ORIGINAL ARTICLE

Hepatocellular carcinoma and the risk of de novo malignancies after liver transplantation – a multicenter cohort study

Sarah Shalaby[1], Martina Taborelli[2], Alberto Zanetto[1], Alberto Ferrarese[1], Francesca D'Arcangelo[1], Martina Gambato[1], Marco Senzolo[1], Francesco Paolo Russo[1], Giacomo Germani[1], Patrizia Boccagni[3], Giuseppe Maria Ettorre[4], Umberto Baccarani[5], Augusto Lauro[6], Laura Galatioto[7], Maria Rendina[8], Raffaella Petrara[9], Anita De Rossi[9], Francesco Nudo[10], Luca Toti[11], Giovanni Fantola[12], Giovanni Vennarecci[4], Andrea Risaliti[5], Antonio Daniele Pinna[6], Salvatore Gruttadauria[7], Alfredo Di Leo[8], Massimo Rossi[10], Giuseppe Tisone[11], Fausto Zamboni[12], Umberto Cillo[3], Pierluca Piselli[13], Diego Serraino[2], & Patrizia Burra[1], Italian Transplant and Cancer Cohort Study

1 Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, Padua, Italy
2 Cancer Epidemiology Unit, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy
3 Hepatobiliary Surgery and Liver Transplantation Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, Padua, Italy
4 Division of General Surgery and Liver Transplantation, S. Camillo Hospital, Rome, Italy
5 Department of Medicine, University of Udine, Udine, Italy
6 Liver and Multiorgan Transplant Unit, S. Orsola-Malpighi University Hospital, Bologna, Italy
7 Department of Gastroenterology and Hepatology, Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (ISMETT), University of Pittsburgh Medical Center, Palermo, Italy
8 Department of Emergency and Organ Transplantation, Gastroenterology Section, University Hospital, Bari, Italy
9 Oncology and Immunology Section, AIDS Reference Center, Department of Oncology and Immunology, University of Padua, Padua, Italy

SUMMARY

Patients with hepatocellular carcinoma (HCC) are at high risk of second primary malignancies. As HCC has become the leading indication of liver transplant (LT), the aim of this study was to investigate whether the presence of HCC before LT could influence the onset of de novo malignancies (DNM). A cohort study was conducted on 2653 LT recipients. Hazard ratios (HR) of DNM development for patients transplanted for HCC (HCC patients) were compared with those of patients without any previous malignancy (non-HCC patients). All models were adjusted for sex, age, calendar year at transplant, and liver disease etiology. Throughout 17 903 person-years, 6.6% of HCC patients and 7.4% of non-HCC patients developed DNM (202 cases). The median time from LT to first DNM diagnosis was shorter for solid tumors in HCC patients (2.7 vs 4.5 years for HCC and non-HCC patients, respectively, $P < 0.01$). HCC patients were at a higher risk of bladder cancer and skin melanoma. There were no differences in cumulative DNM-specific mortality by HCC status. This study suggests that primary HCC could be a risk factor for DNM in LT recipients, allowing for risk stratification and screening individualization.

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Key words
de novo malignancies, liver transplant, post-transplant neoplastic screening, post-transplant survival, solid tumors

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Correspondence
Patrizia Burra MD, PhD, Professor, Head of the Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Padua University Hospital, Via Giustiniani 2, Padua, PD 35128, Italy.
Tel.: +39-49-8212892;
fax: +39-49-8218727;
e-mail: burra@unipd.it

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10 Department of General Surgery and Organ Transplantation, Umberto I Policlinic, Sapienza University, Rome, Italy
11 UOC Transplant Unit, Department of Surgery, Tor Vergata University, Rome, Italy
12 Department of Surgery, General and Hepatic Transplantation Surgery Unit, A.O.B. Brotzu, Cagliari, Italy
13 Department of Epidemiology, National Institute for Infectious Diseases L. Spallanzani, Rome, Italy

Introduction

De novo malignancies (DNMs) are a leading cause of death in liver transplant (LT) recipients [1-8], and survival probability of LT recipients, who develop a DNM, is generally lower than that of nontransplanted patients with the same tumor [9,10].

