predictive power in this set. Another study analyzed 12,600 genes of which a set of 95 genes provided a molecular signature that distinguished between early HCC components and their noncancerous liver tissues, and a set of 92 genes distinguished between progressed and early HCC components [18]. Of these genes, the most abundantly upregulated genes in early HCC components ($P < 0.001$) was heat-shock protein 70 (HSP70). In another profiling study in HCC, the authors identified several clones specifically expressed during malignant cell proliferation by screening a complementary DNA library constructed from a human primary liver cancer [19]. One clone was identified as the glutamine synthetase (GS) transcript, its expression is tightly regulated during development, especially in the hepatic lobule. A recent study evaluated 52 surgically removed non-malignant nodules (15 LRNs, 15 LGDNs, 22 HGDNs) and 53 HCCs (10 early, 22 grade 1, and 21 grade 2-3) or HSP70, GPC3, and GS [20]. The 3-marker panel, when at least 2 of them were positive, the sensitivity and specificity for the detection of eHCC-G1 were respectively 72% and 100%; the most sensitive combination was HSP70+/GPC3+ (59%) when a 2-marker panel was used. This was the first confirmation that these 3 genes may lead to the pathological differentiation between dysplasia and early HCC. The main drawback of this pilot study was that it required surgical specimens.

In this issue of the Journal, Di Tommaso and colleagues evaluated the panel consisting of HSP70, GPC3 and GS in liver biopsy specimens for the distinction between high-grade dysplasia and well-differentiated HCC in liver biopsy specimens, [21]. This is a retrospective study from the pathology files in 2 Italian centers and one Korean center. The specimens were collected with 18- to 19-gauge needles. There were large regenerative nodules ($n = 13$), low-grade dysplastic nodules ($n = 21$), high-grade dysplastic nodules (HGDN) ($n = 50$), very-well differentiated HCC (VWD) ($n = 17$), well-differentiated HCC (WD) ($n = 40$) and moderately–poorly differentiated HCC (MPD) ($n = 35$). The pathological diagnosis was based on internationally-recognized criteria. The expression was determined by immunohistochemistry with positive GPC3 and HSP70 showing more than 5% of hepatocytes immunoreactive. GS staining of lesional areas of strong and diffuse immunoreactivity was considered positive. When at least 2 markers were positive the sensitivity, specificity, positive predictive value and accuracy was 58.7%, 100%, 100%, 78.4%, respectively, for differentiating HCC from non-malignant nodules. When using at least one marker showing immunoreactivity, the sensitivity increased to 93.5% but the specificity decreased to 85.7%. However, the most important part of the study was evaluating the performance of the markers for differentiating HGDN and VWD HCC. When at least 2 markers were positive the sensitivity, specificity, positive predictive value and accuracy was 49.1%, 100%, 100%, 72.9%, respectively. Overall GPC3 had the best performance for differentiating HGDN from VWD HCC with a sensitivity of 61.4%, specificity of 92%, and accuracy of 75.7%. The authors conclude that 2 out 3 immunoreactivity are useful to detect VWD HCC, with GPC3 having the best performance of all the markers studied.

There are some important points that need to be taken into account. One, the patients with HCC included in this study had tumor sizes ranging between 2 and 5 cm in diameter and did not include patients with very early HCC. Dynamic cross-sectional imaging or contrast-enhanced ultrasound can lead to the diagnosis of HCC in the majority of these patients without the need for liver biopsy. Further study in those with very early HCC needs to be performed. Second, specimen size is of utmost importance for a successful interpretation of a liver biopsy [21] as well as for performing immunohistochemistry and other molecular studies. Di Tommaso and colleagues do not indicate the size of the samples in their study and at which specimen size immunohistochemistry tests yields accurate estimates of cancer risk. Lastly, as with other immunohistochemistry studies, an objective measure of positive staining may lead to more accurate results rather than relying on subjective criteria [22], and perhaps more sensitive antibodies may improve the performance as well.

However, this important study validates in liver biopsy specimens, critical for the diagnosis of very early

HCC among those in a surveillance program, a panel of markers selected as part of independent studies evaluating the genomic signature of patients with early HCC. As the genomic signature of VWD HCC is further clarified, it is likely that a more sensitive and specific panel will be developed. The next step is to prospectively evaluate the AASLD criteria for diagnosing very early HCC (1–2 cm in diameter on imaging) in patients with cirrhosis and to determine whether this panel (or a refined one) can improve the diagnostic accuracy. These are exciting times in field of HCC, in which genomics and proteomics are increasingly studied in the development of clinical tools for surveillance, diagnosis, prognosis and for measuring response to treatment.

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