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CASE REPORT

Occurrence of Diffuse, Poorly Differentiated Hepatocellular Carcinoma During Pegylated Interferon Plus Ribavirin Combination Therapy for Chronic Hepatitis C

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Summary

Interferon therapy is indicated for the treatment of chronic hepatitis C and prevention of hepatocellular carcinoma. We describe the case of a 66-year-old Italian woman who received pegylated interferon α-2a plus ribavirin combined therapy for HCV-related chronic liver disease. Preliminary hematochemical, ultrasound and bioptic investigations did not show liver cirrhosis or hepatocarcinoma. After 24 weeks of treatment transaminase serum levels were in the normal range and circulating HCV-RNA was undetectable by PCR qualitative assay. On week 46 a serious adverse event occurred, with rapid transaminase increase, severe hyperpyrexia, and abdominal pain, leading to interruption of interferon and ribavirin. Liver biopsy was repeated and it revealed poorly differentiated hepatocellular carcinoma. Only palliative care could be performed and the patient died of liver failure within 2 months. The present case underlines that hepatocellular carcinoma can be misdiagnosed in spite of laboratory and instrumental follow-up. More sensitive tools are needed for tumor detection, to avoid IFN impairment of the liver, even though it eradicates HCV.

Key words: **Chronic hepatitis C, hepatocellular carcinoma, pegylated interferon α-2a, ribavirin.**

INTRODUCTION

Chronic infection by hepatitis C virus (HCV) is the most common cause of hepatocellular carcinoma (HCC) in many countries [1-3]. A large cohort study [4] carried out in Italy showed a significant presence of HCC among patients affected by viral hepatitis. Specifically, HCV-related liver cirrhosis accounts for about 4% risk per year of developing HCC [5-6], and the tumor incidence in North America, Europe and Japan is increasing, mainly as a consequence of HCV diffusion during the previous decades [1]. Although the mechanisms by which the virus promotes hepatocarcinogenesis are not completely clear yet, interferon (IFN) therapy seems of benefit in preventing HCC, owing to antimitogenic and antioxidative properties, both in sustained virological responders and in transient biochemical responders [7-9]. Since tumor diffusion significantly limits an effective removal of lesions [4], procedures for disclosing primary nodules in cirrhotics have been carefully standardized by major scientific societies [10]. Nevertheless, early diagnosis of HCC is often arduous in clinical practice. Herein we present a recent case in which widespread HCC was unexpectedly latent until triggered during antiviral therapy for chronic hepatitis C, after escaping all preliminary investigations that the patient had undergone. A second element of interest is represented by the particular imaging features of HCC, which appeared in a diffuse, disseminated form with hypovascular pattern.

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CASE REPORT

A 66-year-old Italian woman was referred to our Department for chronic hepatitis C, virus genotype 1b, diagnosed in 1992. On the basis of anamnestic data, no history of blood transfusions, intravenous drug use or alcohol consumption was recorded, and duration of illness was unknown. Immunoserologic blood tests excluded coinfections by hepatitis B virus (namely HBs and HBe antigens, anti-HBs, anti-HBe, and anti-HBc antibodies were negative), and by human immunodeficiency virus. The patient showed good clinical state, with body mass index 18.9 Kg/m^2, and was free of symptoms suggesting liver impairment. In November 2003 ultrasound-guided liver biopsy indicated moderate necroinflammatory activity, portal fibrosis and early periportal bridging fibrosis, namely A2 F2 chronic hepatitis, according to the Metavir scoring system [11]. Neither hepatocellular steatosis, nor iron storage were seen within the specimen, which measured 11 mm in length and contained 7 portal tracts. Serum alanine transaminase (ALT) and aspartate transaminase (AST) were 214 and 180 IU/L, respectively, and viral load was >500,000 IU/mL. Abdominal ultrasound disclosed unhomogeneously increased liver echotexture, without parenchymal nodules.