Etiology of liver disease, infection with oncogenic viruses, recipient's age, and lifestyle habits have been previously investigated as potential risk factors for specific DNMs, creating the basis for individualized post-LT screening protocols [11-16]. However, it has rarely been adequately emphasized that a history of hepatocellular carcinoma (HCC) before LT could be involved in the development of DNM, and such recipients have often been excluded from data analyses [8]. As HCC has become the leading indication for LT [17], it is crucial to investigate whether transplanted HCC patients warrant specific post-LT DNM screening programs.

Previous studies have shown that the risk of cancer survivors developing second primary malignancies is higher than the risk of cancer in the general population, and this holds true for patients with HCC as well [18-20]. A retrospective study conducted in Western countries reported that 7.3% of patients with HCC developed at least one extrahepatic secondary malignancy [21], and their incidence had previously been estimated at around 3.5–8% in the USA [22], 2.4% in Spain [21], 0.7–1.9% in Japan [23,24], and 1.6% in Taiwan [25]. The most common secondary malignancies associated with HCC are colorectal and genitourinary cancers in Europe [21,26-28] adding gastric cancer in USA [19,22,29] and Asia [23,24]. However, limited evidence is available for assessing whether this issue is relevant in the post-transplant setting. In recent papers, patients with HCC undergoing LT were found to carry a greater risk of developing DNM, not only compared with the corresponding general population [30], but also with nontransplanted HCC patients [31]. Furthermore, patients with pretransplant hepatic malignancies (HCC, combined HCC-cholangiocarcinoma and intrahepatic cholangiocarcinoma) were found to be at higher risk for extrahepatic DNMs than other LT recipients in a Korean monocentric study [32]. A recent analysis by the Scientific Registry of Transplant Recipients also found a positive association between HCC and DNM risk, but data to examine their impact on survival was lacking [7]. However, risk assessments of LT recipients without a history of malignancy prior to LT are still scarce.

The aim of this study was therefore to establish whether the presence of HCC before LT, irrespectively of a patient's liver disease etiology, could modify the onset of DNM after LT. Furthermore, the impact of HCC on survival after LT was examined, focusing on DNM-related survival.

Materials and methods

Patients and follow-up

This multicenter cohort study was based on clinical and epidemiological data collected in 3121 patients who underwent LT between 1985 and 2014 at nine Italian centers (a complete list of the participating centers has been reported in Table S1). Exclusion criteria were considered as follows: a diagnosis of cancer other than HCC prior to LT or within 90 days afterward (48 patients); a previous transplant (23 patients); a shorter than 90 days follow-up after LT (365 patients); age at transplant below 18 years (32 patients). Based on these criteria, 468 of the 3121 LT recipients were excluded. Patients were treated with immunosuppressive therapy according to attending physician's choice.

Person-years (PYs) at risk of DNM were computed from 90 days after LT to the date of death, cancer diagnosis, or last follow-up, whichever came first. After a cancer was diagnosed, patients did not contribute further follow-up time to PYs at risk for the type of cancer in question. Nonetheless, these patients continued to contribute follow-up time for other types of cancer.

At each of the participating centers, a trained study coordinator retrieved pertinent information from patients' clinical charts and checked its quality (completeness and accuracy). Data were obtained by means of a standardized questionnaire, which included personal details (e.g., age at transplant, sex, area of origin,

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place of residence), transplant details (e.g., date of LT, transplant center, underlying disease, infections, donor status, immunosuppressive regimen), and follow-up data.

This study was approved by the Ethical Committee of Padua University Hospital (Prot. 4231/AO/17).

De novo neoplasms

DNMs were defined as neoplasms developing after LT in patients negative at pretransplant screening for the concerned cancer, or related premalignant lesions/conditions. Nonmelanoma in situ skin cancers (NMSC) were not considered in the present analysis as the information concerning them was not recorded homogeneously by the different participating centers. Frequently during follow-visits, clinicians do not record as DNM the in situ NMSC, as they rarely impact on patients' survival or require invasive treatments.

Observed DNMs were coded according to the International Classification of Diseases and Related Health Problems, 10th revision (ICD-10). Diagnosis of DNM was always established by histology on biopsies or surgical specimens of the tumor. Date of biopsy or surgical procedure was designated as the date of cancer diagnosis. Cancer diagnoses were actively sought by reviewing patients' clinical charts updated at each follow-up visit. Vital status was also actively checked, and the cause of death was recorded. Information on cancer and on vital status was actively elicited either from clinical records, cancer registries (when available), or the census bureau of the town of residence.

