TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Long-term outcome of 'long-term liver transplant survivors'

Angel Rubín,[1,2] Cristina Sánchez-Montes,[1] Victoria Aguilera,[1,2] Fernando San Juan,[3] Isabel Ferrer,[1] Angel Moya,[3] Eva Montalva,[3] Eugenia Pareja,[3] Rafael López-Andujar,[3] Martín Prieto[1,2] and Marina Berenguer[1,2,4]

1 Liver Transplantation and Hepatology Unit, La Fe Hospital, Valencia, Spain
2 Ciberehd (Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas), Valencia, Spain
3 Liver Transplantation Surgical Unit, La fe Hospital, Valencia, Spain
4 Faculty of Medicine, University of Valencia, Valencia, Spain

Keywords
de novo tumors, liver transplantation, long-term outcome, metabolic complications, renal insufficiency.

Correspondence
Marina Berenguer, Servicio de Medicina Digestivo., Hospital La Fe, C/ Bulevar Sur s/n., Valencia 46026, Spain.
Tel.: +34 961244000, ext 440226;
fax: 34 96 1246257;
e-mail: mbhaym@teleline.es

Conflict of interest
None to declare.

Received: 5 June 2012
Revision requested: 18 July 2012
Accepted: 20 April 2013
Published online: 28 May 2013

doi:10.1111/tri.12118

Summary

There are few studies focusing on long-term complications in liver transplant (LT) recipients. The aim of this study was to define the outcome of LT recipients having survived at least 10 years from LT. Of 323 adult LT done between 1991 and 1997, the 167(52%) alive >10 years post-LT (baseline time) formed the study population. Long-term outcome measures included the following: immunosuppression, metabolic complications [obesity, arterial hypertension (AH), diabetes, dislypidemia], cardiovascular events (CVE), chronic renal dysfunction-CRD, and *de novo* tumors. Median age at LT was 50 years. Most common indication was postnecrotic cirrhosis (89%), mostly because of HCV (46%). At study-baseline (10 years post-LT), 29% were obese and AH, diabetes, dislypidemia, and CRD were present in 75%, 30%, 42%, and 36%, respectively. In most cases, these complications were already present 1 year post-LT; less than one quarter developed them onward. The 6 year cumulative survival since baseline reached 84% (n = 24 deaths), with most deaths related to recurrent graft diseases (mostly HCV) followed by *de novo* tumors or CVE. 1, 3, 5 and 10 years cumulative rates of CVE and *de novo* tumors since baseline were 2%, 5%, 10% and 17%, and 1%, 3%, 6% and 13%, respectively. Chronic renal impairment was independently associated with survival and development of CVE since baseline. The medium-term survival of 'long-term survivors', i.e. patients alive 10 years after LT is good, but metabolic complications and CRD are common and continue to increase afterwards. Cardiovascular events and *de novo* tumors increase gradually over time and represent a major cause of late mortality.

Introduction

As a result of improvements in surgical techniques, immunosuppression regimes and management of infections, survival following liver transplantation (LT) is significantly better in recent years. Currently, median survival is about 90% at 1 year and 60% at 10 years [1]. Most studies to date have focused on short- and medium-term patient and graft survival, so that main causes of patient mortality and graft dysfunction are now clearly established and include, in the first years, infections and recurrent diseases while in the medium- and long-term nonhepatic causes such as *de novo* tumors or cardiovascular events (CVE) [2–4].

There are few studies aimed at evaluating the clinical status of 'long-term survivors', that is patients surviving beyond the first 10 years post-transplantation [5,6]. The main aim of this study was, hence, to define the clinical outcome of liver transplant (LT) recipients having survived at least 10 years from surgery; most specifically, we aimed to: [1] determine their clinical complications, specifically metabolic complications associated with cardiovascular risk, such as diabetes mellitus (DM), arterial hypertension

(AH), obesity, and dyslipidemia (DL), as well as chronic renal dysfunction (CRD) and *de novo* tumors; [2] establish the impact that these complications have on the development of CVE and patient survival; and [3] define risk factors associated with the development of these complications, with the final aim of developing strategies to prevent their occurrence.

To reach these aims, a retrospective analysis of our prospectively maintained transplant database was undertaken.

Patients and methods

Patients

A retrospective analysis of the LT database established in 1991 and maintained prospectively since then was performed. Variables that were not available in the database were searched manually in the patient charts. This was followed by phone call and/or actual visits to the patients to confirm the information gathered in the charts as well as to obtain information that was not in the charts.

Inclusion criteria were as follows: adult patients transplanted in our center between 1991 and 1997 with a minimum survival of 10 years since transplantation and with available long-term follow-up data. Retransplanted patients with a minimum survival of 10 years since retransplantation were also included. There were no live-donor LT nor organs procured from donation after cardiac death.

Methods

'Ten years after transplantation' was considered the 'Baseline of the study'. An analysis of patient survival and causes of mortality since baseline was performed. The date of the most recent hospital visit was considered the last follow-up among surviving patients (to a maximum set in August, 2011) while the date of death was used for those who had died.

Cardiovascular events, *de novo* tumors (excluding skin tumors) and CRD since baseline were evaluated. Variables that could impact survival as well as the development of these complications were analyzed including the following: (i) patient demographics (age and gender); (ii) pretransplantation data, such as transplant indication, history of tobacco use, body mass index (BMI), AH, diabetes, obesity, DL, chronic renal insufficiency, Child-Pugh and MELD scores and primary vs retransplantation status; (iii) donor data (age, gender, cause of death, BMI, obesity, steatosis); (iv) immediate post-transplant-related data (immunosuppression, rejection) and (v) medium- and long-term variables evaluated at different time points (first, 5th and 10th year), including risk factors for CVE (smoking, obesity, AH, diabetes and DL), alcohol intake, renal function, and *de novo* tumors. Protocol liver biopsies were performed in all patients at 1, 3, and 5 years post-LT. In addition, hepatitis C virus (HCV) (+) patients had 7 and 10 years available liver biopsies.

Causes of death are those listed as the primary cause of death by the physician involved in the care of patient.

Definitions

The following definitions were used:

1. Diabetes mellitus (OMS criteria): fasting glycemia $\geq$126 mg/dl or $\geq$200 mg/dl at any time during the day, in at least three consecutive tests, or the need for antidiabetic agents [7].

2. Arterial hypertension (OMS criteria): systolic blood pressure of $\geq$140 mmHg and/or diastolic blood pressure of $\geq$90 mmHg, in at least three consecutive evaluations or the need for antihypertensive treatment [8].

3. Dyslipidemia: fasting cholesterol and/or triglycerides levels higher than 240 mg/dl and 150 mg/dl, respectively, in at least in three consecutive blood tests or the need for anti-lipidemic agents [9].