The patient fulfilled inclusion criteria for combination therapy with pegylated IFN (PEG-IFN) and ribavirin (RBV) [12], and was scheduled to receive PEG-IFN α-2a 180 μg weekly s.c. and RBV 1,000 mg daily *per os* for 48 weeks.

After 24 weeks of therapy serum transaminases lowered to the level of within normal range (namely <40 IU/L), and circulating HCV-RNA was undetectable by Cobas Amplicor polymerase chain reaction (PCR) qualitative assessment (sensitivity: 100 IU/mL).

On week 46 the patient was hospitalized in our Department because of serious disease with continuous fever, persistent abdominal pain, rapid weight loss, and asthenia. HCV viral load was still below 100 IU/mL, but ALT and AST increased up to 441 and 1,321 IU/L, respectively, hence PEG-IFN plus RBV administration was withdrawn. Blood cultures, urinalysis, and autoantibodies resulted negative, alpha-fetoprotein serum level was in the normal range (namely <10 IU/mL), chest X-ray, abdominal X-ray, and upper digestive endoscopy investigations did not show pathologic findings. Abdominal ultrasonography and contrast-enhanced abdominal computed tomography (CT) scan identified unhomogeneous liver texture, with partial thrombosis of portal, suprahepatic and inferior caval veins, mild splenomegaly, and ascites. Laboratory tests excluded specific coagulation diseases, and anticoagulant heparin therapy did not provide significant improvement. Since anti-cytomegalovirus (CMV) IgM antibodies were positive, with blood CMV-DNA equivalent to 1,690 genomes/mL by nested PCR assay, we started specific antiviral treatment with the nucleoside analogue gancyclovir 5 mg/Kg b.i.d. i.v. for 3 weeks, till CMV clearance was accomplished. Nevertheless the patient's condition did not improve.

Abdominal CT scan was performed again 2 months after the previous one and this time it revealed multicentric, infiltrative nodules of the liver, of various sizes, mostly smaller than 1 cm (*Figure 1*), hypo-attenuating in all phases of the examination with unchanged thrombosis in portal, suprahepatic and inferior caval veins (*Figure 2*). A further ultrasound-guided random liver biopsy collected a sample of 29 mm in length, leading to diagnosis of poorly differentiated HCC. Only palliative care was achievable and the patient died of liver failure within 2 months.

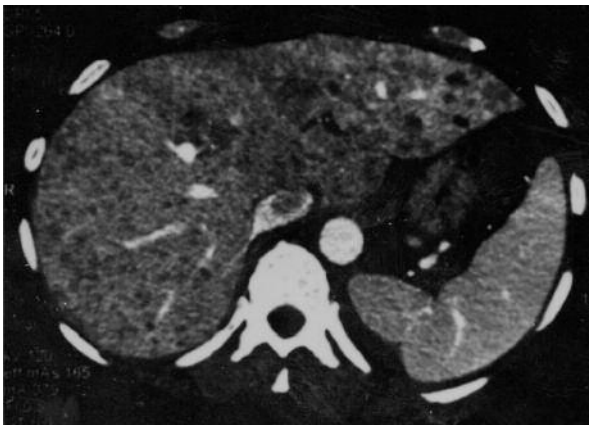

FIGURE 1 - Contrast-enhanced abdominal CT scan showing innumerable, hypo-attenuating various-sized focal lesions of the liver.

DISCUSSION

Although IFN therapy for chronic hepatitis C, both with standard and pegylated drugs, is commonly thought to prevent or delay HCC [5,7-9,13], in the present case severity of disease unexpectedly increased during treatment and concomitantly with viral clearance. On the grounds of its local diffuseness, the tumor was seemingly pre-existing, however neither laboratory tests nor ultrasound investigation had diagnosed it previously, and despite unhomogeneous liver echotexture with hypo- and hyperechoic scattered areas, nevertheless focal lesions were not identifiable. Alpha-fetoprotein serum level was normal, but this diagnostic tool has low sensitivity since only 60% of tumors actually produce enough of this marker to be detected [14]. On the other hand, the liver biopsy before treatment reported moderate fibrosis, without histological features of cirrhosis or nodular proliferation. The small sample obtained on that occasion could explain a serious underestimation of disease, since biopsies shorter than 15 mm in length are susceptible to over 35% risk of diagnostic inaccuracies [15].