Statistical analysis

To account for competing risks of death, the cumulative DNM incidence by time elapsing after LT was estimated using a competing risk approach with nonparametric estimators. Hazard ratios (HRs) of DNM development and corresponding 95% confidence intervals (95%CIs) for patients transplanted for HCC (HCC patients) were estimated and compared with those of patients undergoing LT without any previous malignancy (non-HCC patients) using Fine and Gray's regression models. All models were adjusted for sex, age at transplant (continuous variable), calendar year of transplant (<2000, ≥2000), and liver disease etiology.

The time from LT to DNM was calculated as median and interquartile range (IQR), and the Mann–Whitney U test was used to assess the difference between the HCC and non-HCC groups.

Overall survival probabilities were estimated with the Kaplan–Meier method, and log-rank test was used to compare survival rates. The cumulative incidence analytical method for competing risk data was applied to both DNM-specific and non-DNM-specific mortality. Differences in the cumulative incidence between the groups were assessed with Gray's test.

All statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA, version 9.4), and statistical significance was assumed for $P < 0.05$ (two-tailed).

Results

Patients

Overall, 2653 LT recipients were followed up for a total of 17 903 PYs of observation [median follow-up: 5.6 years (IQR: 2.6–10.1)]. In this cohort, 946 were HCC patients [median age: 56 years (IQR: 50–61)], and 1707 were non-HCC patients [median age: 51 years, (IQR: 46–57)]. Liver disease related to alcohol and viral infections (HBV/HCV) were the most common etiologies in both groups (Table 1).

Immunosuppression was based on calcineurin inhibitors in all patients, mostly involving tacrolimus (70.3%), associated with mTOR-inhibitors in 10.6% of patients. In particular, mTOR-inhibitors were administered more frequently to HCC patients (16.7% vs 7.1% of non-HCC patients; $P < 0.01$; Table 1).

De novo neoplasms

The median follow-up was shorter for HCC patients [3.6 years (IQR: 2.0–6.8)] than for non-HCC patients [6.6 years (IQR: 3.0-11.2)] ($P < 0.01$). During the period of observation, 202 DNM were diagnosed in 189 LT recipients: 62 (6.6%) of 946 HCC patients developed DNM (64 cases), while 127 (7.5%) of 1707 non-HCC patients developed DNM (138 cases). The baseline characteristics of patients who developed DNM are listed in Table 2.

The cumulative incidence of DNM rose steadily over the follow-up period: the 5- and 10-year cumulative risks were, respectively, 6.8% and 9.2% for HCC patients, and 4.5% and 8.0% for non-HCC patients (Fig. 1a). In both groups, DNM most frequently involved head and neck, bronchi and lungs, or colon-rectum, or they were non-Hodgkin lymphomas. In 111 (11.7%) patients transplanted for HCC, their cancer recurred after LT (median time from LT to recurrence: 10 months); and 2 of these patients developed extrahepatic DNM as well (one head and neck cancer and one bladder cancer).

Table 1. Distribution of 2653 liver transplant (LT) recipients according to pretransplant HCC status and selected characteristics.