4. Chronic renal dysfunction: creatinine levels higher than 1.5 mg/dl in at least three consecutive blood tests for at least 30 days. This cut-off was chosen to be able to compare the data with that of prior published studies [4,10,11].

5. Overweight and obesity (OMS criteria): BMI greater than 25 or 30 kg/m^2, respectively [12].

6. Alcohol consumption: Pre-LT, Significant alcohol consumption was defined as >50 g/day for a period of >5 years or >80 g/day for a shorter period. This information was obtained from chart review, most specifically from the pretransplantation protocol used in our center. All patients with alcoholic cirrhosis fulfilled criteria for abstinence from alcohol for greater than 6 months and were evaluated by a psychiatrist prior to transplantation. Post-transplantation alcohol intake was divided into mild, moderate, or severe [13].

7. Tobacco use: information regarding the number and years of tobacco use was obtained from chart review and personal patient contact.

8. Cardiovascular events were defined as ischemic cardiomyopathy (myocardial infarction or angina with pathological coronary angiography), cerebrovascular disease (thrombosis or hemorrhagic stroke demonstrated on computed tomography or magnetic resonance imaging) and peripheral vascular disease (occlusive or sub-occlusive arterial disease). CVE in patients with sepsis or hemorrhage were excluded.

9. *De novo* tumors: skin tumors and recurrent hepatocellular carcinoma (HCC) were excluded. Skin malignancies were excluded because of high prevalence of these tumors in our geographical area and the high likelihood of their

underestimation as most are discovered and treated in other centers.

Immunosuppression protocol

Immunosuppression at our centre in this period consisted of calcineurin inhibitor –CNI (mainly cyclosporine - Csa), azathioprine (AZA) and methylprednisolone therapy. Target whole blood trough levels of Csa were 250–350 the first month, 150–250 ng/ml the second and the third months, 100–150 ng/ml until the end of the first year, and 100 ng/ml thereafter. Target whole blood trough levels of tacrolimus (Tac) were 5–15 ng/ml the first 3 months, 5–10 ng/ml thereafter. In patients with development of renal dysfunction, mycophenolate mofetil (MMF) was used from 1995 together with reduction in the CNI dose. Methylprednisolone was initiated with a 1-gm intravenous bolus immediately after the reperfusion of the graft and then tapered progressively to 20 mg/day (day 6) and was discontinued 6–12 months after LT. AZA was discontinued within 6 months post-LT. Recurrent hepatitis C has been treated with interferon-based therapies which have evolved over time [14].

Statistical analysis

All data were analyzed using the statistical package SPSS (version 15.0 SPSS Inc, Chicago, IL). The chi-squared test was used for comparing qualitative variables and Student *t*-test and Mann–Whitney *U*-test for comparing quantitative continuous variables. Quantitative variables which were normally distributed were expressed as mean values $\pm$ 1 S.D and those non-normally distributed were expressed as median values (range). Significance testing was two-sided and set at $P < 0.05$. Survival curves were analyzed using the Kaplan-Meier curves and compared with the log-rank. Cox-regression was used to assess the independent factors associated with (i) survival; (ii) development of CRD; (iii) development of *de novo* tumors; and (iv) development of cardiovascular events.

Results

Study population

Overall transplanted population: between 1991 and 1997, 323 liver transplants were performed in adults ($\geq$ 14 years of age) in our center, with a median follow-up since transplantation of 11.5 years (range: 0–20 years). No patient had combined liver–kidney transplantation and all except one were Caucasian. Of these, 156 patients (48.3%) dying before the 10th post-transplant year – at a median of 2.25 (range: 0–10) years – were excluded (Table 1). Most deaths occurred during the first year (37%) – similar to what has been recently reported in the Pittsburg's series [15]. The causes of these early deaths were: (i) liver-related causes (n = 45; 29%), including recurrence of nontumoral primary diseases (n = 34), hyperacute rejection (n = 1), chronic rejection (n = 6), and primary graft failure (n = 4); and (ii) extra-hepatic causes (n = 111, 71%) such as tumors – including HCC recurrence- (n = 36), infections (n = 31), CVE (n = 2) and others (n = 22).

Table 1. Causes of mortality of the patients who died in the first 10 years after liver transplantation (n = 156).

Timing of death	
Intra-operative death	5 (3.2%)
In-hospital death	33 (21.2%)
In follow-up	118 (75.6%)
Causes of death	
Recurrence liver disease	
Non-HCC	34 (22%)
HCC	9 (6%)
Rejection	7 (4.5%)
Primary graft failure	4 (2.5%)
Infections	31 (20%)
De novo malignancy	28 (18%)
Cardiovascular events	22 (14%)
Others	21 (13%)

HCC, hepatocellular carcinoma.

The cause-specific probability of death over time (by primary cause) of all the 323 transplants performed between 1991 and 1997 is shown in Fig. 1. While infections were the leading cause of death in the first 2 years, hepatic causes

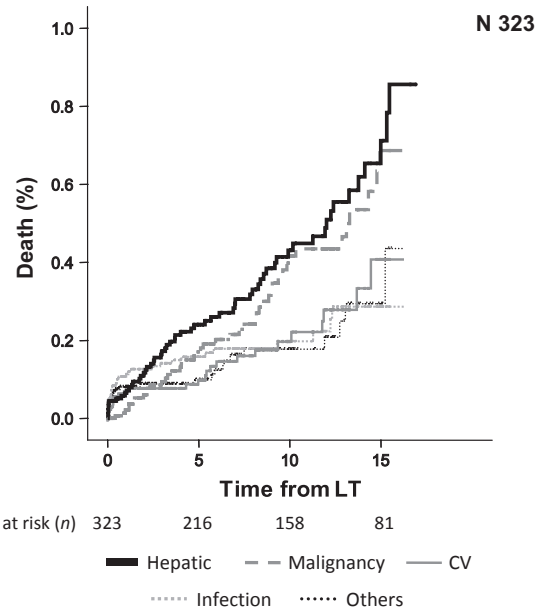

Figure 1 Cumulative incidence of cause-specific probability of death from LT.

were the most common thereafter and overall, with increased cumulative rate overtime. After the 5th year, *de novo* tumors become the second most frequent cause of patient mortality.

'Long-term survivors alive 10 years post-LT': of the 167 patients with a minimum survival of 10 years, nine additional cases were excluded because of lack of data (lost to follow-up), so that the final cohort comprised 158 LT recipients surviving beyond 10 years from transplantation. Their median age at baseline was 60.4 years (24–75). The main features of these patients at time of liver transplantation (including transplant demographics, indication for liver transplantation and donor-related factors) and data regarding immunosuppression/rejection are shown in Table 2. The median age at transplantation was 50 years and 67% were men. Most common indication for transplantation was postnecrotic cirrhosis (89%), mostly caused by HCV (46%). The majority of the patients were Child B (53%). Before LT, there were 65 smokers (41%), more in men than in women (54% vs 15%, $P < 0.001$), whereas a history of alcohol intake was reported by 54 patients (34%), also significantly more frequent in men than in women (44% vs 17%, $P = 0.001$). After transplantation, 20% of patients were active smokers ($n = 32$), without differences in gender, whereas alcohol intake was reported by 19 patients (12%).