Cirrhosis and HCC are the ultimate stages of long-lasting hepatic damage by viruses or other injuries,

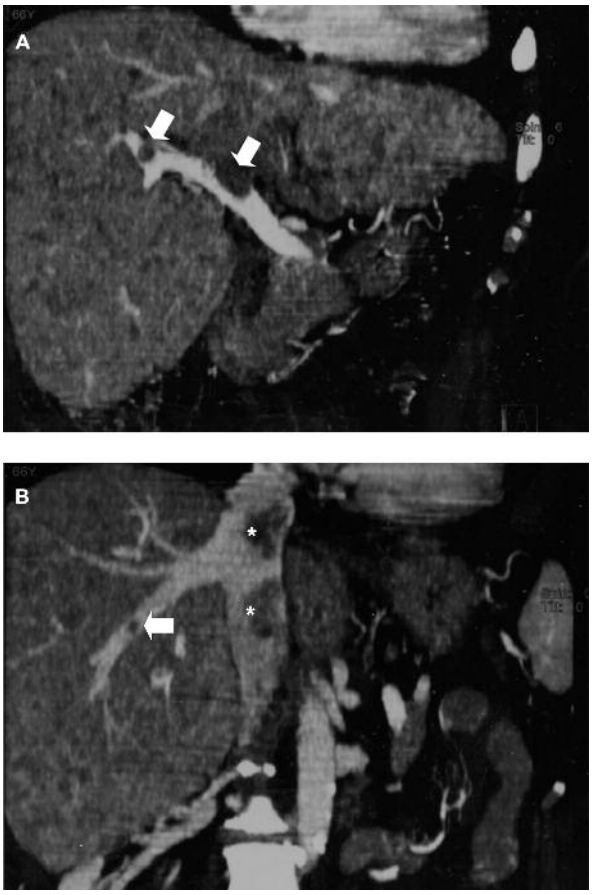

FIGURE 2 - Contrast enhanced abdominal CT scan images (coronal reconstruction). A) Various localizations of portal vein thrombosis (arrows). B) Thrombosis of the middle suprahepatic vein (arrow), and inferior caval vein (asterisks).

which induce progression of liver fibrosis and parenchymal disruption [11,16]. Although advanced hepatic alterations should be identified by imaging techniques, ultrasonography may lack specificity and sensitivity to discriminate diffuse parenchymal abnormalities in the absence of portal hypertension [17], as in the first ultrasound examination of this case. Previously the patient felt rather well, without suffering from any symptoms till she was administered PEG-IFN and RBV. Liver impairment occurred only after portal thrombosis and ascites had occurred, but HCC remained misdiagnosed for a longer time, probably because of its anomalous appearance. Moreover, the active CMV infection acted as a confounding factor and concealed the actual cause of hepatic failure.

The latest abdominal CT scan showed multicentric nodules, heterogeneous in size, shape and attenuation pattern, prevalently hypo-attenuating in all phases of the examination. Such a pattern appeared not specific for liver malignancy, since even benign lesions can mimic the described pattern on CT, and similar findings have been reported in multiple hepatic abscesses due to CMV infection after orthotopic liver transplantation [18], or caused by various opportunistic pathogens in patients with acquired immunodeficiency syndrome [19]. Most premalignant lesions in chronic liver disease such as regenerative and dysplastic nodules are multiple and hypovascular [20-21].