	HCC patients (*N* = 946) *N* (%)	Non-HCC patients (*N* = 1707) *N* (%)
Male	806 (85.2)	1182 (69.2)
Median age at LT, years (IQR)	56 (50-61)	51 (43-57)
Calendar year at LT		
<2000	72 (7.6)	468 (27.4)
≥2000	874 (92.4)	1239 (72.6)
Etiology of liver disease		
Virus (HBV/HCV)	659 (69.7)	910 (53.3)
Alcohol abuse	85 (9.0)	220 (12.9)
Alcohol abuse and virus (HBV/HCV)	159 (16.8)	267 (15.6)
Cholestatic/autoimmune disease	3 (0.3)	137 (8.0)
Metabolic disease	11 (1.2)	81 (4.8)
Miscellaneous	29 (3.1)	92 (5.4)
Indication for LT		
HCC	796 (84.0)	0 (0.0)
Acute liver failure	0 (0.0)	91 (5.3)
Decompensated Cirrhosis	0 (0.0)	1555 (91.1)
HCC and Decompensated cirrhosis	151 (16.0)	0 (0.0)
Miscellaneous	0 (0.0)	61 (3.6)
Area of residence		
Northern Italy	219 (23.3)	429 (25.3)
Central Italy	232 (24.7)	396 (23.3)
Southern Italy	484 (51.5)	858 (50.5)
Abroad	4 (0.4)	16 (0.9)
Status of the donor		
Alive	44 (4.6)	57 (3.3)
Deceased	902 (95.4)	1649 (96.7)
BMI*		
<25	309 (43.4)	595 (53.1)
25–<30	320 (44.9)	408 (36.4)
≥30	83 (11.7)	118 (10.5)
Diabetes at LT*		
No	565 (73.8)	1091 (80.0)
Yes	201 (26.2)	273 (20.0)
Smoking habits*		
Never	140 (28.7)	339 (43.2)
Former	172 (35.3)	225 (28.7)
Current	175 (35.9)	220 (28.1)
Ever use of Cyclosporine*		
No	680 (76.3)	960 (60.0)
Yes	211 (23.7)	639 (40.0)
Ever use of Tacrolimus*		
No	157 (17.6)	468 (29.3)
Yes	734 (82.4)	1131 (70.7)
Ever use of mTOR inhibitors*		
No	742 (83.3)	1485 (92.9)
Yes	149 (16.7)	114 (7.1)

*The sum does not add up to the total because of missing values; percentages were calculated within the group of patients in whom these variables were available.

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Table 2. Distribution of 189 liver transplant (LT) recipients with de novo neoplasm according to pretransplant HCC status and selected characteristics.

	HCC patients (*N* = 62) *N* (%)	Non-HCC patients (*N* = 127) *N* (%)
Male	52 (83.9)	98 (77.2)
Median age at LT, years (IQR)	58 (53-62)	52 (43-57)
Calendar year at LT		
<2000	7 (11.3)	61 (48.0)
≥2000	55 (88.7)	66 (52.0)
Etiology of liver disease		
Virus (HBV/HCV)	39 (62.9)	52 (40.9)
Alcohol abuse	5 (8.1)	29 (22.8)
Alcohol abuse and virus (HBV/HCV)	16 (25.8)	20 (15.8)
Cholestatic/autoimmune disease	0 (0.0)	11 (8.7)
Metabolic disease	1 (1.6)	6 (4.7)
Miscellaneous	1 (1.6)	9 (7.1)
Indication for LT		
HCC	50 (80.6)	0 (0.0)
Acute liver failure	0 (0.0)	7 (5.5)
Decompensated Cirrhosis	0 (0.0)	114 (89.8)
HCC and Decompensated cirrhosis	12 (19.4)	0 (0.0)
Miscellaneous	0 (0.0)	6 (4.7)
Area of residence		
Northern Italy	12 (19.3)	41 (32.3)
Central Italy	21 (33.9)	30 (23.6)
Southern Italy	29 (46.8)	56 (44.1)
Status of the donor		
Alive	2 (3.2)	2 (1.6)
Deceased	60 (96.8)	125 (98.4)
BMI*		
<25	22 (45.8)	40 (51.9)
25–<30	24 (50.0)	25 (32.5)
≥30	2 (4.2)	12 (15.6)
Diabetes at LT*		
No	34 (66.7)	81 (77.9)
Yes	17 (33.3)	23 (22.1)
Smoking habits*		
Never	10 (27.8)	12 (24.0)
Former	14 (38.9)	25 (50.0)
Current	12 (33.3)	13 (26.0)
Ever use of Cyclosporine*		
No	40 (66.7)	55 (48.3)
Yes	20 (33.3)	59 (51.8)
Ever use of Tacrolimus*		
No	14 (23.3)	39 (34.2)
Yes	46 (76.7)	75 (65.8)
Ever use of mTOR inhibitors*		
No	44 (73.3)	96 (84.2)
Yes	16 (26.7)	18 (15.8)

*The sum does not add up to the total because of missing values; percentages were calculated within the group of patients in whom these variables were available.