The main immunosuppressive agent immediately after LT was Csa used in 97.5% of cases and discontinued in 14% of cases at a median of 11.5 years. In turn, steroids were used in 100%, discontinued in 95% at a median of 1.5 years since surgery (mainly per protocol) and re-introduced in 25% of patients at a median of 6 years post-transplantation (mainly in the context of autoimmunity or rejection). MMF was introduced in 31% of the patients after a median of 8 years since transplantation, mostly in the setting of renal impairment. Fifteen patients were switched to tacrolimus (Tac), 12 because of gingival hypertrophy. Globally, more than two-third of recipients were on Csa (59%) or Tac (4%) or MMF (9%) monotherapy, and 21% were on concomitant steroid therapy.

The main characteristics of the patients at baseline, that is, 10 years from transplantation are shown in Table 3. These patients were followed for five additional years, ranging from 0.2 to 10 years. Twenty-two percent had developed cirrhosis, mainly because of recurrent hepatitis C, by the time they had reached the 10th year post-LT.

Table 2. Characteristics of the 158 patients at time of liver transplantation who were alive 10 years later and included in this study.

Demographics	
Median follow-up from LT (years)	15 (10–20)
Median follow-up after 10 from LT (baseline; years)	5.1 (0.2–10)
Re-LT	6 (4%)
Age at LT (years)	50 (14–65)
Gender (men)	106 (67%)
Body mass index at LT	24.5 (17–35)
Active smokers	65 (41%)
Child A/B/C (%)	18/53/29
Indication to liver transplantation	Underlying etiology (n, %)
Post-necrotic cirrhosis	*140 (88.6%)*
Viral cirrhosis	89 (56%)
Hepatitis C virus (HCV) alone	70 (44%)
Hepatitis B virus (HBV) ± hepatitis D virus (HDV)	16 (10%)
HBV + HCV	3 (2%)
Alcoholic cirrhosis (OH)	38 (24%)
Mixed cirrhosis (OH + HCV)	16 (10%)
Criptogenic cirrhosis/Autoinmune cirrhosis	7 (4.5%)/6 (4%)
Cholestasic cirrhosis	*13 (8.2%)*
Primary biliary cirrhosis/cistic fibrosis	12 (7.5%)/1 (0.5%)
Others	*3 (1.8%)*
Malignancy	*22 (14%)*
Hepatocellular carcinoma (HCC)	20 (12.5%)
Biliary tract tumor	1 (0.6%)
Carcinoid metastases	1 (0.6%)
Donor characteristics	
Age (years)	28 (8–70)
Gender (men)	106 (67%)
Overweight/obesity	50 (32%)/7(4.5%)
Steatosis	
No/not available	98 (62%)/32 (20.3%)
Mild/moderate/severe	23 (14.6%)/5 (3.2%)/0
Initial inmunosupression	
Cyclosporine (Csa)	154 (97.5%)
Tacrolimus (Tac)	4 (2.5%)
Azathioprine (AZA)	155 (98%)
Steroids	158 (100%)
Rejection	
Acute (first month)/(late)	52 (33%)/22 (14%)
Chronic	6 (4%)
Steroids bolus/OKT3 administration	53 (33.5%)/5 (3%)
Changes in inmunosupression during follow-up	
Change to tacrolimus	15 (9.5%)
Gingival hypertrophy	12 (80%)
Time from LT (y)	6 (0.5–11.5)
Mycofenolate introduction	50 (31.5%)
Cause: nefrotoxicity	45 (90%)
Time from LT (y)	8 (1.5–16.5)
Rapamycin introduction	1 (0.5%)
Time from LT (y)	10
Cyclosporine withdrawal	22 (14%)
Cause: nefrotoxicity	21 (95%)
Time from LT (y)	11.5 (6–16.5)

Patient survival and causes of late mortality (since baseline)

Since baseline, 24 of the 158 'long-term survivors' (15%) have died with a cumulative survival of 98%, 94%, 87%, and 84% at 1, 3, 5, and 6 years, respectively. The main

Table 2. continued

Steroids withdrawal	150 (95%)
Time from LT (y)	1.6 (0.1–12)
Azathioprine withdrawal	148 (94%) *
Time from LT (y)	0.5 (0.1–12.5)
Steroids reintroduction	39 (25%)
Time from LT (y)	6 (1–15)

*Per protocol. LT, liver transplantation; y, years.

Table 3. Characteristics of the patients at baseline (10 years from LT).

Median follow-up since baseline (y)	5.1 years (0.2–10)
Age	60 (24–75)
Gender (male)	106 (67%)
Overweight	72 (46%)
Obesity	46 (29%)
DM	48 (30%)
Dyslipidemia	66 (42%)
Arterial hypertension	118 (75%)
Renal insufficiency	57 (36%)
Hemodyalisis	9 (6%)
Graft cirrhosis	34 (22%)
HCV graft cirrhosis	32 (20%)
Alcohol intake	19 (12%)
Active smokers	32 (20%)
Inmunosupression	
Csa	94 (59.5%)
Csa + PDN	9 (6%)
Csa + MMF	9 (6%)
Csa + MMF + PDN	10 (6.3%)
Tac	6 (4%)
Tac + MMF + PDN	3 (2%)
Tac + MMF	2 (1%)
Tac + PDN	3 (2%)
MMF	14 (9%)
MMF + PDN	7 (4.5%)
SRL + MMF + PDN	1 (0.5%)

HCV, hepatitis C virus; Csa, Cyclosporine; PDN, prednisone; MMF, mycophenolate mofetil; Tac, tacrolimus; SRL, sirolimus.

causes of mortality were hepatic in 40% (all but one because of recurrent disease) and nonhepatic in 60%, particularly *de novo* tumors and cardiovascular diseases.

The cause-specific probability of death over time (by primary cause) since baseline is shown in Fig. 2. The major cause was liver related with a progressive increase over time, followed by mortality caused by *de novo* tumors.

Table 4 reflects the univariate analysis of risk factors associated with long-term survival. In the multivariate Cox regression analysis, age at baseline > 60 years (RR: 2.74; 95% CI: 1.11–6.77; $P = 0.030$), hemodialysis at 10 years post-LT (RR: 5.5; 95% CI: 1.9–15.4; $P = 0.001$) and post-transplant graft cirrhosis (RR 3.19, 95% CI: 1.43–7.14; $P = 0.005$) impacted significantly long-term survival.