Several papers report primary tumors with different presenting symptoms in cirrhotic or even non-cirrhotic livers [22-26]. In the present case clinical discomfort, including fever and abdominal pain, could suggest not only HCC and portal thrombosis, but also viral or fungal superinfections, other than adverse events due to IFN intake [12]. Only a further liver biopsy accomplished the diagnosis of poorly differentiated HCC, which was consistent with neoplastic portal thrombosis.

A paper by Yamaura *et al.* [27] reviewed 24 cases, including one admitted to the authors' Hospital, of Japanese patients with HCC detected after IFN treatment and virological response. All patients, except one, were sustained virological responders, namely with non-detectable circulating HCV-RNA for at least 24 weeks after the end of treatment [12]. The HCC sizes at detection ranged from 10 to 90 mm, mean 30.6 mm. In order to exclude the possibility of microscopic HCC before IFN treatment, the authors discriminated between patients (n = 13) with >2-year time interval, and patients (n = 11) with <2-year time interval from antiviral therapy to tumor diagnosis [27]. However, the former group had larger HCC mean size (37.1 *vs.* 22.2 mm), with diameter >25 mm in 8/13, and previously covert neoplastic lesions cannot be excluded in everyone, since the tumor volume doubling time is likely to range from 90 to 204 days [2].

The present case shows interesting peculiarities in comparison with other literature reports, because the tumor appeared just after disappearance of viral load, when IFN was still ongoing, in a patient who had not many risk factors for HCC, aside from HCV infection [6]. From the CT imaging, it should be emphasized that the given pattern is unusual, in particular for a widely disseminated and poorly differentiated HCC: most of these tumors can be detected on arterial phase as hyper-enhancing areas. It is well known that HCC nodules enhance in proportion to the degree of contrast agent uptake and the hepatic arterial supply. On the other hand the surrounding hepatic parenchyma is mainly supplied by the portal inflow (70-75%). When the arterial supply is not sufficient to enhance the nodule more than the surrounding parenchyma, the HCC will be less attenuating. Moderately or poorly differentiated HCCs are almost solely supplied by the hepatic artery, due to the disappearance of the portal tracts in the sclerotic derangement [28-30]. On the contrary, in some cases of moderately or well differentiated HCC, nodules will present as persistently less attenuating in all the various phases [31]. Such tumors are believed to have an arterial supply insufficient to improve the degree of the enhancement more than the surrounding parenchyma. Probably they possess the same degree

of hepatic artery and portal venous supply compared with the surrounding liver parenchyma.

The hepatocarcinogenesis in this case had almost certainly begun prior to antiviral treatment, and the possibility that subclinical tumor was present before IFN therapy is presumed under such circumstances [2]. Recently Kobayashi *et al.* [32] performed a retrospective study of 1,124 patients treated with IFN for HCV-related chronic hepatitis, reporting that HCC developed after virus clearance in 3.5% of sustained virological responders. Nonetheless, as regards the present case, in light of early HCC occurrence during antiviral therapy, the most likely hypothesis is that multiple neoplastic foci already existed and it was not possible to visualize them because of limitations of the instruments used. In this patient the development of HCC seems not to have been inhibited by IFN administration. On the other hand, HCV might retain its pro-oncogenic potential despite a complete block of its replication machinery. Recent studies indicate that the virus genome is able to trigger the host genes regulating cell proliferation and differentiation, along various pathways and signaling cascades [33-35].

The present case exemplifies the contention that HCC in HCV-related chronic liver disease may be present even during or shortly after successful antiviral treatment. Therefore neoplastic degeneration should always be suspected when the patient's condition dramatically worsens, and imaging techniques show atypical features. Conventional screening modalities before PEG-IFN and RBV are susceptible to misdiagnosis, and patients run the risk of undergoing useless intake of poorly tolerated drugs. Novel tools are needed to enhance detection of HCC, and to avoid impairment of the liver by IFN therapy, even though the viral infection is removed.

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