Onset timing of de novo neoplasm malignancies

The median time elapsing from LT to diagnosis of the first DNM was 2.4 years (IQR: 1.2–4.0) and 4.1 years (IQR: 1.6–7.8) in patients with and without HCC, respectively ($P < 0.01$). When differentiating by DNM macrotype (solid tumors, hematological malignancies, and

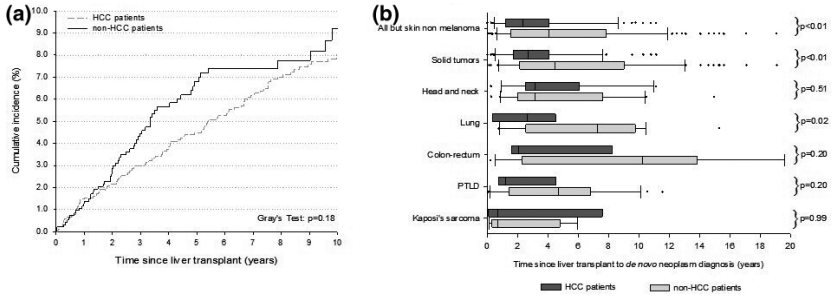

Figure 1 Cumulative de novo neoplasm (DNM) incidence by time since liver transplant (LT) (a) and time since LT to DNM diagnosis (b). Time since liver transplant to de novo neoplasm diagnosis according to HCC status and selected cancer types/sites. In each box plot, the left boundary represents the value of the 25th percentile (Q1); the right boundary represents the value of the 75th percentile (Q3); median values are marked within the boxes by vertical bars; the left and right vertical bars outside boxes (whiskers) represent the most extreme values within Q1-1.5(Q3-Q1) and Q3 + 1.5(Q3-Q1), respectively; the points outside whiskers are outliers. $P < 0.05$ denotes significant differences obtained by Mann–Whitney *U* test.

Kaposi's sarcoma), there was only a significant difference in time to onset of DNM for solid tumors, which occurred significantly earlier in HCC patients [2.7 years (IQR: 1.7–4.0) vs 4.5 years on non-HCC patients (IQR: 2.2–9.0, $P < 0.01$], as shown in Fig. 1b. Among the most common solid DNMs, a significant difference emerged for lung cancer, with a median time to occurrence of 2.7 years (IQR: 0.4–4.1) in HCC patients as opposed to 7.3 years (IQR: 2.8–9.7) in non-HCC patients ($P = 0.02$).

When considering the era of transplantation separately, there was a trend for an higher risk for DNM in HCC patients undergoing transplantation after year 2000 compared to those transplanted earlier, which however was not statistically significant ($P = 0.053$, data not shown).

Risk assessment

Table 3 shows the association between HCC status and DNM risk. No significantly stronger association with the risk of any DNMs emerged for HCC patients as with the non-HCC group (HR = 1.2, 95%CI 0.9–1.6). When subgroup analyses were run for the main types/sites of DNM, a significantly higher risk in HCC patients than in non-HCC patients emerged for bladder cancer (HR = 12.8, 95%CI 1.0–160.1) and skin melanoma (HR = 3, 95%CI: 1.3–7.1).

Survival analysis

Overall, 235 HCC patients (24.8%) and 369 non-HCC patients (21.6%) died during the follow-up. Non-HCC patients' overall survival was markedly better than that of HCC patients with 5- and 10-year survival probabilities of 86% and 77%, respectively, in the former, and 76% and 66% in the latter ($P < 0.01$; Figure S1A). As expected, the worst survival among HCC patients was observed for those whose HCC recurred recurring after LT (median survival: 1.8 years; 95%CI: 1.4–2.7) with 5- and 10-year survival probabilities of only 20% and 11%, respectively, against 84% and 74% of HCC patients who remained disease-free ($P < 0.01$, Figure S1B).

Table 4 shows the distribution of the LT deceased recipients according to cause of death and HCC status. Considering only the patients who developed DNM, most deaths were due to these DNM in both HCC and non-HCC patients (80.0% and 73.9%, respectively). No differences in cumulative DNM-specific mortality emerged by HCC status, with a 5-year cumulative risk of 43.7% for non-HCC patients, and 37.9% for HCC patients (P for Gray's test = 0.12). A similar pattern was observed for non-DNM-specific mortality, the 5-year cumulative risk being 9.6% for non-HCC patients and 11.1% for HCC patients (P for Gray's test = 0.42; Fig. 2).

