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ORIGINAL ARTICLE

A changing etiologic scenario in liver transplantation for hepatocellular carcinoma in a multicenter cohort study from Latin America

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Abbreviations: AFP, alpha-fetoprotein; CC, cryptogenic cirrhosis; CI, confidence interval; CT, computerized tomography; HCC, hepatocellular carcinoma; HR, hazard ratio; IQR, interquartile range; LT, liver transplantation; MC, Milan criteria; MELD, model for end stage liver disease; MRI, magnetic resonance imaging; MVI, microvascular invasion; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; SHR, subhazard ratio; TACE, trans-arterial chemoembolization; TTR, time to recurrence; WL, waiting list.

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KEYWORDS
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Summary
Background and aim: Non-alcoholic fatty liver disease (NAFLD) is an increasing cause of hepatocellular carcinoma (HCC) and liver transplantation (LT). Our study focused on changing trends of liver related HCC etiologies during the last years in Latin America.
Methods: From a cohort of 2761 consecutive adult LT patients between 2005 and 2012 in 17 different centers, 435 with HCC were included. Different periods including years 2005—2006, 2007—2008, 2009—2010 and 2011—2012 were considered. Etiology of liver disease was confirmed in the explant.
Results: Participating LT centers per country included 2 from Brazil ($n = 191$), 5 transplant programs from Argentina ($n = 98$), 2 from Colombia ($n = 65$), 4 from Chile ($n = 49$), 2 from Mexico ($n = 12$), and 1 from Peru ($n = 11$) and Uruguay ($n = 9$). Chronic hepatitis C infection was the leading cause of HCC in the overall cohort (37%), followed by HBV (25%) and alcoholic liver disease (17%). NAFLD and cryptogenic cirrhosis accounted for 6% and 7%, respectively. While HCV decreased from 48% in 2005—06 to 26% in 2011—12, NAFLD increased from 1.8% to 12.8% during the same period, accounting for the third cause of HCC. This represented a 6-fold increase in NAFLD-HCC, whereas HCV had a 2-fold decrease. Patients with NAFLD were older, had lower pre-LT serum AFP values and similar 5-year survival and recurrence rates than non-NAFLD.
Conclusion: There might be a global changing figure regarding etiologies of HCC in Latin America. This epidemiological change on the incidence of HCC in the world, although it has been reported, should still be confirmed in prospective studies.

Introduction

Hepatocellular carcinoma (HCC) is currently the second most common cause of cancer related death worldwide [1]. Data from liver transplant centers in Europe and the United States have shown that approximately 20—30% of all liver transplants (LT) are indicated for hepatocellular carcinoma (HCC) [2,3].

Although chronic hepatitis B (HBV) and C viral (HCV) infections account for most cases of HCC, non-alcoholic fatty liver disease (NAFLD) has been recently reported as an increasing etiology of HCC and a leading cause of LT in the United States [4]. Heterogeneous data has been reported to date related to etiologies, management and selection criteria of LT for HCC in Latin America [5—10]. Chronic hepatitis C infection (HCV) and alcoholic liver disease have been described as the most frequent causes of HCC in this region [11], although hepatitis B virus (HBV) chronic infection accounts as a leading cause in some regions of Brazil [12].

Despite increasing prevalence of fatty liver in developed countries, resulting from obesity and diabetes, information related with the burden of NAFLD related HCC in Latin America is lacking. The aim of this study was to evaluate recent changing trends in etiologies of HCC and LT in a multicenter cohort from Latin America, evaluating further the impact of etiology on clinical outcomes after transplantation.

Patients and methods

Study design, setting and participating centers

This study was conducted including a multicenter Latin American cohort of consecutive adult patients (> 17 years of age) who underwent a first LT between June 1 2005 and June 1 2012 in 17 different LT centers and were prospectively followed-up after transplantation.

Eligibility criteria and study variables

Criteria for inclusion required patients to be adult cirrhotic or non-cirrhotic recipients with confirmed HCC in the explanted liver. Patients were excluded if (1) other tumors than HCC were confirmed in the explanted liver, (2) had incidental HCC (patients with tumors discovered on final pathology without a preceding imaging diagnosis), (3) extrahepatic or macrovascular tumor invasion were observed

during pre-transplant evaluation and (4) had a previous liver transplant.

