CASE REPORT

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# The Onset of *de novo* Hepatocellular Carcinoma after Liver Transplantation can be both of Donor and Recipient origin. A Case Report

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## ABSTRACT

The occurrence of *de novo* hepatocellular carcinoma after liver transplantation is a rare event with only few cases reported in the literature. In a post liver transplantation setting distinguishing between a *de novo* hepatocellular carcinoma from recurrence should be tested with molecular analysis such as fluorescent in situ hybridization (for sex chromosomes) or microsatellite analysis. Nevertheless, a certain degree of epithelial chimerism between recipient and donor tissues could be responsible for the development of *de novo* hepatocellular carcinoma of recipient origin. We report two cases of *de novo* hepatocellular carcinoma after liver transplantation. The first one occurred in a patient receiving transplantation for hepatitis C related cirrhosis and hepatocellular carcinoma. A *de novo* hepatocellular carcinoma developed five years after transplantation and microsatellite analysis revealed the donor origin of the neoplasia. The second one occurred in a patient who received transplantation for secondary sclerosing cholangitis. Hepatocellular carcinoma was found six years after transplantation. Both microsatellite analysis and fluorescent in situ hybridization revealed the recipient origin of the tumor, potentially due to tissue chimerism.

**Key words:** *de novo* HCC – epithelial chimerism – HCC recurrence – hepatocellular carcinoma – liver transplantation – microsatellite analysis.

**Abbreviations**: CEPX/Y: centromeric probes for chromosome X and Y; FISH: fluorescent in situ hybridization; HBcAb: antibodies anti hepatitis B core antigen; HBIG: hepatitis B immune globulin; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; LT: liver transplantation; STR: short tandem repeats; US: ultrasound.

## INTRODUCTION

Recurrence of hepatocellular carcinoma (HCC) after liver transplantation (LT) is a well-studied phenomenon that can occur in up to 20% of patients, despite the adoption of a strict allocation policy (such as the Milan criteria) in the last 15 years [1]. Recurrence of HCC usually develops early after LT and is strictly dependent on tumor grading and presence of microvascular invasion [2, 3]. In addition, LT recipients are exposed to a higher risk of developing other *de novo* malignancies as a consequence of immunosuppression associated with exposure to oncogenic factors, especially in long-term survivors [4, 5].

The onset of *de novo* HCC after LT is a rare event with only 15 cases reported in the literature until now [2, 6]. In most of these cases, the *de novo* origin has been assumed on the basis of HCC development several years after LT or of the absence of cancer at explant histologic examination. For a correct interpretation of the HCC origin, genetic analysis of recipient/donor tissue by fluorescent in situ hybridization (FISH) for sex chromosomes or by microsatellite analysis has to be provided [7]. In addition, a certain degree of epithelial chimerism between recipient and donor tissues has been observed, meaning that hepatocytes or cholangiocytes of recipient origin can be found in the allograft due to recipient stem cells proliferation. This phenomenon could explain the development of *de novo* HCC of recipient origin [8].

Here we describe two cases of *de novo* HCC after LT in whom the origin (donor or recipient) was proved either by FISH or microsatellite analysis.

## CASE REPORT 1

A 54 year-old man affected by hepatitis C virus (HCV)-related cirrhosis and HCC, within Milan criteria, received LT in November 2008. The histological examination of the native liver showed a single tumor of 3 cm, grade G2 according to Edmondson and without any evidence of micro-vascular invasion. The patient received an immunosuppressive regimen based on calcineurin inhibitors (Tacrolimus) and tapered doses of prednisone. The donor was a white male who died from a cerebral hemorrhage. Serum virology showed anti-HBc positivity and a prophylactic treatment with lamivudine and Hepatitis B immune globulin (HBIG) was immediately adopted after LT. After an uneventful post-operative course, the patient was discharged and a regular follow-up with biochemical tests, ultrasound (US) examination and periodic computed tomography (CT) scan was adopted.