Discussion

This study did not identify differences in terms of overall risk of developing DNMs in patients transplanted due to HCC compared with non-HCC patients, and no differences emerged in cumulative DNM-specific mortality by HCC status. However, an increased risk was observed for melanoma skin cancer and bladder cancer in HCC patients.

HCC has become the leading indication for LT [17], and its prevalence is predicted to grow even more in parallel with the global increase of nonalcoholic steatohepatitis and its intrinsic risk of HCC [33]. In this cohort, 946 patients were transplanted for HCC (35.7%

Table 3. Hazard Ratios (HR)* and 95% confidence intervals (CI95%) for de novo neoplasms†.

Type/Site	ICD-10 codes	HCC patients No. of cases	Non-HCC patients¶ No. of cases	HR (95% CI)
Kaposi's sarcoma	C46	4	9	1.5 (0.5-4.8)
Post-transplant lymphoproliferative diseases‡		8	28	0.7 (0.3-1.7)
NHL	C82-85, C96	6	24	0.6 (0.2-1.4)
Solid tumors‡		50	92	1.3 (0.9-1.8)
Head and neck	C00-14, C30-32	13	20	1.4 (0.6-3.1)
Bronchus and lung	C34	8	19	0.8 (0.4-1.9)
Colon-rectum	C18-20	4	16	0.6 (0.2-2)
Bladder	C67, D09.1, D30.3, D41.4	7	1	12.8 (1.0-160.1)
Esophagus	C15	0	7	–
Skin melanoma	C43	4	3	3.0 (1.3-7.1)
Stomach	C16	1	6	0.5 (0.1-4.2)
Liver	C22	0	6	–
All but non-melanoma skin‡		62	127	1.2 (0.9-1.6)

*Estimated using Fine-Gray proportional hazard model adjusted for sex, age at transplant, calendar year at transplant, and etiology of liver disease.

†As some patients were diagnosed with more than one malignancy, the sums can exceed the total. For patients diagnosed with more than one malignancy within the same ICD-10 group (e.g., colon-rectum ICD-10 codes: C18-20; head and neck: C00-14, C30-32), only the first one was considered.

‡It includes sites/types with <5 observed cases, which were not shown in table.

¶Reference category.

Table 4. Distribution of deceased liver transplant recipients according to cause of death.

	All (N = 604)			Patients with DNM (N = 91)		
	HCC patients (N = 235)		Non-HCC patients (N = 369)	HCC patients (N = 22)		Non-HCC patients (N = 69)
Cause of death	Without recurrence N (%)	With recurrence N (%)	N (%)	Without recurrence N (%)	With recurrence N (%)	N (%)
De novo neoplasm	16 (11)	0 (0.0)	51 (13.8)	16 (80.0)	0 (0.0)	51 (73.9)
HCC recurrence	–	86 (96.6)	–	–	2 (100)	–
Cardiovascular disease	19 (13.0)	0 (0.0)	62 (16.8)	1 (5.0)	0 (0.0)	2 (2.9)
Liver disease recurrence	37 (25.3)	0 (0.0)	65 (17.6)	1 (5.0)	0 (0.0)	1 (1.5)
Infection	26 (17.8)	1 (1.1)	58 (15.7)	0 (0.0)	0 (0.0)	0 (0.0)
Other	20 (13.7)	0 (0.0)	49 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	28 (19.2)	2 (2.3)	84 (22.8)	2 (10.0)	0 (0.0)	15 (21.7)

of the whole cohort, and 41% of those transplanted since the year 2000), representing a significant share of LT recipients.

The cumulative risk of DNMs following LT in HCC patients was 9.2% at 10 years, and these cohort patients were at a statistically significant greater risk of bladder cancer and skin melanoma than non-HCC transplanted patients.

HCC has been previously described as a pre-LT risk factor for *de novo* NMSC [34-36]. The protocol did not consider NMSC, but these patients reported a higher risk of malignant melanomas, that is, those skin cancers with the strongest impact on survival. As it is a first, this specific association deserves further investigation in other cohorts with different characteristics in order to draw definite conclusions.