Recipient characteristics, pre-transplant tumor characteristics and serum α-fetoprotein (AFP) levels were obtained at listing. Etiology of liver disease was histologically confirmed in the explanted liver specimen in all patients. The primary diagnosis of liver disease included HCV (anti HCV positivity), HBV (hepatitis B surface antigen positivity), alcoholic liver disease (alcohol intake exceeding 30 g/day), NAFLD, cryptogenic cirrhosis (CC), cholestatic liver diseases (i.e., primary biliary cholangitis, primary and secondary sclerosing cholangitis), autoimmune hepatitis and other causes including metabolic diseases or miscellaneous causes (e.g., hereditary hemochromatosis, Wilson disease, toxic liver disease). Patients were classified presenting NAFLD if all other etiologies of liver disease were ruled out, a past history of ultrasonographic fatty liver was present and > 10% steatosis was observed in the explanted liver. Patients with cryptogenic cirrhosis or unknown etiology were not included as NAFLD as proposed by other authors [4].

Subjects with HCC diagnosis based on imaging criteria were classified according to Milan (MC) [13], University of California San Francisco (UCSF) [14] and the AFP model criteria [15], depending on size and number of lesions detected on pre-LT computerized tomography (CT) or magnetic resonance images (MRI) and serum AFP values. All patients were staged before LT excluding any metastatic localization assessed with lung CT and bone scintigraphy. Transplantation for patients exceeding MC was discussed at each transplant center on a case-by-case basis [16]. Site-specific organ allocation policies were also registered.

Tumor treatment before transplantation was decided in each transplant center and reviewed including trans-arterial chemoembolization (TACE), radiofrequency ablation (RFA), percutaneous ethanol injection (PEI) and liver resection.

Explanted liver findings included: confirmation of etiology of liver disease, macroscopic and microscopic evaluation of each nodule, number and diameter (cm) of each, presence of microvascular invasion (mvi), and degree of tumor differentiation according to Edmonson-Steiner grading system [17]. Finally, Milan and up to seven criteria were also applied to the explanted liver specimen [18].

Maintenance immunosuppression regimen was recorded, such as Tacrolimus (Tac), Cysclosporine A (CsA) or mammalian target of Rapamycin inhibitors (mTOR, sirolimus or everolimus) with or without sodium/mophetil micophenolate (MMF), at the 3rd, 12th and 24th month after transplant.

Study end-points

Our study focused on changing trends of etiologies related with HCC comparing different periods including years 2005—2006, 2007—2008, 2009—2010 and 2011—2012. A subgroup analysis per etiologies and per country was performed post-transplant outcomes (5-year survival and recurrence rates). All patients were followed-up until death or last outpatient visit.

Post-LT follow-up for HCC recurrence consisted of one CT or MRI, bone scintigraphy and serum AFP assay every 6 months, as recommended [19]. Recurrence was determined on the basis of imaging criteria plus serum AFP or by biopsy. Time to recurrence (TTR) was considered a robust clinical outcome measure and calculated as the time in months elapsed between transplantation and diagnosis of recurrence.

All procedures followed were in accordance with guidelines for observational studies [20]. This study was approved by each LT center ethic committee. It complied with the ethical standards (institutional and national) and with Helsinki Declaration of 1975, as revised in 2008.

Statistical analysis

Categorical data were compared using Fisher's exact test or Chi-Square (X2) test (2-tailed). Continuous variables were compared with Student's *t*-test or Wilcoxon rank-sum test according to their distribution, respectively. A multivariate Cox regression analysis, with hazard ratios (HR) and 95% confidence intervals (95% CI) for identifying baseline pre and post-transplant risk variables for 5-year mortality and recurrence was carried out evaluating potential confounding variables. Dummies for ordinal or categorical variables were assessed. Variables with a *P*-value < 0.15 after the univariate analysis were included in the multivariate model, generated by stepwise backward elimination evaluating *P*-values (Wald test) and considering adjusted HR with confounding variables (> 20% of change in crude HR). From the univariate analysis, 1 variable per 10 events were included in the multivariate analysis. Adjustment of each final model was evaluated with proportional hazards through graphic (log-minus-log curves, cumulative hazard cox regression curves and smoothed hazard estimates) and statistical evaluation (Schoenfeld residual test). Calibration was assessed by comparison of observed and predicted curves and evaluation of the goodness of fit of the model by Harrell's c-statistic index. Finally, a competing risk analysis with death (competing event = death before recurrence) and recurrence was done with calculation of subhazard ratios (SHR) and 95% CI. Kaplan Meier survival curves were compared using the log-rank test (Mantel-Cox). In order to evaluate if any changing etiologic scenario related with NAFLD could have been the result of changing listing practices or increasing number of LT centers versus a real change in disease epidemiology, a sensitivity analysis was performed with a multivariate logistic regression analysis evaluating potentially confounding factors related with NAFLD with corresponding adjusted odds ratios (OR) and 95% CI. Collected data were analyzed with STATA 10.0.