The patient developed an early HCV recurrence confirmed by a biopsy two months later (January 2009). Peg-interferon and ribavirin treatment was immediately started, but discontinued after few months because of severe thrombocytopenia, without any viral response. In the subsequent years, the patient developed clinical signs of cirrhosis with a transient elastography (Fibroscan) examination (July 2010) showing a liver stiffness of 31.6 kPa (compatible to F4 according to Metavir score), the development of ascites and repeated episodes of encephalopathy, treated with medical therapy. During this time, and until January 2014, there was no evidence of a tumor.

From June to December 2013 the patient received Sofosbuvir and ribavirin therapy, but relapsed about two weeks after discontinuation, despite an initial early viral clearance. In January 2014, at a routine US examination, a single hypo-echoic nodule of about 20 mm in segment VIII was observed. A CT scan examination was immediately performed showing the presence of three nodules, located in segment VIII, of 18mm, 10mm and 6mm, each with radiological features typical for HCC. A US-guided biopsy of the larger nodule confirmed the diagnosis of HCC grade G2.

In order to distinguish between recurrence of the previous HCC from the onset of a *de novo* HCC a genomic evaluation by microsatellite analysis was conducted on the biopsy specimen (neoplastic tissue compared with donor liver tissue and peripheral blood of the patient) which confirmed the donor origin of cancer (Table I). The HCC was treated with trans-arterial chemoembolization in February 2014 with a complete radiological response after three months. In March 2014, the patient was listed for a re-LT.

## CASE REPORT 2

A 44 year-old man affected by sclerosing cholangitis secondary to congenital vascular alteration (Ivemark syndrome) associated with congenital portal cavernoma received an LT in February 2007. Since the portal vein was not suitable for portal reconstruction, a cavo-portal hemi-transposition was created during surgery. Within 72 hours from the LT, an early hepatic artery thrombosis occurred requiring urgent re-transplantation. The biliary tract was reconstructed with an end-to-side hepatico-jejunostomy. The first donor was a white female who had died from a cerebral hemorrhage; serum virology showed anti-HBc positivity and a prophylactic treatment with lamivudine plus HBIG was immediately adopted after the first LT. The second donor was again a female who had died with cerebral hemorrhage. The patient received an immunosuppressive regimen based on Tacrolimus and tapered doses of prednisone. After a subsequent uneventful post-operative course, the patient was discharged and a regular follow-up with biochemical tests and US examination was adopted.

In the following years, the patient had a period of relative good health except for recurrent episodes of cholangitis and anemia due to chronic gastrointestinal bleeding. In October 2013 (6 years after LT) a 35 mm hypoechoic lesion in the liver III segment was diagnosed at a routine US examination. Liver function tests and US examination excluded the presence of cirrhosis. The subsequent hepato-specific contrast magnetic resonance imaging showed the presence of a multi-focal disease of uncertain origin (suspected for a lymphoma). The patient underwent a liver biopsy and the histologic examination revealed an HCC (G3 according to Edmonson).

In this case, the genetic evaluation conducted on the neoplastic tissue (compared with the donor liver tissue and the peripheral blood of the patient) by microsatellite analysis and FISH (female donor) indicated the recipient origin of the tumor (Table II).

Because of the tumor burden, the patient was proposed for Sorafenib treatment. To date he is alive and on regular oncologic follow-up.

**Table I.** Results of genotyping test for the 54 year-old man.

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|---|
| Genotyping Test to establish the donor/recipient origin of the tumor was performed by *test AmpF/STR Identifiler (Life Technologies)* on DNA extracted from the following samples: |
| - blood sample of the patient (recipient) |
| - histologic sample of HCC developed in the recipient liver |
| - histologic sample of the donor liver |
| 16 loci were analyzed. 8 of them were not informative; the other 8 loci showed the complete matching of HCC and Donor genomic profile. |