More interestingly, genitourinary cancers (apart from prostate cancer) are more frequent, and carry a worse prognosis in LT recipients than in the general population [37-39]. Bladder cancer risk is increased in a

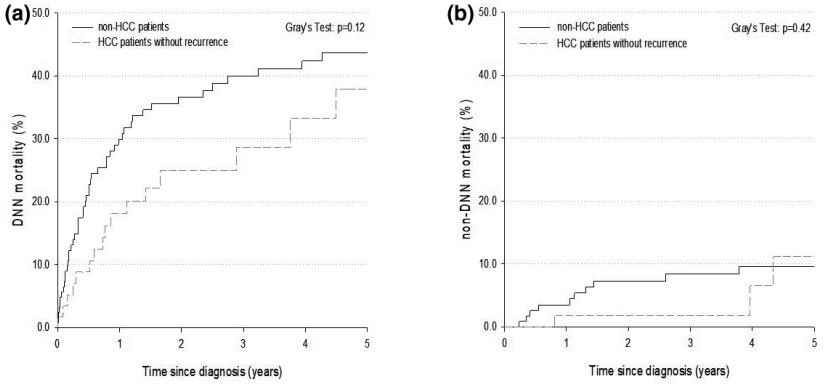

Figure 2 Cumulative de novo neoplasm (DNM) (a) and non-DNM (b) mortality by time since diagnosis and HCC status.

number of studies, with a range of SIR value from 1.5 to 2.4 [40-42], and it was noted to develop late (10 years) after LT in one cohort [43]. Although no specific studies have been conducted on the topic, there have been several reports of common pathogenic pathways linking HCC to bladder cancer, involving *β-catenin* mutants resistant to ubiquitylation-mediated proteasomal degradation [44], and *BAF* complex [45]. In a recent Korean monocentric study, patients with pretransplant hepatic malignancies, which were mainly represented by HCC, were at higher risk to develop for extrahepatic DNM than other LT recipients. Bladder cancer was again showing between the most common malignancies in this particular group of patients, together with lymphoproliferative disease, lung cancer, stomach cancer, and colorectal cancer [32]. While malignancies like melanomas and stomach cancers can be related to geographical epidemiology, the association between HCC and bladder cancer both pre- and post-LT has been repeatedly confirmed in epidemiological studies conducted in different countries [21-25,30,31], so the role of common risk factors should be investigated in dedicated studies. Smoking could be one of the common risk factors. In our cohort, we dispose of this information for only half of the patients, and this highlights the importance of raising awareness between transplant hepatologists to screen for this variable both during patients evaluation in the pre-LT setting and during the follow-up after transplantation.

An earlier onset of solid DNMs in HCC patients compared with non-HCC ones was also noticed in this cohort. Among the most common solid DNMs, this difference in timing of occurrence appeared mainly in lung cancer. In a recent study in approximately 90 000 LT recipients, 26% of DNM-related deaths were due to lung cancer [4], with a reported one-year survival of 37.5% [38]. Only limited data pooling the clinical characteristics of patients who develop lung cancer after LT are available to date, however, HCC could be an important variable in this setting, concerning in particular early-onset DNMs. Actually, also Heo and colleagues identified it as a frequently- and early-occurring cancer in transplanted HCC patients when compared to non-transplanted ones [30]. All our statistical analysis were corrected by year of transplant; however, we conducted also a separate analysis considering era of transplantation which, however, did not impact on the risk of DNMs our cohort (data not shown). Nevertheless, statistical significance was borderline, therefore, the effect of the year of transplantation could be significant in larger cohorts with larger number of events.

In our cohort, almost all patients diagnosed with a DNM after LT died of their secondary cancer, alongside any cases of recurrent HCC. Most of these malignancies show a more aggressive behavior in LT recipients, who are less responsive to treatment and have worse survival rates than in the general population. The early onset of DNM may thus spell an earlier mortality. It could be speculated that "early" diagnosis of DNM in HCC patients could, at least in part, derive from their post-LT surveillance for HCC recurrence. However, not all patients were uniformly screened for HCC recurrence after transplant as this was a multicenter study including patients transplanted along a wide time-span. Anyhow, no differences in cumulative DNM-specific mortality emerged by HCC status, so no evidence of a better survival emerged for such patients. In other words, previous and current screening protocols, even in HCC patients, seemed to be scarcely effective for this purpose. Further studies including analysis of histological status of DNM at diagnosis, and considering smaller, homogenously screened, HCC populations could better define this particular issue.

