TITLE PAGE

Title: Long-term outcomes (beyond 5 years) of liver transplant recipients - a transatlantic multicentre study

Short title: Post-transplant long-term outcomes

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Abbreviations:

LT	liver transplantation
HCV	hepatitis C
HBV	hepatitis B
NAFLD	non-alcohol-related liver disease
ALD	alcohol-related liver disease
HCC	hepatocellular carcinoma
CVD	cardiovascular disease

ABSTRACT

The long-term (>5 year) outcomes following liver transplantation (LT) have not been extensively reported. The aim was to evaluate outcomes of LT recipients who have survived the first 5 years. A multicentre retrospective analysis of prospectively collected data from 3 high volume LT centres (Dallas-USA, Birmingham-UK, and Barcelona-Spain) was undertaken. All adult patients, who underwent LT since the inception of the programme to 31 December 2010, and survived at least 5 years since their LT were included. Patient survival was the primary outcome. A total of 3682 patients who survived at least 5 years following LT (long-term survivors) were included. Overall, median age at LT was 52 years (IQR 44–58); 53.1% were males; and 84.6% were Caucasians. 49.4% (n=1820) died during a follow-up period of 36828 person-years (mean follow-up 10 years). 80.2% (n=1460) of all deaths were premature deaths. Age-standardised all-cause mortality as compared to general population was 3 times higher for males and 5 times higher for females. On adjusted analysis, besides older recipients and older donors, predictors of long-term mortality were malignancy, CVD and dialysis. Implementation of strategies such as non-invasive cancer screening, minimising immunosuppression and intensive primary/secondary cardiovascular prevention could further improve survival.

KEYWORDS

Liver Transplantation

Long-term outcomes

Post-transplant mortality

Post-transplant malignancy

1. INTRODUCTION

Organ transplantation remains a significant medical advance in human history [1,2]. Liver transplantation (LT) remains the curative treatment for acute fulminant liver failure, decompensated cirrhosis irrespective of the aetiology and hepatocellular carcinoma (HCC). Without the advent of LT, the prognosis of these conditions would remain dismal. Recipient outcomes have seen a remarkable improvement since the first successful human liver transplantation in 1963 by Starzl et al [3], owing to the advances in surgical techniques, optimisation of peri- and post-operative management, organ preservation and immunosuppressive strategies.

Recipient survival rates of 90% at 1-year (short-term) and 80% at 5-years (medium-term) has become the accepted norm rather than the exception [4-6] – a significant achievement compared to 30% and 20% at 1- and 5-years of LT recipients prior to 1985 [7]. Long-term outcomes (beyond 5 years) of LT recipients have not however been studied or reported as extensively as the short- and medium-term outcomes. A previous UK study reported a loss of 7 life years in recipients transplanted between 1985 – 2003 who survived more than 6 months post-LT, compared to age- and sex-matched population [8]. Similarly, a population-based Nordic study reported a 21% lower survival rate at 10 years in recipients transplanted between 1985 – 2009 who survived more than 1 year post-LT, compared to the general population [9]. Both studies included recipients who were within 5 years of LT, a period where disease recurrence such as HCC, hepatitis C and transplant-related complications are common thus potentially impacting upon survival rates both directly and indirectly.

Short- and medium-term survival rates remain high (80 to 90%) [4-6] and further substantial improvements remain difficult to achieve, but sought-after. However, improvement in the longer-term survival beyond 5 years post-LT is realistic and achievable world-wide, and a better understanding of the true morbidity and mortality of these long-term survivors is vital towards this.

The aim of this international multicentre study was to evaluate survival outcomes of recipients who survived the first 5 years following LT and to understands potential avenues for improving survival.

2. METHODS

2.1. STUDY POPULATION

This was a multicentre, retrospective analysis of prospectively collected data from 3 tertiary transplant centres – Baylor Scott & White Annette C. and Harold C. Simmons Transplant Institute at Baylor University Medical Centre, Dallas, TX, USA; The Liver Unit, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK; and Transplant Programme, Hospital Universitario Valle d'Hebrón, Barcelona, Spain. All adult patients (age 18 or over at the time of transplantation) who underwent LT since the inception of the LT programme in the respective centres to 31 December 2010 and survived 5 years or more since their first LT were eligible for inclusion. Those who underwent LT and died within the first 5 years of their first LT, those transplanted at less than 18 years of age and those who underwent combined organ transplantation were excluded.