**Table II.** Results of genotyping tests for the 44 year-old man.

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| A. Genotyping Test to establish the donor/recipient origin of the tumor was performed by test AmpF/STR Identifiler (Life Technologies) on DNA extracted from the following samples: |
| - blood sample of the patient (recipient) (sample A) |
| - histologic sample of HCC developed in the recipient liver (sample B) |
| - histologic sample of the donor liver (sample C) |
| 16 loci were analyzed. 2 of them were not informative; the other 14 loci showed the complete matching of HCC and Recipient genomic profile. |
| B. FISH test developed with CEPX/Y DNA probe KIT on sample B showed a male chromosomal profile -XY- for the HCC cells and a female chromosomal profile -XX- of the perilesional hepatocytes (donor liver tissue) denoting the recipient origin of the HCC. |

CEPX/Y centromeric probes for chromosome X and Y; FISH, fluorescent in situ hybridization; STR, short tandem repeats.

## DISCUSSION

In these case reports we described two representative cases of *de novo* HCC. In the first patient, the onset of the new HCC was related to the recurrence of HCV hepatitis post LT and its rapid evolution to cirrhosis. The donor HBcAb positivity can probably be considered as a contributory factor causing the tumor development. Molecular analyses led to the certain distinction between the pre-LT tumor and the post-LT one.

In the second patient, the occurrence of *de novo* HCC has a less immediate explanation. Tumor genesis can be triggered by chronic inflammatory stimulation secondary to repeated cholangitis (even though those never lead to cirrhosis development); the presence of the cavo-portal hemi-transposition could also be invoked, through the altered hepatic blood inflow, especially the absence of portal flow, which could induce hepatocyte proliferation [9]. The evidence of the recipient origin of the neoplasia from molecular analysis is potentially linked to the epithelial chimerism given by recipient stem cell proliferation into hepatocytes under flogistic stimulation. Also, an environmental exposure to carcinogenic factors cannot be excluded, since the patient was resident in an Italian area of a high rate of air pollution.

In the post-transplant setting, the distinction between a recurrent HCC from the development of a *de novo* one is of remarkable importance, considering the differences in management and outcomes of the two conditions. A recurrent HCC should be considered a metastasis of the original tumor reflecting a systemic dissemination of the tumor; those patients may benefit from all surgical, medical or radiological treatments for the HCC, as in the pre-transplant setting, but re-transplantation is contraindicated and the prognosis is really poor, with about 22% chance of survival at 5 years, with a median of less than one year after diagnosis [3, 10, 11]. Conversely, the development of a *de novo* HCC can allow even re-transplantation, such was the case in our first patient. Considering the few cases described to this date, not enough data is available to formulate a prognosis for the onset of a *de novo* HCC, nonetheless there is no reason to avoid the same therapeutic management of the non-transplant setting [12].

## CONCLUSION

The distinction between HCC recurrence and the development of a *de novo* one should be considered a key point in the management of the patient after liver transplantation. A recurrence means a metastatic expression of the previous hepatocarcinoma, a condition that avoids a re-LT possibility for the patient. The development of a *de novo* HCC instead should be managed as in the pre-transplant setting offering the patient all kind of treatments, including re-transplantation. According to this point of view, molecular analysis acquires a major role, giving us a certain distinction between recurrence and the onset of a new hepatocarcinoma through donor/recipient genetic data. On the other hand, it seems that liver transplantation inevitably exposes tissues to a certain degree of chimerism, so that each situation should be assessed as unique, considering the possibility of a *de novo* carcinoma of recipient origin, especially when it occurs many years after LT in a patient with no evidence of cancer at time of transplantation (as in our case 2).

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**Authors' contribution:** C.C. contributed to the conception and design of the report, acquisition of data, writing and drafting of the manuscript. M.R.T. and Al.C. followed the patients in the Gastroenterological and Surgical Unit, contributed to the conception and design of the report and drafting of the manuscript. E.G. performed molecular analysis. An. C. performed all ultrasound examinations and US guided biopsies. F.B: critically revised the whole work.

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