Hepatocellular carcinoma after sustained response to interferon in non-cirrhotic hepatitis C: Flaws in the cure, or a clue to the flaws?

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Hepatocellular carcinoma (HCC) is among the three most common fatal malignancies in the Asian-Pacific region. Most cases are attributable to chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alone or in combination, while male sex and increasing age are two other invariable risk factors. Most cases of HCC complicating chronic viral hepatitis are also associated with cirrhosis. This proportion is 80% in the case of hepatitis B and possibly higher for hepatitis C; some authors have stated ‘almost all cases’ of HCC complicating hepatitis C have cirrhosis.

The notion that HCC develops in hepatitis C largely as a complication of cirrhosis has given rise to the concept that successful antiviral treatment in non-cirrhotic cases should, by preventing transition to cirrhosis, also prevent development of HCC. To date, the bulk of evidence has supported this concept. However, in this issue of the Journal, Miyano and colleagues report a case of HCC developing in an elderly man 4.5 years after apparent complete eradication of HCV infection with interferon treatment. An even more perplexing observation was that the patient had only mild hepatic fibrosis before treatment and that the liver showed no evidence of cirrhosis at the time of tumour resection. Is this case, to use an old English pun, simply ‘the exception that proves the rule’? Before accepting this, we should reconsider what we know about HCV, the liver disease it causes, and how this leads to hepatic carcinogenesis. The outcome in this case also provokes us to question how perfect is the concept that sustained normality of liver tests and non-detectability of HCV-RNA in blood and liver is really a cure of hepatitis C. Is this concept flawed? If so, does this mean we should be more intensive in clinical follow up of individuals with a sustained viral response to treatment, perhaps as suggested by Miyano et al., subjecting them to repeated hepatic imaging in a relentless search for early cancers?

Conventional wisdom is that the mechanisms of hepatic carcinogenesis are likely to differ between HBV and HCV. Hepatitis B virus is a DNA virus that inserts randomly into host genomic DNA with the potential to cause mutations of potential oncogenic significance. Further, some regions of the HBV genome, such as the HBx gene, can transactivate expression of c-myc or other proto-oncogenes in experimental systems. Despite these findings, it seems likely that the major factors linking chronic HBV infection to the development of HCC arise from the chronic hepatic inflammation and disturbed hepatocellular proliferation that characterize the pathobiology of chronic viral hepatitis, especially when cirrhosis is established.

In contrast to HBV, HCV is an RNA virus. Its replication does not involve DNA intermediates and, thus, mitogenic integration into host DNA is not possible. However, there is increasing evidence that proteins encoded by the HCV genome can regulate transcription of cellular genes concerned with mitogenesis and apoptosis. For example, the non-structural protein NS3 has been shown to transform NIH 3T3 cells. The hepatitis C core protein can also, under certain conditions, transactivate expression of c-myc or other proto-oncogenes, suggesting a potential role in the development of HCC.
circumstances, transform rat embryo fibroblasts and inhibit c-myc-mediated apoptotic cell death. It can promote cell growth through repression of p53 transcription, and by repressing p21 transcription.

Thus, a picture is emerging of HCV as a virus with potential to interfere with the cell biological responses pertinent to carcinogenesis, stimulation of cell proliferation and suppression of apoptotic cell death. An enticing, more direct demonstration of such oncogenic potential now comes from studies of transgenic mice expressing the HCV core protein. These animals show hepatic steatosis early in life, and after 16 months develop hepatic adenomas that evolve into HCC.