Demographic and clinical data were extracted from electronic transplant database and supplemented with manual chart review. This study was approved by the local Research Ethics Boards at the respective institutions (Dallas, USA – IRB# 009-261; Birmingham, UK – CARMS 13119; Barcelona, Spain - PR(AG)155/2016 and PR(AG)598/2021).

2.2. LIVER TRANSPLANTATION POLICIES AND INDICATIONS

Selection of patients in Dallas, USA, Birmingham, UK and Barcelona, Spain follows the national or regional policies governed by the Organ Procurement and Transplantation Network/ United Network for Organ Sharing (OPTN/UNOS), National Health Service Blood and Transplant (NHSBT) and the Spanish Society of Liver Transplantation (SETH)/Catalan Transplant Organization (OCATT), respectively.

Broadly, the indications for LT in all 3 centres include acute fulminant liver failure, decompensated cirrhosis of any aetiology and hepatocellular carcinoma (deemed suitable for LT via local multidisciplinary team or tumour board). The specific details of LT indications are summarised in supplementary table 1. Transplant evaluation including rigorous psychosocial assessment was undertaken in all 3 centres, followed by the decision to list a patient for LT taken at a Multi-Disciplinary Team meeting. Unlike in Dallas, USA and Birmingham, UK where there was no age limit for prospective LT candidates, 68 years of age was recognised as the upper limit in Barcelona, Spain during the study period.

2.3. POST-TRANSPLANT LONG-TERM MANAGEMENT

2.3.1. Immunosuppression

Long-term immunosuppression regimen was similar in all 3 centres. Long-term monotherapy with calcineurin inhibitor (primarily tacrolimus or cyclosporine in a minority) was the standard of care in the mainstay of patients. Long-term dual therapy (calcineurin inhibitor and mycophenolate or azathioprine or low-dose steroid) or long-term triple therapy was used in selected groups of patients (e.g., patients transplanted for autoimmune-mediated liver diseases or patient with a history of recurrent acute cellular rejection or chronic rejection). Sirolimus or everolimus, a mammalian target of rapamycin inhibitor, was used as a calcineurin sparing agent, usually in combination with another immunosuppressant.

2.3.2. Out-patient follow up

In all 3 centres, LT recipients were followed up more frequently in the immediate post-LT period followed by relatively less frequently thereafter as long as there were no ongoing LT-related concerns. Post-LT management was not transferred to primary care physicians. However chronic conditions that were not related to LT were primarily managed by primary care physicians with input from the transplant team, where needed.

In Dallas, USA, LT recipients were reviewed twice weekly for 6 weeks, followed by every 2 weeks for 12 weeks, then monthly for the first year and yearly thereafter. All LT recipients are followed up at the Dallas transplant centre. In Birmingham, UK, LT recipients were reviewed weekly for 6 weeks, every 2 weeks for 3 months, then 6 weekly for the 6 months followed by 4 monthly for the first year. After the first year of LT, the care of stable recipients was transferred to the referring hepatologist at the recipient's local hospital, where possible, with 6-12 monthly review at joint outreach clinics attended by a transplant physician from Birmingham and local hepatologist (care closer to home). In Barcelona, Spain, LT recipients were reviewed weekly during the first month, 2 weekly for 3 months, monthly during the next 3 months, and every 2 - 3 months for two years and every six months thereafter, irrespective of the post-LT duration.

2.3.3. Surveillance for disease recurrence

Patients who underwent LT for HCC or those found to have 'incidental' HCC on explants underwent secondary surveillance with 3 – 6 monthly contrast-enhanced dynamic computerised tomography or magnetic resonance imaging up to 5 years following transplantation in 2 centres. In Birmingham, UK a bespoke protocol was discussed with patients having after LT for HCC and implemented where appropriate via multidisciplinary team discussions.