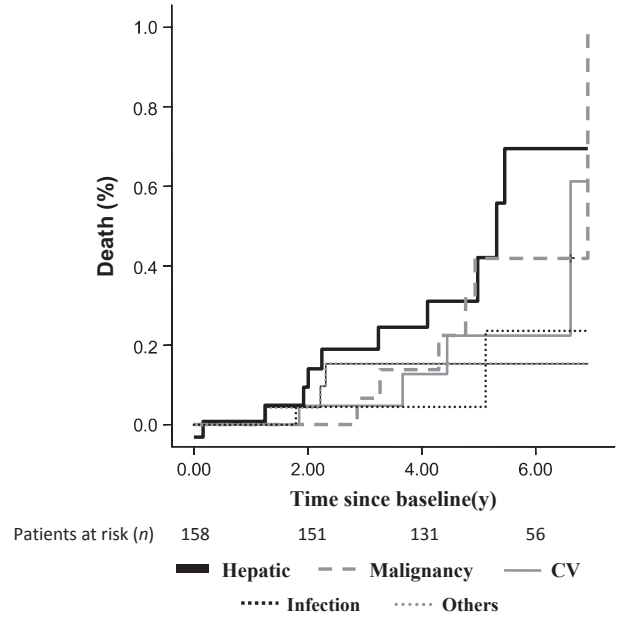

Figure 2 Cumulative incidence of cause-specific probability of death since baseline (10 years after LT).

Development of CVE and *de novo* tumors since baseline

The cumulative incidence of CVE and *de novo* tumors since baseline is shown in Fig. 3.

Twenty-seven CVE occurred in 27 patients (17%), 16 since baseline (ischemic cardiomyopathy in 11, cerebrovascular disease in three and peripheral vascular disease in two) between 0.18 and 8.1 years (median 3.1 years), with a 1, 3, 5 and 10 years cumulative rate of CVE since baseline of 2%, 5%, 10% and 17%, respectively.

All but two of these patients had ≥ 2 cardiovascular risk factors. The majority (87.5%) were men with a median age of 65 years (55–74).

Interestingly, the curve representing the development of CVE after 10 years since transplantation could not be predicted by the curve of CVE developing since LT (Fig. 4) with a significantly greater increase than predicted after 10 years. In the univariate analysis, factors significantly predictive of CVE since baseline were as follows: a family history of coronary heart disease, alcohol abuse pre-LT, a history of diabetes or renal impairment pretransplantation, CNI withdrawal, MMF introduction, steroids reintroduction, and hyperlipidemia and renal insufficiency at any time points post-transplantation (Table 5). In the multivariate analysis, the only factors independently associated with CVE development since baseline were a family history of ischaemic cardiopathy (RR: 10.6, 95% CI: 3.6–31.1; $P < 0.001$), alcohol abuse pre-LT (RR: 3.8, 95% CI: 1.14–12.4; $P = 0.03$) and renal insufficiency at any time points

Table 4. Univariate analysis of baseline (10 years post-LT) factors evaluated for association with survival since baseline.

	(*P*)	RR (95% CI)
Pre-LT factors		
Age >50 years (*n* = 71)	0.228	1.51 (0.78–2.95)
Gender (male, *n* = 106)	0.920	1.19 (0.60–2.37)
LT indication (HCV, *n* = 74)	0.168	1.92 (0.97–3.82)
Alcohol abuse pre-LT (*n* = 56)	0.288	1.29 (0.65–2.54)
Tumoral etiology (*n* = 22)	0.160	1 (0.38–2.55)
Family history of coronary heart disease (*n* = 15)	0.168	0.60 (0.21–1.74)
Smoking pre-LT (*n* = 65)	0.997	1.46 (0.74–2.87)
Pre-LT overweight (*n* = 59)	0.996	0.98 (0.49–1.99)
Pre-LT obesity (*n* = 17)	0.071	2.9 (1.3–6.47)
Pre-LT arterial hypertension (*n* = 8)	0.907	1.2 (0.29–5.04)
Pre-LT diabetes mellitus (*n* = 21)	0.389	1.77 (0.80–3.90)
Pre-LT dyslipemia (*n* = 15)	0.307	1.66 (0.49–5.57)
Pre-LT renal insufficiency (*n* = 9)	0.572	1.24 (0.29–5.26)
Donor factors		
Donor age >40 years (*n* = 48)	0.414	1.41 (0.70–2.80)
Inmunosupression		
Acute rejection (*n* = 74)	0.318	1.15 (0.74–1.80)
Chronic rejection (*n* = 6)	0.939	0.83 (0.11–6.11)
CNI withdrawal (*n* = 22)	0.914	0.73 (0.25–2.09)
Mycophenolate mofetil introduction (*n* = 50)	0.589	0.87 (0.41–1.85)
Steroids withdrawal (*n* = 150)	0.051	0.34 (0.10–1.15)
Steroids reintroduction (*n* = 39)	0.599	1.05 (0.45–2.44)
Factors at any time post-LT related with survival		
Age at baseline >60 years (*n* = 81)	0.024	2.23 (1.1–4.5)
Smoking post-LT (*n* = 32)	0.788	1.57 (0.70–3.55)
Alcohol post-LT (*n* = 19)	0.640	0.58 (0.14–2.46)
Obesity post-LT (*n* = 62)	0.340	0.69 (0.31–1.52)
Arterial hypertension post-LT (*n* = 131)	0.163	0.50 (0.15–1.66)
Diabetes mellitus post-LT (*n* = 107)	0.055	0.41 (0.19–0.86)
Dyslipemia post-LT (*n* = 99)	0.591	0.75 (0.32–1.72)
Renal insufficiency post-LT (*n* = 77)	0.362	0.57 (0.16–0.57)
Hemodialysis at 10 years post-LT (*n* = 9)	0.004	3.56 (1.35–9.40)
De novo tumor (*n* = 22)	0.123	0.53 (0.24–1.18)
Graft cirrhosis (*n* = 34)	0.002	2.65 (1.31–5.32)
Re-Liver transplantation (*n* = 6)	0.260	0.58 (0.08–4.30)
Cardiovascular event post-LT (*n* = 16)	0.713	0.80 (0.31–2.06)

LT, liver transplantation; HCV, hepatitis C virus; CNI, calcineurin inhibitors.

post-transplantation (RR: 3.83, 95% CI: 1.16–12.6; $P = 0.027$).