Results

Participating centers and patients characteristics

From a total of 2761 consecutive adult LT patients in 17 different centers, 435 patients with HCC were included (Fig. 1). Participating LT centers per country included 2 from Brazil (*n* = 191, 43.9%), 5 transplant programs from Argentina (*n* = 98, 22.5%), 2 from Colombia (*n* = 65, 14.9%), 4 from Chile (*n* = 49, 11.3%), 2 from Mexico (*n* = 12, 2.8%), and 1 from Peru (*n* = 11, 2.5%) and Uruguay (*n* = 9, 2.1%). Table 1 describes the overall cohort.

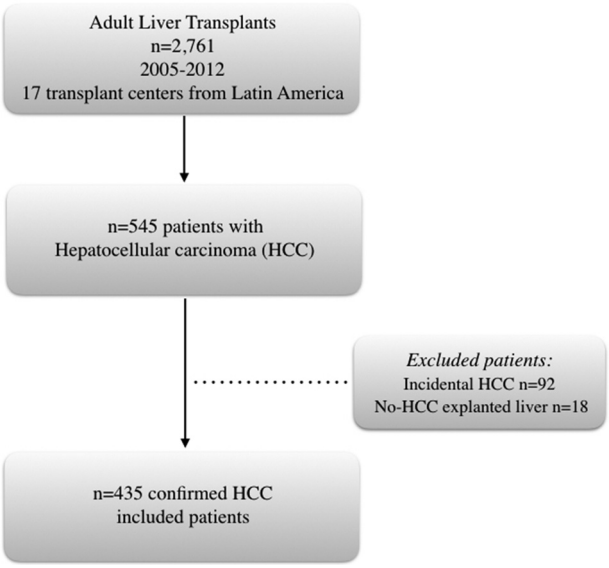

Figure 1 Inclusion and exclusion criteria flow chart.

Changing trends in etiologies: towards an increasing NAFLD related HCC

Viral related HCC including HCV and HBV were the most frequent causes of liver disease. Chronic HCV was the main etiology in the overall cohort (36.6%, *n* = 159), followed by HBV (25.3%, *n* = 110), alcoholic liver disease (16.8%, *n* = 73), cryptogenic cirrhosis 7.4% and non-alcoholic fatty liver disease 5.7% (Table 2). Three patients had HCV-HBV chronic co-infection. Six patients had HCC in a non-cirrhotic liver; 2 of these patients had chronic HBV infection and the rest of them were cryptogenic.

Between years 2005 and 2012 the number of liver transplants increased, as well as the number of active transplant centers. Number of HCC transplanted patients for each year was as follows: 2005 (*n* = 22), 2006 (*n* = 34), 2007 (*n* = 42), 2008 (*n* = 61), 2009 (*n* = 61), 2010 (*n* = 82), 2011 (*n* = 87) and 2012 up to June (*n* = 46).

From years 2005 to 2012, viral related transplanted HCC (HCV + HBV) decreased from 66% to 50% of the overall cohort per periods, respectively. Chronic hepatitis C infection was the leading cause of HCC in every period considered. Although the relative frequency of HCC HCV decreased from 2005, a sustained increase in the absolute number of HCV was observed (Table 2, Fig. 2). This figure represents a main decease in the overall proportion of HCC related HCV-cirrhosis in this cohort, from 48.2% in 2005 to 25.6% in 2011—2012. Whereas alcoholic liver disease was mainly stable, NAFLD increased from 1.8% in years 2005—2006 to 12.8% in 2011—2012, accounting for the third cause of HCC following viral and alcoholic liver disease (Fig. 2B). NAFLD related HCC presented a 6-fold increase

Table 1 Patients' baseline characteristics and comparative analysis between NAFLD and non-NAFLD HCC patients.