There are few human studies of the pathobiology of hepatocyte proliferation and cell death in hepatitis C in relation to development of HCC. Our recent results showed that livers of patients with HCV-related cirrhosis who subsequently developed HCC were more likely to exhibit Ki-67, a marker of hepatocyte proliferation, than those who did not. Further, those with evidence of previous HBV infection (i.e. positive for antibodies to HBV core antigen (anti-HBc)) were more likely to have Ki-67 positivity. This finding is intriguing because coinfection of HBV and HCV appears particularly carcinogenic, accounting for 20–30% of HCC cases in Europe, Australia and 15% in the USA. A recent Japanese study found HBV transcripts in at least 30% of HCV-related HCC among patients who were negative for hepatitis B virus surface antigen (HBsAg) in serum. This indicates an alternate way in which hepatocytes could be transformed, other than directly from HCV. More such human studies are needed to complement the very exciting work in transgenic and other experimental models.

So how strong is the evidence that HCV-related HCC is really a complication of cirrhosis? Most studies from Europe, North America and Australia have found that HCC is strongly, almost exclusively associated with cirrhosis in hepatitis C. For instance, all patients in an American post-transfusion hepatitis C follow-up study who developed HCC had cirrhosis, and in an Australian cohort of patients with diverse risk factors for HCV, the only ones to develop HCC were those with established cirrhosis or grade 3 fibrosis (bridging fibrosis with architectural distortion) at entry. In Italy, the annual cumulative risk of developing HCC is approximately 1% in HCV patients without cirrhosis and 3–10% in those with cirrhosis, depending on the presence of cofactors such as alcohol and HBV infection.

The above studies could actually underestimate the importance of cirrhosis for evolution of HCC because transition to cirrhosis may occur after the time of enrolment into follow-up studies. In contrast, several more recent studies tend to give a biased impression because they have been confined to retrospective analysis of cohorts of patients who have already developed cirrhosis and some patients may have been included in more than one study. It should be noted that individual cases of HCV-related HCC in the absence of cirrhosis have been observed; most cases have been associated with bridging fibrosis, but very rare cases have been in patients with minimal or no hepatic fibrosis.

The viral, environmental and host factors that influence hepatic carcinogenesis in response to chronic HCV infection may differ geographically. This could influence both the natural history of chronic hepatitis C and the propensity to develop HCC. The rate of HCC complicating hepatitis C appears to be particularly high in Japan. The difference is not well explained by major genotypic differences in HCV, because in Japan and in other countries HCC is most often (but by no means exclusively) associated with genotype 1b. However, more complex structural differences in regions of the viral genome, such as NS5A have been found in Japanese isolates of HCV genotype 1b compared to type 1b isolates from European subjects.

Given these geographic differences in virus structure and host response, are they relevant to HCC complicating less severe (non-cirrhotic) chronic hepatitis C? In one large cohort study from Japan that was uncontrolled for effects of disease severity and interferon treatment, Kasahara et al. found that fibrotic severity (or presence of cirrhosis) was not a significant risk factor for HCC. This may not be an entirely reproducible finding because in even larger cohorts of otherwise similar Japanese patients both Imai et al. and Ikeda et al. found a strong influence of fibrotic severity. In other work from the latter group, duration of HCV infection, acquisition of HCV from blood transfusion, alcohol intake, fibrotic stage of hepatitis and functional indices of severe liver disease were independently associated with the rate of HCC. The importance of alcohol has also been noted by others. What is apparent from these large studies is that, in Japan, the development of HCC in a non-cirrhotic liver may not be such a rare finding. In the future, clearer documentation of the frequency of cirrhosis in HCV-related HCC between countries and in relation to viral determinants, age and gender would contribute to understanding the aetopathogenesis of liver cancer in hepatitis C.

A key question is whether interferon treatment is effective in reducing the most lethal complication of HCV infection, development of liver cancer. In the one randomized, controlled trial of interferon for hepatitis C patients with cirrhosis, therapy was associated with a reduced rate of development of HCC, from 17 to 4% over 2–7 years. This much discussed study has sometimes been misinterpreted as indicating that interferon therapy, irrespective of treatment outcome, can reduce the risk of HCC in patients with HCV-related cirrhosis. Bonino et al., Serfaty et al. and Benvegnu et al. did find such an effect, but Niederau et al. and Fatovich et al. did not. Further analysis of the cases reported by Nishiguchi et al. has revealed that only patients with a sustained antiviral response to interferon have substantial protection from HCC (S Nishiguchi, pers. commun., 1996); a response/relapse outcome to treatment appears to delay onset of HCC, but cases start to accrue at 2–5 years after treatment.