This study has some limitations that need to be mentioned. First of all, despite the relatively large sample considered, the study was still underpowered for the purpose of detecting any associations for specific DNM types/sites, so our results should be interpreted with caution. The lack of complete information on some variables (i.e., smoking habit, occupation, and obesity which are not routinely screened during LT evaluation or follow-up, pre-LT HCC treatments, use of different immunosuppressive schedule, post-transplant HCC recurrence surveillance protocols, indication for the use of m-TORi and immunosuppressant dosages) known to be associated with the risk of specific tumors also needs to be borne in mind. Most importantly, besides the known importance to screen for smoking habit, the presence of metabolic syndrome both in the pre and post-transplant setting should be routinely assessed as its prevalence is rapidly increasing, and will surely impact both the rate of HCC in the context of nonalcoholic steatohepatitis and related DNMs. It is also possible that not all DNMs were not completely and accurately identified in some cases, as cancer diagnoses were obtained from patients' clinical records. That said, the close clinical follow-up of these patients should be a guarantee of the completeness of cancer reporting. With regard to DNM occurrence timings, it may be speculated that an "early" diagnosis of DNM in HCC patients could derive from their post-LT screening for HCC recurrence. However, even though European guidelines currently recommend to screen patients after LT through CT-scan or MRI every 3 months during the first year and every 6 months thereafter, not all patients were screened according with this protocol, mostly when considering elder transplants, so we could not compare subgroups of patients in these terms in our analysis. Furthermore, all the results need to be validated in external cohorts. Lastly, even though the association between HCC and bladder cancer seems to be frequently reported in different cohorts, both in the pre- and post-transplant context, direct pathophysiological studies should be performed to verify the presence of a common tumoral hit. Despite these limitations, these findings highlight the need to consider a strict follow-up of HCC patients after LT, not only for disease recurrence but also for solid DNMs occurrence. Further investigations in this field are indeed necessary to better stratify HCC patients. For instance, our study did not look at pretransplant HCC treatments (such as radio- and chemotherapy), which might have contributed further to HCC patients' predisposition to cancer after LT.

In conclusion, in this cohort, patients with HCC prior to their transplant were found at higher risk of skin melanoma and bladder cancer than LT recipients without any previous malignancy. Furthermore, solid DNMs seemed to occur earlier in HCC patients, and DNM-related survival seemed not to benefit from current screening strategies. These findings are even more interesting when considering that these patients were more frequently treated with m-TORi, which are known for their antitumoral properties [46]. Our results suggest that HCC should be considered among other known potential risk factors during post-transplant DNM risk stratification and surveillance individualization. The amount of information that was retrieved from this large cohort of LT recipients will enable comparison with cohorts from other centers to build up solid epidemiological studies to address this very actual issue. If our results are confirmed by further studies, a strict follow-up for patients transplanted for HCC would be advisable, combining the abdominal imaging already used to screen for HCC recurrence, with contrast-enhanced second-level thoracic imaging techniques, urine cytology, and strict dermatological follow-up. Smoking history should always be recorded during risk assessment, mostly in HCC patients who appear to be at higher risk for smoke-correlated cancers. As prophylactic measures patients should be firmly instructed to quit or not start smoking, as well as using high-barrier sun protection and avoid prolonged UV exposure.

Authorship

SS and MT: study concepts and design, collection of data, analysis and interpretation of data, draft of the manuscript. AZ: collection of data, critical revision for important intellectual content, and draft of the manuscript. AF, FDA, MG, MS, FPR, GG, PB, EGM, BU, LA, GL, RM, PR, DRA, NF, TL, FG, VG, RA, PAD, GS, DLA, RM, TG, ZF, and UC: collection of data. PP: collection of data, analysis and interpretation of data, and critical revision for important intellectual content. DS and PB: study concept and design, study supervision, critical revision for important intellectual content, and final approval of the version to be published.

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Conflict of interest

The authors have no conflict of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Survival outcome after liver transplantation.

Table S1 Highlights.

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