Variable	Overall *n* = 435	NAFLD *n* = 25 (5.7%)	non-NAFLD *n* = 410 (94.2%)	*P*
Age, years (± SD)	57 ± 9	60 ± 4	56 ± 8	0.03
Gender, male, *n* (%)	356 (81.8)	19 (76.0)	337 (82.2)	0.43
Months on waitlist, median (IQR)	3.0 (1.0—9.0)	3.0 (1.0—11.0)	3.0 (1.0—9.0)	0.93
Child Pugh A/B/C, *n* (%)	145 (33.3)/192 (44.3)/98 (22.4)	8 (32.0)/12 (48.0)/5 (20.0)	137 (33.4)/180 (44.1)/93 (22.5)	0.92
MELD, (± SD)[a]	16.7 ± 7.7	16 ± 8	16 ± 7	0.97
Supplementary MELD points, *n* (%)	293 (67.4)	17 (68.0)	276 (67.3)	0.94
AFP level at listing, ng/mL				
≤ 100 ng/mL, *n* (%)	330 (75.9)	24 (96.0)	306 (75.3)	0.06
101—1000 ng/mL, *n* (%)	79 (18.2)	1 (4.0)	78 (19.2)	
> 1000 ng/mL, *n* (%)	22 (5.1)	0 (0.0)	22 (5.4)	
Within Milan, *n* (%)	354 (81.4)	21 (84.0)	333 (81.2)	0.73
Within UCSF, *n* (%)	394 (90.6)	23 (92.0)	371 (90.5)	0.80
AFP model ≤ 2, *n* (%)	353 (81.1)	23 (92.0)	330 (80.9)	0.16
Treatment before LT, *n* (%)	193 (44.4)	14 (56.0)	179 (43.7)	0.23
TACE	143	10	133	
RFA	38	2	36	
TACE + RFA	8	2	6	
Liver resection	4	—	4	
Explanted liver features				
Within Milan, *n* (%)	277 (63.7)	18 (72.0)	259 (63.2)	0.37
Within up to 7, *n* (%)	358 (82.3)	22 (88.0)	336 (81.9)	0.44
Microvascular invasion, *n* (%)	77 (17.7)	3 (12.0)	74 (18.0)	0.44
Macrovascular invasion, *n* (%)	12 (2.8)	0 (0.0)	12 (2.9)	0.36
Nuclear grade > II, *n* (%)	79 (18.2)	1 (4.0)	78 (19.0)	0.34

MELD: Model for end stage liver disease; NAFLD: non-alcoholic fatty liver disease.
[a] Laboratory MELD score before liver transplant.

Table 2 Underlying etiologies of liver disease in liver transplant patients per year (frequencies).

	HCV	HBV	Alcohol	NAFLD	CC	Cholestasis	Autoimmune	Other	Total
2005	14	3	3	0	2	0	0	0	22
2006	14	7	6	1	3	2	1	0	34
2007	19	10	4	0	3	1	2	3	42
2008	22	24	8	4	2	3	0	0	61
2009	22	11	11	1	6	3	3	5	61
2010	34	24	12	2	6	1	1	2	82
2011	28	16	20	7	5	3	2	6	87
2012	6	15	9	10	5	1	0	0	46

CC: cryptogenic cirrhosis; *Cholestasis: primary biliary cholangitis, primary and secondary sclerosing cholangitis. HBV: hepatitis B virus; HCV: hepatitis C virus; ** HFE: hemochromatosis; HCC: hepatocellular carcinoma; LT: liver transplantation; NAFLD: non-alcoholic fatty liver disease. Note: Year 2012 was included up to June.

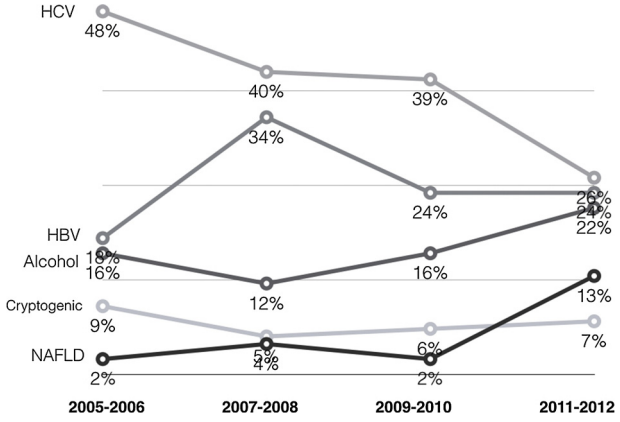

Figure 2 Hepatocellular carcinoma and liver related etiologies from years 2005 to 2012. Note: from years 2005 to 2011, viral related liver disease decreased from 66% to 50%. NAFLD related HCC presented a 6-fold increase between 2005—2006 and 2011—2012, whereas HCV related HCC had a 2-fold decrease during the same period.

between 2005—2006 and 2011—2012, whereas HCV related HCC had a 2-fold decrease during the same period.

Changes in major etiologies per country from 2005 to 2012 were evaluated. Chronic hepatitis C virus infection was the most frequent etiology of liver disease in Argentina (46.9%), Mexico (83.3%), Peru (36.4%) and Uruguay (33.3%). Hepatitis B accounted for most of the cases in Brazil (44.5%) and was the third leading cause of HCC in Colombia (18.5%) and Argentina (12.2%). Alcoholic liver disease and NAFLD were the first causes of liver disease in Colombia (24.6%) and Chile (26.5%), respectively.

In some countries, NAFLD increased during the study period, Argentina (26%), Chile (37%) and Peru (50%). Hepatitis B remained the leading cause of HCC and LT in Brazil (46%), followed by HCV (27%). Alcoholic liver disease increased during the last years in Colombia (35%), whereas in Mexico and Uruguay, HCV remained the leading cause of HCC (Supplementary Table).