In turn, the cumulative incidence of *de novo* tumors since baseline increased from 1% at 1 year to 13% at 10 years. Twenty-two patients developed *de novo tumors*, 10 (6%) after the 10th year since LT. The main tumors were solid-organ tumors ($n = 7$) followed by lymphoproliferative disorders ($n = 3$, all HCV +). In the univariate analysis, factors significantly predictive of *de novo* tumors since baseline were having a tumor as the indication for transplantation (RR: 4.7, 95% CI: 1.3–17.2; $P = 0.02$), DM pre-LT

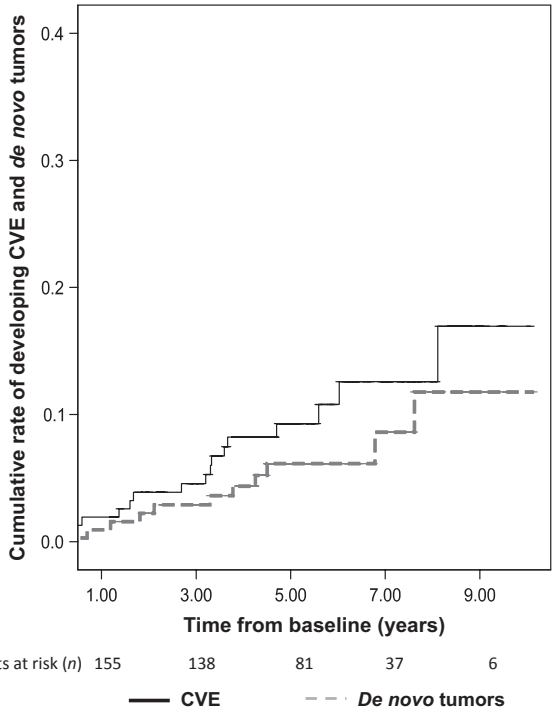

Figure 3 Cumulative rate of cardiovascular events (CVE) and *de novo* tumors since baseline (10 years from LT).

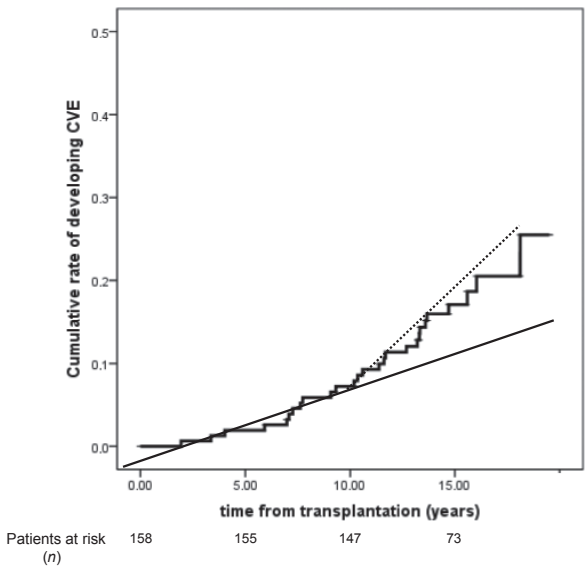

Figure 4 Cumulative rate (thick line) of cardiovascular events (CVE) since transplantation.

(RR: 3.75, 95% CI: 1.05–13.31; $P = 0.028$) and DM post-LT (RR: 4.62, 95% CI: 1.2–17.8; $P = 0.026$). In the multivariate analysis, the only factors independently associated

Table 5. Risk factors of cardiovascular events, since baseline (10 years from LT) (Univariate).

	(*P*)	RR (CI 95%)
Pre-LT factors		
Age >50 years at LT	0.76	1.16 (0.43–3.139)
Sex (male)	0.08	0.29 (0.06–1.27)
HCV etiology	0.9	0.94 (0.35–2.53)
Alcohol abuse pre-LT	0.001	5.75 (1.83–18.10)
Smoking pre-LT	0.302	1.63 (0.60–4.37)
Family history of ischaemic cardiopathy	0.001	9.4 (3.3–26.9)
Pre-LT obesity	0.68	0.66 (0.08–5.06)
Pre-LT arterial hypertension	0.058	3.81 (0.86–16.97)
Pre-LT diabetes mellitus	0.014	3.45 (1.20–9.95)
Pre-LT dyslipemia	0.73	0.80 (0.10–6.07)
Pre-LT renal insufficiency	0.004	5.3 (1.49–18.83)
Inmunosupression		
Chronic rejection	0.052	3.91 (0.88–17.34)
Change to tacrolimus	0.575	1.52 (0.34–6.77)
CNI withdrawal	0.030	3.07 (1.05–8.93)
Mycophenolate mofetil introduction	0.040	2.70 (1.01–7.28)
Steroids withdrawal	0.359	21.8 (0.1–5.12)
Steroids reintroduction	0.041	1.80 (0.67–4.85)
Factors at any time post-LT		
Smoking post-LT	0.33	2.12 (0.73–6.17)
Alcohol post-LT	0.9	1.15 (0.26–5.06)
Obesity post-LT	0.468	0.81 (0.26–1.90)
Arterial hypertension post-LT	0.147	0.25 (0.03–1.89)
Diabetes mellitus post-LT	0.2	0.47 (0.17–1.24)
Dyslipemia post-LT	0.07	0.33 (0.09–1.16)
Renal insufficiency post-LT	0.021	0.28 (0.09–0.89)
Graft cirrhosis	0.247	0.32 (0.04–2.44)
Re-Liver transplantation	0.955	1.06 (0.13–8.23)

LT, liver transplantation; HCV, hepatitis C virus; CNI, calcineurin inhibitors.

with *de novo* tumors were tumoral etiology of LT (RR: 4.85, 95% CI: 1.3–17.9; $P = 0.018$) and DM post-LT (RR: 4.74, 95% CI: 1.22–18.5; $P = 0.025$).

Risk factors associated with CVE in patients alive 10 years after LT: evolution over time

The development since transplantation of the different risk factors known to be associated with CVE, including obesity, diabetes, AH, and hyperlipidemia as well as the rate of chronic renal dysfuntion is shown in Table 6. Except for hyperlipidemia, the rate of all these complications increased over time ($P < 0.001$).

Ten years from transplantation:

- Only 13 patients (8%) had no risk factors. In turn, 20% of patients had one risk factor, and 71% had ≥ 2 risk factors (37% had ≥ 3 risk factors).
- Twenty-nine percent of these long-term survivors were obese and 46% were overweight. Only 1 of 16 patients with obesity pre-LT and nine of 59 with overweight pre-LT normalized their BMI after LT. At the time of last follow-up, the median BMI had increased from 24.3 kg/m^2 (range, 15–35.4) pre-LT to 27.5 (range 18.3–37) and median body weight had increased 7.5 kg.
- Diabetes mellitus was present in 30% of patients (40% of them had pre-LT diabetes), with 85% of them requiring pharmacological treatment (59% with insulin, 41% with oral antidiabetics, none on double therapy). Of note, 17% of these patients were on steroids at this time-point.
- Dyslipidemia was present in 42% of patients (46% had hypertriglyceridemia, 24% hypercholesterolemia and 30%

Table 6. Evolution over time of renal insufficiency, arterial hypertension, diabetes mellitus, obesity and hyperlipidemia.

