



## Hepatic Oncology

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“despite any controversy regarding whether DAA increase the risk of *de novo* HCC occurrence after achieving SVR, there is an urgent need for a risk stratification strategy that combines baseline characteristics of patients, the pre-existing HCC risk factors and serum biomarkers before and after DAA treatment protocols”

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The perspective of hepatitis C virus (HCV) therapy has dramatically changed over the years after the introduction of direct-acting antiviral (DAA) therapy, which increased the sustainable viral response rate (SVR) up to 90% with better tolerance and effectiveness in clinical practice as compared with interferon-based regimens [1]. However, despite the excellent efficacy and extensive studies, alarming reports from two retrospective studies, conducted in 2016 in Spain and Italy [2,3], suggested an increased risk of hepatocellular carcinoma (HCC) occurrence and recurrence after DAA treatment in patients, triggering the debate on the safety profile of DAA and its correlation to HCC development. Nonetheless, some criticisms regarding the absence of control groups, sample size or short-follow-up periods have been raised in some studies.

A meta-analysis conducted by Waziry *et al.* in 2017 estimated no significant HCC occurrence cases in both DAA-treated or interferon-treated patients following SVR [4]. Indeed, several reports underlined the efficacy of DAA in significantly reducing the risk of HCC in patients with SVR as compared with those with either treatment failure or no treatment [1,5,6]. However, what emerges from those studies is that the DAA-induced SVR reduces the risk of HCC occurrence, without eliminating it. This includes patients with other risk factors, such as age, gender and cirrhosis. In particular, patients with cirrhosis with long exposure to the virus, can still be considered at risk, even after the achievement of SVR. Hamdane *et al.* described how epigenetic alterations induced by chronic HCV infection persist even after viral clearance and were further associated with HCC risk [7]. Liver alteration was also evident in early studies conducted by Kono *et al.*, observing sustained abnormal ALT and AFP levels, especially in F3–F4 patients (F3: severe fibrosis, characterized by fibrotic bridging across lobules, between portal areas and between portal areas and central veins; F4: cirrhosis), even after the achievement of SVR. Multivariate analysis identified pre-treatment low albumin levels and fibrosis 4 (FIB-4) index as independent predictive factors for the sustained AFP after SVR. At the same time, the fatty liver presence was associated with both sustained abnormal AFP and ALT levels after SVR [8] suggesting that the persistence of hepatocyte damage and regeneration mechanisms might lead to HCC development. The oxidative stress present in the fatty liver might also be responsible for DNA damage that foster carcinogenesis [8]. Confirming the results of Kono *et al.*, Watanabe *et al.* identified FIB-4 index  $\geq 4.0$  and albumin  $\leq 3.8$  g/dl at the beginning of DAA treatment and a FIB-4 index  $\geq 4.0$  and AFP  $\geq 6.0$  at the end of DAA treatment as independent predictors for HCC occurrence [9]. Moreover, despite the correlation between HCC risk with fibrosis index, recent data suggested the presence of liver steatosis in HCV patients as a major predictor of mortality and HCC occurrence in patients who achieved SVR following DAA treatment regardless of fibrosis [10].

Other studies suggested alternative serum biomarker candidates associated with HCC occurrence, focusing on the alteration of the immunological profiles during and after DAA-induced HCV elimination [11]. In 2017, Debes *et al.* identified a set of circulating immune mediators measured before DAA treatment: MIG, IL-22, TRAIL, APRIL, VEGF, IL-3, TWEAK, SCF and IL-21A. These markers were able to identify patients who developed *de novo* HCC [12]. Subsequent studies reported a significantly higher levels of IL-4 and IL-13 before and after DAA treatments in patients who developed HCC, suggesting a pro-oncogenic immune profile in those patients at risk.

The alteration of inflammatory and anti-inflammatory balance might affect antitumor surveillance contributing to the development of HCC. For example, the persistence of T-reg and the decrease of natural killer group 2 member D cells, after DAA treatments, might be one of the causes of early HCC occurrence after SVR, leading to the proliferation of undetectable dysplastic nodules already present within the damaged liver [13]. Also, the increase in VEGF and liver Ang2 found in the serum of DAA-treated patients might further sustain the tumor growth by promoting neo-angiogenesis [14,15]. These alterations in the immunological status and the consequent changes in cytokines and chemokines profiles before and after DAA treatment might represent a marker which could aid in the identification of patients at risk of HCC development.

Circulating miRNA might represent a promising tool to assess pre DAA-treatment HCC-risk and post DAA-treatment surveillance. In a retrospective study, we evaluated the association of circulating miRNA biomarkers with the risk of HCC occurrence and suggested that patients who develop HCC had a pre-existing risk, even before the DAA therapy initiation [16]. The alterations of circulating miRNAs were associated to HCC risk before therapy initiation and persisted after viral clearance [16], suggesting that the risk of HCC occurrence may be identifiable even before initiation of DAA therapy.

Other studies evaluated the potential use of alternative circulating biomarkers for patients' surveillance. Serum sphingolipids, for instance, were able to predict *de novo* HCC in HCV cirrhotic patients with SVR with an accuracy greater than AFP [17]. In another study, Yasui *et al.* showed that the level of WFA<sup>+</sup>-M2BP might be used as post-DAA treatment biomarker for assessing the risk of HCC development [18]. WFA<sup>+</sup>-M2BP is secreted by hepatic stellate cells and can induce Mac-2 expression in Kupffer cells, resulting in the activation of stellate cells and the production of fibrogenic chemokines, actively contributing in the pro-oncogenic environment [19].

Based on the previously discussed studies, circulating biomarkers hold promise as useful tools to be included in risk-predicting algorithms for better patient stratification. The benefit of being noninvasive approach, easy to measure and cost effective, thus facilitating serial sampling and the monitoring of dynamic changes during patients' follow-up, demonstrate the importance of circulating biomarkers for setting the basis of personalized evidence-based clinical strategies.

Since SVR achievement does not eliminate the risk of HCC, current clinical guidelines still recommend surveillance to be conducted in each individual. However, despite the proven benefits, this strategy might not be feasible in a universal setting. It was estimated that 6-month programs of surveillance with ultrasound and AFP in cirrhotic cases cost 753,226 USD, a sum totally unfeasible for low-income countries [20]. Therefore, a more specific and cost-efficient surveillance system is needed to stratify patients according to their risk factors, such as advanced fibrosis and cirrhosis, for instance, especially in consideration that the vast majority of HCC occurrence episodes after SVR were observed within 12–24 months [1,3]. Thus, despite any controversy regarding whether DAA increase the risk of *de novo* HCC occurrence after achieving SVR, there is an urgent need for a risk stratification strategy that combines baseline characteristics of patients, the pre-existing HCC risk factors and serum biomarkers before and after DAA treatment protocols.

However, despite this enormous effort in the biomarker research field, we are conscious that only few are currently utilized in decision-making algorithms. Considering the great complexity of the field, there is a crucial need to develop a shared approach in biomarker discovery and validation studies that accelerates the diffusion of newly discovered biomarkers into clinical practice.

#### Author contributions

All the authors contributed to text conceptualization, writing and proofreading.

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