Patients with NAFLD were older and had similar Child Pugh and MELD scores than those patients without NAFLD. Patients with NAFLD had lower serum AFP values at listing, with almost all the patients having an AFP value below 100 ng/mL. A similar proportion of patients within Milan and extended criteria were observed when compared to non-NAFLD HCC patients. Similar explanted liver features were found between groups (Table 3). Patients with cryptogenic HCC had higher MELD score than that of NAFLD patients (20 ± 6 vs 16 ± 8; $P = 0.011$) and a lower proportion of supplementary MELD points (38% vs 68%; $P = 0.026$). No significant differences were observed regarding age, gender and AFP levels between NAFLD and cryptogenic HCC groups (Table 3).

Changing trends in etiology of HCC: Sensitivity analysis

From a multivariate logistic regression analysis of potentially confounding factors related with NAFLD, there were not any changing site-specific organ allocation policies promoting or precluding any etiology of liver disease during the study period in all countries. Neither gender, age and LT center volume were independently related with NAFLD; whereas the only independent variable related with NAFLD was year of LT OR 2.28 (CI 1.33; 3.91).

Impact on post-transplant outcomes regarding etiologies of HCC

Overall, median follow-up in the overall cohort was 38.0 months (IQR 16.0—64.0 months). Patient survival rates at 1, 3 and 5 years were 77.7%, 67.4% and 64.8% ($n = 153$ deaths), respectively. Cumulative incidence of recurrence at 1, 3 and 5 years were 7.1%, 12.2% and 14.2% ($n = 62$ recurrences) with a median TTR of 12.5 months (IQR 6.0—26.0 months). No significant differences were observed regarding survival and recurrence rates between NAFLD and non-NAFLD HCC patients (Fig. 3A), HBV (Fig. 3B) and HCV (Fig. 3C).

Pre-transplant Cox regression model for HCC recurrence for the overall cohort is shown on Table 4. Etiologies of liver disease were not associated with HCC recurrence. Adjusted HR in the multivariate Cox regression model showed that the adjusted effect on 5-year HCC recurrence of the AFP model at listing was a HR of 2.44 (CI 1.32;4.52) after backward elimination considering confounding effect of treatment before LT and Milan criteria at listing (Table 4). Proportional hazard assumption was kept in the model ($P = 0.78$) and Harrell's c-statistic concordance for this model was 0.64. Competing risk analysis with death before recurrence

Table 3 Comparative analysis between NAFLD and cryptogenic HCC patients.

Variable	NAFLD $n=25$	Cryptogenic $n=32$	P
Age, years ($\pm$SD)	60 ± 4	58 ± 11	0.57
Gender, male, *n* (%)	19 (76.0)	21 (72.4)	0.76
Months on waitlist, median (IQR)	3.0 (1.0—11.0)	1.5 (0.0—5.0)	0.22
Child Pugh A/B/C, *n* (%)	8 (32)/12 (48)/5 (20)	7 (24)/14 (48)/8 (28)	0.73
MELD, ($\pm$SD)*	16 ± 8	20 ± 6	0.011
Supplementary MELD points, *n* (%)	17 (68.0)	11 (37.9)	0.026
AFP level at listing, ng/mL			
$\leq$100 ng/mL, *n* (%)	24 (96.0)	27 (93.1)	0.53
101—1000 ng/mL, *n* (%)	1 (4.0)	1 (3.4)	
> 1000 ng/mL, *n* (%)	0 (0.0)	1 (3.4)	
Within Milan, *n* (%)	21 (84.0)	23 (79.3)	0.66
Within UCSF, *n* (%)	23 (92.0)	26 (89.7)	0.77
AFP model $\leq$2, *n* (%)	23 (92.0)	25 (86.2)	0.49
Treatment before LT, *n* (%)	14 (56.0)	9 (31.0)	0.06
Explanted liver features			
Within Milan, *n* (%)	18 (72.0)	21 (72.4)	0.97
Within up to 7, *n* (%)	22 (88.0)	23 (79.3)	0.38
Microvascular invasion, *n* (%)	3 (12.0)	6 (20.7)	0.35
Macrovascular invasion, *n* (%)	0 (0.0)	0 (0.0)	—
Nuclear grade > II, *n* (%)	1 (4.0)	2 (7.7)	0.88

AFP: Alpha-fetoprotein; MELD: Model for End stage Liver Disease; NAFLD: non-alcoholic fatty liver disease.