	Pre-LT	1st year	5th year	10th year
Body mass index	25 (17–35.4)	27.3 (17–40)	27.6 (17.7–40.5)	27.5 (18.3–37)
Overweight	59 (37%)	77 (49%)	74 (47%)	72 (46%)
Obesity	17 (11%)	38 (24%)	41 (26%)	46 (29%)
Arterial hypertension	8 (5%)	107 (68%)	109 (69%)	118 (75%)
Diabetes mellitus	21 (13%)	35 (22%)	38 (24%)	48 (30%)
Dyslipidemia	15 (9.5%)	73 (46%)	59 (37%)	66 (42%)
Hypercholesterolemia	7 (4.5%)	6 (3.8%)	5 (3.2%)	16 (10.1%)
Hypertriglyceridemia	4 (2.5%)	48 (30.4%)	33 (21%)	31 (19.6%)
Mixed	4 (2.5%)	19 (12%)	21 (13.3%)	20 (12.7%)
Renal insufficiency	9 (6%)	43 (27%)	45 (28.5%)	57 (36%)
Hemodialysis	0%	0%	1 (0.6%)	9 (6%)
Cardiovascular risk factors (*n*)				
None	63%	11%	14%	8%
1	31%	25%	30%	20%
2	5%	38%	25%	35%
≥ 3	1%	26%	31%	37%

LT, liver transplantation.

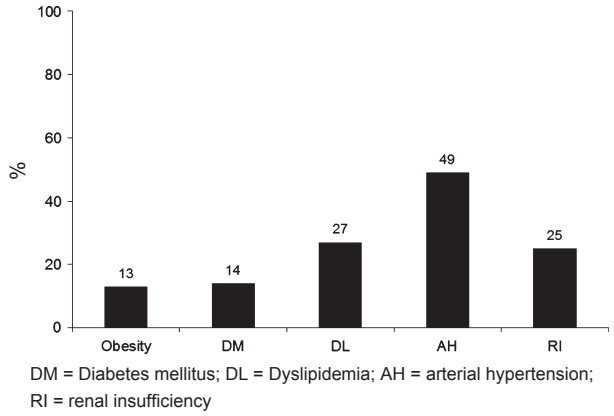

Figure 5 Proportion of patients without clinical complications one year post-LT who developed them at 10 years post-LT.

mixed) with 43% of them receiving pharmacological treatment (most statins).

- Arterial hypertension was present in 75% of patients, all except one, on pharmacological treatment (60% on monotherapy with calcium-chanel blockers, and 10% on a double antihypertensive drug regimen). Only one patient with AH prior to LT normalized the arterial pressure after LT.
- Chronic renal dysfunction was present in 36%; of these, 15% were on hemodialysis.
- No difference in gender was found for any of these complications.

The prevalence of most of these risk factors increased significantly after compared with before liver transplantation, particularly AH. Interestingly, most patients who had risk factors 10 years post-LT had developed these risks during the first year after surgery. However, there were still a substantial number of patients without risk factors 1 year post-LT who had these clinical conditions present 10 years after transplantation, particularly AH (Fig. 5). For instance, of 119 patients without obesity at 1 year, 13% had it at 10 years. Of 123 non1-year diabetics, 14% had diabetes at 10 years. Of 85 nonhyperlipidemic at 1 year, 26% had hyperlipidemia at 10 years. Of 51 patients without AH at 1 year, 49% had AH at 10 years, and finally, of 115 without chronic renal dysfuntion at 1 year, 24% developed this complication by year 10, possibly determining the increase in AH in long-term survivors.

Discussion

There are many studies focusing on 5–10 year survival since transplantation but few that have analyzed the outcome of 'long-term survivors', alive 10 years after transplantation. Our transplant program started 20 years ago so we decided to evaluate the outcome of these long-term survivors, in an effort to understand their mortality and main comorbidities, and in doing so, to develop strategies that may ameliorate their long-term outcome. The main findings of our study can be summarized as follows: (i) the medium-term survival of 'long-term survivors' alive 10 years from transplantation is good with 84% surviving six additional years; (ii) Liver-related deaths continue to be the first specific cause of death after the 10th year; (iii) Metabolic complications, such as diabetes, AH, obesity and hyperlipidemia are very common 10 years post-transplantation, and continue to increase afterwards, rising along with increased life expectancy after LT; (iv) Cardiovascular events occur in 17% of these long-term survivors. In addition, several cardiovascular risk factors coexist in many patients, which increases the risk of CVE; (v) Chronic renal dysfunction is very frequent among long-term survivors, present in about one-third of patients at 10 years; (vi) *De novo* tumors increase gradually over time and represent a major cause of late mortality.

The following comments can be made regarding our results:

(i) In terms of survival, medium-term survival of 'long-term survivors' is good, and altogether, nonhepatic conplications, including malignancies, CVE and infections, represent the most frequent causes of death during follow-up (median 15.1 years from LT). Our results are similar to those recently published by Watt et al. using the UNOS database [4]. Interestingly, we did not find any association between pretransplant risk factors, such as diabetes, AH, obesity, DL and renal insufficiency and survival of 'long-term survivors'. While this could be because of the small number of patients with each of these risk factors (see Table 3), it might also reflect the preselection of a 'healthier population'.

(ii) Liver-related deaths continue to be the first specific cause of death in the long term, mostly related to recurrent HCV. Indeed, several series have shown that graft cirrhosis develops in approximately 25% of recipients (range: 8–44%) after 5–10 years of follow-up and this percentage is likely to increase with increased duration of follow-up [16,17].

(iii) Regarding post-transplantation metabolic complications, our study focusing on a selected population of 'potentially healthier recipients' (alive at 10 years post-LT) confirms the results of previous studies with shorter follow-up [18].

Arterial hypertension was the most common medical complication, present in three quarters of patients. It is interesting to note that despite the frequent changes in immunosuppression during the follow-up, these complications remain very common if not increased. A possible explanation is that these changes were not made early enough post-transplantation, and in addition, specific treatments of these metabolic complications were not applied in all cases.

Interestingly, we recently published a multicenter Spanish study on control of blood pressure hypertension in almost 1000 LT recipients [19] and we found that almost one-third of the patients known to be hypertensive have an inadequate control of their blood pressure. Another remarkable result in our study was that 25% of patients never diagnosed as hypertensive after transplantation had increased blood pressure at the time of the study.

During the beginning of the 1990s (the early years of our transplant activity), the protection of the graft against rejection was the main aim, and high doses of immunosuppressive drugs were typically used. It is likely that the improved knowledge regarding their toxic profile, particularly the potential benefit associated with steroid avoidance and minimization of calcineurin inhibitors, will result in a reduction of these metabolic complications in patients transplanted in more recent eras [20].