Similar findings have been reported from uncontrolled analyses of patients with hepatitis C of varying severity. Thus, we and others have not observed cases of HCC among more patients with a sustained response to interferon followed for more than 5 years. The implication is that elimination of HCV infection pre-
vents development of HCC by arresting necroinflammatory activity and partially reversing hepatic fibrosis. 40,41 In Japan, Shindo et al. confirmed that sustained response to interferon (particularly if defined on the basis of non-detection of HCV-RNA) did arrest progression of liver disease to cirrhosis and response/reapse slowed such progression. 42 No sustained responder developed HCC compared with 16% of non-responders. Similar findings of no HCC among sustained responders were reported by Miyajima and colleagues, 43 even in those with cirrhosis. 44 In comparison with a large number of untreated controls (albeit non-randomized), Imai et al. found an approximate 95% reduction in the rate of HCC among sustained responders to interferon, 35 but no reduction in non-responders. Finally Okanoue et al. found that a sustained response to interferon greatly reduced the risk of HCC in patients with grade 2 fibrosis, but not in those with stage 3 fibrosis or cirrhosis. 45

Given that in Japan more than 250,000 patients with hepatitis C have been treated with interferon, the reported sustained response rates of between 25 and 35% would have produced approximately 70,000 sustained responders. If there is a 95% reduction in the rate of HCC formation by a sustained response to interferon, the annual rate of HCC in this group (compared with approximately 1% per annum in untreated subjects) might be 0.05% or 350 cases each year. Cases of HCC occurring after clearance of HCV with interferon therapy have been reported in this journal 46 and elsewhere in the literature. 47,48 Miyano et al. also bring three other cases to our attention that hitherto had been published only in abstract form in Japanese. In some of these cases, de novo development of HCC was suggested by the absence of detectable lesions by imaging for 4 years or more before diagnosis. Interestingly, the five Japanese cases were all in men, four over the age of 65 years, and none had cirrhosis although three had bridging fibrosis. Studies into the epidemiological associations of these cases of non-cirrhotic HCC found in association with chronic HCV infection would be of interest, particularly including environmental factors, such as alcohol and dietary content of aflatoxins and phytoestrogens. Further investigation into biological aspects of the cancers themselves and the surrounding liver could reveal clues to the flaws in cell growth regulation that lead to these unusual cancers long after HCV elimination.

In summary, the paradigm that HCC occurs only in hepatitis C cases when cirrhosis has developed may not be as absolute as we have come to accept, particularly in countries like Japan where additional host or environmental factors could play a more important role. However, the accumulating weight of evidence from retrospective studies is that a response to interferon therapy and, particularly a sustained viral response, substantially lowers HCC risk, although it does not completely abolish the chance of subsequent development of HCC. Without such a sustained antiviral response, interferon does not demonstrably alter the risk of HCC, although it may delay onset if a temporary treatment response has been obtained, much as it delays progression of fibrosis. 41

Application of more effective antiviral therapy for hepatitis C in its advanced fibrotic stages still carries potential to impact further on cancer risk. Despite this, there will be a few patients who have a sustained antiviral response to interferon who will develop HCC, particularly, but perhaps not exclusively, among those with established cirrhosis. In most countries, this flaw in the concept of cure is not likely to justify the expense of routine screening for HCC in non-cirrhotic individuals after a sustained response to interferon. However, the continuing experience from Japan where special expertise and organization of hepatic ultrasonographic services in special centres facilitates such monitoring will be watched with great interest by the rest of the world.

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