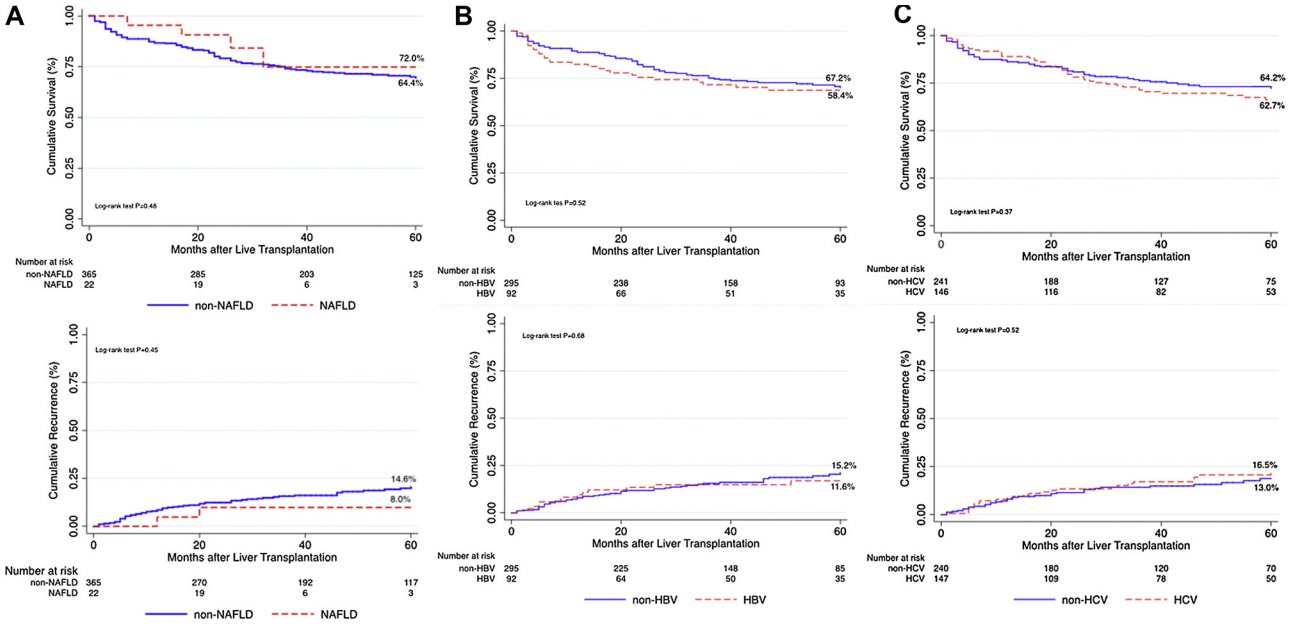

Figure 3 Tumor recurrence and patient survival rates according to etiologies of liver disease: NAFLD (Panel A), HBV (Panel B) and HCV (Panel C) (Kaplan Meier; log-rank test). Note: survival and recurrence rates were not significantly different between patients with NAFLD and non-NAFLD, HBV and non-HBV, and HCV and non-HCV, respectively.

showed that SHR for the AFP model adjusted with confounding variables was 2.11 (CI 1.21; 3,67).

Immunosuppression after transplantation

Drugs most used for initial immunosuppression during the first three months after LT were Tac in 71.0% of the cohort, MMF 49.7% and steroids 89.1%. Three months after LT, 4.4% ($n=16$) of patients were on mTOR inhibitors and it increased to 14.5% ($n=43$) by 2 years follow-up. The use of mTOR inhibitors within the first 3 months after transplantation was related to 5-year recurrence rate of 62.5% (HR 4.95 CI 2.64;9.28). However, patients treated with mTORs during the first three months after transplant had higher proportion of patients exceeding MC, UCSF and AFP score > 2 points at

Table 4 Pre-transplant variables associated with 5-year HCC recurrence after liver transplantation. Univariate Cox regression.

Variable	5-year incidence of recurrence (%)	Unadjusted hazard ratio (95% CI)	*P*	Adjusted hazard ratio (95% CI)	*P*
WL time < 3 months					
Yes (*n* = 261)	13.4	0.84 (0.52;1.38)	0.97		
No (*n* = 156)	16.0	1.18 (0.73;1.94)			
Underlying liver disease			0.29		
Hepatitis C (*n* = 158)	16.5	1.29 (0.79;2.10)	0.58		
Hepatitis B (*n* = 112)	11.6	0.84 (0.46;1.55)	0.45		
NAFLD (*n* = 25)	8.0	0.58 (0.14;2.39)			
Treatment before LT					
Yes (*n* = 193)	19.2	1.83 (1.09;3.05)	0.02	—	—
No (*n* = 242)	10.3	0.53 (0.32;0.88)			
Milan Criteria at listing					
Within (*n* = 354)	11.3	0.37 (0.22;0.63)	0.0001		
Exceeding (*n* = 81)	27.2	2.59 (1.54;4.36)		—	—
UCSF Criteria at listing					
Within (*n* = 394)	11.9	0.26 (0.14;0.46)	0.0001	0.41 (0.16;1.08)	0.11
Exceeding (*n* = 41)	36.6	3.81 (2.12;6.82)			
AFP model at listing					
≤ 2 points (*n* = 354)	10.2	0.29 (0.18;0.50)	0.0001		
> 2 points (*n* = 80)	30.0	3.22 (1.91;5.43)		2.44 (1.32;4.52)	0.008
AFP level at listing, ng/mL					
≤ 100 (*n* = 330)	9.6	—	—		
101—1000 (*n* = 79)	17.7	1.82 (0.97;3.43)	0.06		
> 1000 (*n* = 22)	39.1	4.38 (2.10;9.09)	0.0001		