(iv) With the improvement in survival observed in recent years, cardiovascular risk factors (and their consequences), CRD and *de novo* tumors have become a source of major concern in the long-term follow-up. Established cardiovascular risk factors in the general population, such as hypertension, DM, overweight-obesity and DL are very common in LT recipients [4,18,21–29] where the same factors have been linked with CVE [30,31]. For instance, in a study by Johnston et al., LT recipients had a twofold increased risk of cardiovascular deaths and threefold increased risk of ischaemic events as compared to an age and sex-matched population without liver transplantation. [31] In our study, at 10 years post-transplantation, most patients had at least two cardiovascular risk factors, and more than one-third had three risk factors.

Since we had no control group, we compared our results with that of the general Spanish population of similar age (mean age: 65 years). Based on this comparison, we estimated an increased prevalence of obesity, diabetes and DL (twofold increase), AH (75% vs 40–65%), and CRD (threefold increase) with regards to the general population [33].

The cumulative rate of developing CVE was greater than 15% beyond 15 years. Hence, it appears that the risk of developing CVE increases exponentially after 10 years [30,32].

(v) Chronic renal dysfuntion is a frequent complication after liver transplantation, more common than after cardiac or lung transplantation [35], especially in the long term; however, it rarely progresses to end-stage renal disease requiring hemodialysis or renal transplantation. These data are confirmed in our series, where more than a third of the patients had chronic renal impairment, but only 6% developed end-stage renal failure [36,37]. Although infrequent, hemodialysis (present in nine patients) was associated with worse survival [35,36].Consistent with other series, renal insufficiency at 1 year was predictive of long-term renal impairment [36, 37]. Protocols aimed at minimization of renal complications (calcineurin inhibitors minimization, induction therapy, newer non-nephrotoxic immunosuppressive agents) will likely result in a reduction overtime of this complication. In our series, nephrotoxicity was the main reason for calcineurin inhibitors discontinuation with/without introduction of MMF. However, the modification in the immunosuppressive regimen was probably too late to preserve renal function. Furthermore, the most common indication for liver transplantation in our series was HCV-cirrhosis, and HCV has been described as an independent risk factor for renal insufficiency following liver transplantation [38]. In fact, renal insufficiency at 10 years was more frequent in HCV transplanted patients than in uninfected recipients. Finally, chronic renal dysfuntion is another risk factor of cardiovascular disease in the general population as well as in transplanted patients [39,40].In our series, chronic renal dysfuntion at any time post-transplantation was significantly associated with the development of cardiovascular events.

(vi) The incidence of *de novo* tumors in LT recipients ranges from 3 to 16% [18], a percentage significantly higher than that observed in the general population with a twofold to fourfold overall higher cancer incidence [41–43], possibly related to the prolonged immunosuppression. Indeed, in our long-term survivors the incidence of this complication increased significantly over time. Previous studies have shown that this complication is responsible for approximately one-fourth of late deaths. In our study, it was the second cause of mortality among 'long- term survivors' [4,44].

Our study had some limitations, inherent to its retrospective nature and the fact that current immunosuppression protocol and characteristics of transplanted candidates (older, more comorbidities, greater disease severity) do not apply to those performed a couple of decades ago. Furthermore the population of our study was selected by the way the population as a whole was treated in the first 10 years after LT. Based on our findings and recent changes in immunosuppressive protocols, we believe that the long-term complications described in our series will probably be less common in future series that include LT patients transplanted more recently.

Given the low number of CVE and *de novo* tumors since baseline, the results of the multivariate analysis need to be interpreted with caution.

In conclusion, patients who have survived the first 10 years since transplantation have a good outcome. However, significant comorbidities, particularly AH, diabetes, CRD and obesity are present in a significant proportion of these long-term survivors and will possibly have an impact with longer follow-up. As most of these risk factors for CVE are present early post-transplantation, we need to

treat them aggressively at early time points. The older age of the patients undergoing liver transplantation in recent years (in contrast to our series, where 55% of patients were younger than 50) may result in a higher number of transplant recipients developing cardiovascular events; alternatively, the recent changes in immunosuppression protocols put into place in most transplant centers may reduce these complications.

Authorship

AR: designed research/study, performed research/study, contributed important reagents, collected data, analyzed data, and wrote the manuscript. CSM: contributed important reagents, collected data, and analyzed data. VA: designed research/study and contributed important reagents. IF: collected data. FSJ, AM, EM, EP and RLA: contributed important reagents. MP: designed research/study and contributed important reagents. MB: designed research/study, performed research/study, contributed important reagents, analyzed data, and wrote the manuscript.

Acknowledgements

Ciberehd is funded by the Instituto de Salud Carlos III. Marina Berenguer is supported by the 'Programa de intensificación de la actividad investigadora en el Sistema Nacional de Salud' (funded by the Instituto de Salud Carlos III and the Generalidad Valenciana), and has received a grant (PI-050981) from the Instituto de Salud Carlos III.

References

1. Carithers RL Jr. Liver transplantation. American Association for the Study of Liver Diseases. *Liver Transpl* 2000; **6**: 122.
2. Liu LU, Schiano TD. Long-term care of the liver transplant recipient. *Clin Liver Dis* 2007; **11**: 397.
3. Mells G, Neuberger J. Long-term care of the liver allograft recipient. *Semin Liver Dis* 2009; **29**: 102.
4. Watt KD, Pedersen RA, Kremers WK, Heimbach JK, Charlton MR. Evolution of causes and risk factors for mortality post-liver transplant: results of the NIDDK long-term follow-up study. *Am J Transplant* 2010; **10**: 1420.
5. Ciccarelli O, Kaczmarek B, Roggen F, *et al.* Long-term medical complications and quality of life in adult recipients surviving 10 years or more after liver transplantation. *Acta Gastroenterol Belg* 2005; **68**: 323.
6. Abbasoglu O, Levy MF, Brkic BB, *et al.* Ten years of liver transplantation: an evolving understanding of late graft loss. *Transplantation* 1997; **64**: 1801.
7. World Health Organization. *World Health Organization: Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia*. Report of a WHO/IDF Consultation. Part 1.Diabetes mellitus – diagnosis. Geneva: World Health Organization, 2006.
8. Whitworth JA; World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. *J Hypertens*. 2003;**21**:1983.
9. American Association of Clinical Endocrinologists. Medical guidelines for clinical practice for the diagnosis and treatment of dyslipidemia and prevention of atherogenesis 2002 Amended Version. AACE Lipid Guidelines. *Endocr Pract* 2000; **2**: 165.
10. Pfitzmann R, Nüssler NC, Hippler-Benscheidt M, Neuhaus R, Neuhaus P. Long-term results after liver transplantation. *Transpl Int* 2008; **21**: 234.
11. Schmitz V, Laudi S, Moeckel F, Puhl G, Stockmann M, Tran ZV, *et al.* Chronic renal dysfunction following liver transplantation. *Clin Transplant* 2008; **22**: 333.
12. American Association of Clinical Endocrinologist/American College of Endocrinology (AACE/ACE) Obesity Task Force. AACE/ACE Position statement on the prevention, diagnosis, and treatment of obesity. *Endocr Pract* 1998; **4**: 297.
13. Aguilera V, Berenguer M, Rubín A, San-Juan F, Rayón JM, Prieto M, Mir J. Cirrhosis of mixed etiology (hepatitis C virus and alcohol): post-transplantation outcome-Comparison with hepatitis C virus-related cirrhosis and alcoholic-related cirrhosis. *Liver Transpl* 2009; **15**: 79.
14. Berenguer M, Aguilera V, Rubín A, Ortíz C, Jimenez M, Prieto M. Comparison of two non-contemporaneous HCV-liver transplant cohorts: strategies to improve the efficacy of antiviral therapy. *J Hepatol* 2012; **56**: 1310.
15. Jain A, Reyes J, Kashyap R, *et al.* Long-term survival after liver transplantation in 4,000 consecutive patients at a single center. *Ann Surg* 2000; **232**: 490.
16. Wiesner RH, Sorrell M, Villamil F. Report of the first international liver transplant society consensus conference on liver transplantation and hepatitis C. *Liver Transpl* 2003; **9**: S1.
17. Samuel D, Forns X, Berenguer M, *et al.* Report of the monothematic EASL conference on liver transplantation for viral hepatitis (Paris, France, January 12–14, 2006). *J Hepatol* 2006; **45**: 127.
18. Sheiner PA, Magliocca JF, Bodian CA, *et al.* Long-term medical complications in patients surviving > or = 5 years after liver transplant. *Transplantation* 2000; **5**: 781.
19. Martínez-Saldivar B, Prieto J, Berenguer M, *et al.* A Control of blood pressure in liver transplant recipients. *Transplantation* 2012; **93**: 1031.
20. Klintmalm GB, Washburn WK, Rudich SM, *et al.* Corticosteroid-free immunosuppression with daclizumab in HCV (+) liver transplant recipients: 1-year interim results of the HCV-3 study. *Liver Transpl* 2007; **13**: 1521.

21. Bianchi G, Marchesini G, Marzocchi R, Pinna AD, Zoli M. Metabolic syndrome in liver transplantation: relation to etiology and immunosuppression. *Liver Transpl* 2008; **14**: 1648.
22. Seo S, Maganti K, Khehra M, *et al.* De novo nonalcoholic fatty liver disease after liver transplantation. *Liver Transpl* 2007; **13**: 844.
23. Sawyer RG, Pelletier J, Pruett TL. Increased early morbidity and mortality with acceptable long-term function in severely obese patients undergoing liver transplantation. *Clin Transplant* 1999; **13**: 126.
24. Nair S, Verma S, Thuluvath PJ. Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. *Hepatology* 2002; **35**: 105.
25. Moon JI, Barbeito R, Faradji RN, Gaynor JJ, Tzakis AG. Negative impact of new - onset diabetes mellitus on patient and graft survival after liver transplantation: long-term follow up. *Transplantation* 2006; **82**: 1625.
26. Khalili M, Lim JW, Bass N, Ascher NL, Roberts JP, Terrault NA. New onset diabetes mellitus after liver transplantation: the critical role of hepatitis C infection. *Liver Transpl* 2004; **10**: 349.
27. Charco R. Dyslipemia and long-term immunosuppression. *Transplant Proc* 2002; **34**: 124.
28. Charlton M. Obesity, hyperlipidemia, and metabolic syndrome. *Liver Transpl* 2009; **15**(Suppl 2): S83.
29. Desai S, Hong JC, Saab S. Cardiovascular risk factors following orthotopic liver transplantation: predisposing factors, incidence and management. *Liver Int* 2010; **30**: 948.
30. Guckelberger O, Mutzke F, Glanemann M, *et al.* Validation of cardiovascular risk scores in a liver transplant population. *Liver Transpl* 2006; **12**: 394.
31. Mells G, Neuberger J. Reducing the risks of cardiovascular disease in liver allograft recipients. *Transplantation* 2007; **83**: 1141.
32. Johnston SD, Morris JK, Cramb R, Gunson BK, Neuberger J. Cardiovascular morbidity and mortality after orthotopic liver transplantation. *Transplantation* 2002; **73**: 901.
33. Bertomeu V, Castillo-Castillo J. Cardiovascular disease in Spain today. From risk factors to morbidity. *Rev Esp Cardiol Supl* 2008; **8**: 2E.
34. Guckelberger O, Byram A, Klupp J, *et al.* Coronary event rates in liver transplant recipients reflect the increased prevalence of cardiovascular risk-factors. *Transpl Int* 2005; **18**: 967.
35. Ojo AO, Held PJ, Port FK, *et al.* Chronic renal failure after transplantation of anonrenal organ. *N Engl J Med* 2003; **349**: 931.
36. Gonwa TA, Mai ML, Melton LB, *et al.* End-stage renal disease (ESRD) after orthotopic liver transplantation (OLTX) using calcineurin-based immunotherapy: risk of development and treatment. *Transplantation* 2001; **72**: 1934.
37. Cohen AJ, Stegall MD, Rosen CB, *et al.* Chronic renal dysfunction late after liver transplantation. *Liver Transpl* 2002; **8**: 916.
38. McGuire BM, Julian BA, Bynon JS Jr, *et al.* Brief communication: glomerulonephritis in patients with hepatitis C cirrhosis undergoing liver transplantation. *Ann Intern Med* 2006; **144**: 735.
39. Balamuthusamy S, Srinivasan L, Verma M, *et al.* Renin angiotensin system blockade and cardiovascular outcomes in patients with chronic kidney disease and proteinuria: a meta-analysis. *Am Heart J* 2008; **155**: 791.
40. Bahirwani R, Reddy KR. Outcomes after liver transplantation: chronic kidney disease. *Liver Transpl* 2009; **15**(Suppl 2): S70.
41. Haagsma EB, Hagens VE, Schaapveld M, *et al.* Increased cancer risk after liver transplantation: a population-based study. *J Hepatol* 2001; **34**: 84.
42. Åberg F, Pukkala E, Höckerstedt K, *et al.* Risk of malignant neoplasms after liver transplantation: a population-based study. *Liver Transpl* 2008; **14**: 1428.
43. Herrero JI. De novo malignancies following liver transplantation: impact and recommendations. *Liver Transpl* 2009; **15** (Suppl 2): S90.
44. Pruthi J, Medkiff KA, Esrason KT, *et al.* Analysis of causes of death in liver transplant recipients who survived more than 3 years. *Liver Transpl* 2001; **7**: 811.