Normal values: alpha-fetoprotein 0.6—4.4 ng/mL. AFP: alpha-fetoprotein; HCC: hepatocellular carcinoma; LT: liver transplantation; UCSF: University of California San Franc.

listing (43.8%, 37.5% and 40.0%, respectively), and in the explanted liver 50% (*n* = 8) exceeded up to 7 criteria, 50% (*n* = 8) and 33% (*n* = 5) had mvi and dedifferentiated tumors, respectively.

No significant differences were observed regarding use of Tac, CsA and mTOR immunosuppression during the first three months after LT between HCV, HBV and NAFLD subgroup of patients. However, a significantly lower use of MMF in HCV patients was observed (39.1% vs 56.1% non-HCV, *P* = 0.002). Corticosteroids were still included in the immunosuppression scheme at the 12th month of transplantation in 23.8% of HCV patients, 12.5% in HBV, 23.6% in alcoholic liver disease and 9.5% in NAFLD-HCC patients (*P* = 0.003).

Discussion

This multicenter cohort study describes recent trends regarding etiologies of LT for HCC in Latin America. Data from global patterns of etiology of HCC are lacking from this region and not reported previously [1]. First, in this cohort, we observed that there might be a changing figure regarding etiologies of HCC similar to what has been reported in other regions of the world, including a decreasing proportion of HCV and an increasing NAFLD. However, a high number of HBV related HCC was observed, particularly from Brazil, Argentina and Colombia. Second, patients with NAFLD were older, had similar tumor burden, time on the waiting list, were treated in a similar proportion while on the waitlist and had similar pre-LT MELD and Child Pugh scores than non-NAFLD HCC patients. No significant differences were observe upon survival and recurrence rates between NAFLD and non-NAFLD HCC patients. Third, etiology of liver disease was not associated with 5-year HCC recurrence rate. Finally, the use of corticosteroids at the 12th month of transplantation was significantly lower in NAFLD-HCC patients when compared to other etiologies showing a focus on its potential metabolic effects in these patients.

Participating centers included those with the highest number of procedures in each country, as previously shown. More than 2500 procedures are performed in this region every year; including Brazil > 1500, Argentina > 400, Colombia > 200, Mexico > 100, Chile > 70, Peru > 30 and Uruguay > 15. All these countries participated in this study. Although data from Cuba, Ecuador, Costa Rica, Dominican Republic, Venezuela and Panama are lacking; the number of transplants in these countries is very small [21]. Consequently, in this study regional data of LT for HCC are very well represented.

Different published series related to LT for HCC in Latin America showed that the main etiology of HCC was HCV,

and in other series HBV [6—10,21]. In this study, HCV was the main cause of HCC, followed by HBV and alcoholic liver disease. However, what is novelty in our research is that we found that during the last years, HCV related HCC decreased significantly whereas NAFLD increased 6-fold. This picture might be related to the rise in obesity, diabetes and worsening cardiometabolic health worldwide and might have a profound public health implication, as a substantial proportion of fatty liver disease burden can be prevented. Consequently, it seems that during the next years, NAFLD related HCC could represent a rising cause of this tumor in this region, in parallel with a growing burden of this disease worldwide [4,22,23].

We did not considered including cryptogenic cirrhosis as probable NAFLD as reported by other authors and tried to focused on histologically confirmed NAFLD in the explant. In this sense, it has been reported that NAFLD related HCC has been an increasing cause of LT and HCC in the United States [4]. However, the authors considered including obese patients with cryptogenic cirrhosis in NAFLD. Thus, a real or pure NAFLD subgroup could have been lower than that reported. In our series, data regarding diabetes mellitus and body mass index was not recorded, this was the reason why including cryptogenic obese patients was not considered. However, our epidemiological results are in line with what is going on in some developed countries [4,22,23], and in a recently published epidemiological data from Argentina [24], an increasing NAFLD as a public health implication in the upcoming years that Latin America is not precluded from this issue.

This trending change in the main etiologies in this Latin American cohort has two important connotations to take into account. First, the decrease in HCV in proportion but not in absolute terms goes hand in hand with the increase in NAFLD, mainly in Argentina, Chile and Peru. In Brazil, HBV and HCV were the first cause of HCC in the different periods. The decrease in HCV is striking in a region where access to old and new treatments for this chronic viral infection is likely to be lower than in other developed regions of the world. Second, the number of patients per center and per country generated heterogeneity in the results to be considered. However, the trend of an increase in NAFLD was a common factor, except in Brazil.

Finally, although the use of mTOR inhibitors was associated with HCC recurrence, this should be cautiously interpreted. We are not saying that the use of mTORs has influenced the outcome of recurrence if not the opposite. We described how the use of immunosuppressants has behaved in different periods over the post-transplant time. The use of mTORs, describes that patients with ''higher risk'' of recurrence received mTORs since at that time there were published data suggesting a protective effect on the development of recurrence. A recent randomized controlled trial has revealed that sirolimus does not prevent from recurrence at 5 years of follow-up [25]. Considering the metabolic effect of corticosteroids, this might had an influence on the decision to taper steroids more rapidly in NAFLD patients when compared to other etiologies.

We recognize that this study has limitations including first, that in cohort studies with no control group, prognostic factors might be biased. However, a strict revision of the data was centrally requested, investigators who performed the final analysis did not participate in the data collection to avoid differential outcome assessment on exposure and a complete follow-up and outcome assessment was available for all patients included. Second, data regarding body mass index and diabetes mellitus are lacking and this could have leaded a potential selection bias, particularly considering cryptogenic cirrhosis. It would have been interesting to report the prevalence trends of the metabolic risk factors during the study period. However, this limitation does not go against the observation that NAFLD is increasing in this region. In fact, the reported data from other regions of the world still included CC as ''NAFLD'' whereas our selection criterion included ''pure'' NAFLD as already mentioned. Combination of different etiologies (e.g. alcoholic liver disease plus chronic HCV) was not considered in order to include the main factor of chronic liver disease. Finally, although a confirmatory analysis including a more recent period of this changing etiologic scenario is mandatory, we included patients transplanted between 2005 to 2012 in order to have a sufficient follow-up period and second, to perform a comparable analysis including a similar period of time of that considered in previously published data [4]. A sensitivity analysis to evaluate any selection bias or confounder factor regarding the increasing prevalence of NALFD was done. The observed epidemiological trend was not biased by any voluntary restriction of patients with any particular etiology in all LT centers. We further evaluate in a multivariate regression analysis potential indicators of some epidemiological restriction that would have biased the results (age, gender, LT center volume) showing that none of these had and independent relationship with NAFLD per periods.

Finally, although HCC recurrence between subgroups have not been statistically significant, a lower incidence of recurrence in the NAFLD group was observed when compared to HBV and HCV. This difference should be explored in cohorts with greater statistical power by expanding the number of subjects included in order to evaluate whether this difference is or not really statistically significant.

In summary, NAFLD related HCC has been recognized as a growing burden in the United States and some European regions. This figure is similar to what it might happen in Latin America during the next years. While HCV and HBV related cirrhosis were the most frequent causes of HCC, both had decreased in the recent years. Hepatitis C had a 2-fold decreased whereas NAFLD related HCC had a 6-fold increased since 2005. This etiologic scenario is not only changing in high-income countries but also it may be happening in developing ones [26,27]. From a regional perspective, NAFLD might be one of the first HCC related causes in the coming decades, an issue to consider tackling the rise of this disease with effective prevention strategies. However, this observation is probably preliminary and has been observed in a short period over a cohort included with a small number of patients with wide socio-cultural heterogeneity. Also, the epidemiological change observed in these countries is not homogenous in all parts of the world [24], including some European countries [28]. What is certain is that the impact of this epidemiological change on the incidence of HCC in the world, although it has been reported, should still be confirmed in prospective studies.

Author's contributions

Concept and design, statistical analysis, writing of article: Federico Piñero, Marcelo Silva. Data recording, critical review of the manuscript: Paulo Costa, Elaine Cristina de Ataide, Sergio Hoyos Duque, Sebastian Marciano, Margarita Anders, Adriana Varón, Alina Zerega, Jaime Poniachik, Alejandro Soza, Martín Padilla Machaca, Josemaría Menéndez, Rodrigo Zapata, Mario Vilatoba, Linda Muñoz, Martín Maraschio, Luis G Podesta, Lucas McCormack, Adrian Gadano, Ilka SF Fatima Boin, Jose Huygens Parente García.

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Disclosure of interest

The authors declare that they have no competing interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.clinre.2018.03.014.

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