Prior to the introduction of direct-acting antiviral therapies, historically protocol liver biopsies were undertaken on patients who were hepatitis C RNA positive at transplantation to monitor fibrosis progression and guide treatment in Dallas, USA and Birmingham, UK. Liver biopsies were only performed when clinically indicated in Barcelona, Spain.

Routine surveillance for recurrence of autoimmune liver diseases with protocol liver biopsy or surveillance imaging was not undertaken in any centres. In other disease aetiologies, biopsies were performed where clinically indicated post-LT.

2.3.4. Other screening/surveillance programmes

Screening for hypertension, dyslipidaemia and diabetes mellitus was undertaken in the form of regular blood pressure monitoring, lipid profiling and fasting blood glucose or HbA1c monitoring, respectively during post-transplant clinic follow-ups in all 3 centres.

Patients who were transplanted for primary sclerosing cholangitis and intact colon underwent yearly colonoscopy with random biopsies as part of colorectal cancer surveillance in all 3 centres. Both in Dallas, USA and Birmingham, UK routine colonoscopy surveillance was not offered to transplant recipients of non-PSC aetiology; these patients underwent bowel cancer screening and/or colonoscopy surveillance according to national screening programmes with their family physicians and/or local gastroenterologists. Five yearly colonoscopy surveillance was offered to non-PSC aetiology recipients at Barcelona, Spain. All 3 centres offered colonoscopy to symptomatic LT recipients irrespective of the aetiology of liver disease.

General measures of skin cancer prevention such as avoidance of exposure to ultraviolet radiation during sun peak hours, use of sunscreen and hats were part of routine recommendation in all 3 centres. However, dedicated specialist dermatologist reviews were not part of routine post-transplant follow up. Breast and cervical cancer screening were offered to all LT recipients as part of national screening programmes.

2.4. OUTCOME MEASURE

Patient survival was the primary outcome measure, which was defined as the time from LT to death from any cause. Causes of death were broadly categorised into cardiovascular, cancer-related, renal failure, sepsis, transplant-related, disease recurrence and other/unknown. Age-standardised mortality rates (per 1000 person-years) stratified by sex were calculated for all cause and individual causes of death for each centre. Transplant-related deaths were defined as death of recipients due to causes attributable to transplant-related complications (e.g., deaths due to graft failure from ischaemic cholangiopathy or chronic rejection). Deaths due to disease recurrence was defined as death of recipients due to recurrence of primary disease that originally led to liver transplantation

(e.g., deaths due to graft failure from HCV autoimmune liver disease recurrence or alcohol recidivism; deaths due to HCC recurrence).

Mortality rates were compared with respective jurisdiction age-standardised mortality rates. Survivors were censored at the time of their last clinic visit. The country-specific all cause, cardiovascular and cancer-related mortality rates for the general population were obtained from Centers for Disease Control and Prevention (USA), Office for National Statistics (UK) and Eurostat, European Commission (Spain).

Premature death was defined as death that occurred before the average age of death in the respective general population. In USA and UK premature death is defined by death before the age of 75 years, and in Spain, premature death is defined as death before the age of 65 years [10].

2.5. STATISTICAL ANALYSIS

Categorical variables were expressed as frequencies and proportions. All continuous variables were not normally distributed and, as such, were expressed as median values with interquartile range. Demographics, pre-transplant clinical factors, donor characteristics, comorbidities and causes of death were compared across treatment centres using the Chi square test with Fishers exact test for categorical factors, and Kruskal-Wallis rank test for continuous factors. A p value <0.05 (2-tailed) was considered to indicate statistical significance.

For each transplant centre age-standardised mortality rates (per 1,000 person-years), stratified by sex, were calculated by dividing the number of deaths by the number of people in the transplant cohort, weighted by the US standardised population (2000) and the European standardised population (2013), for American and European centres, respectively. Individual age-standardised mortality rates were calculated for each cause of death category.

Median survival was calculated after at least five years post-transplant using Kaplan-Meier methods. Univariable Cox regression was used to assess factors independently associated with overall survival.

These factors included demographics, pre-transplant clinical factors, donor characteristics and comorbidities; transplant recipient and donor age were categorised into groups (18-29, 30-39, 40-49, 50-59, and >60 years). Factors that were significantly associated with overall survival were included in a multivariable Cox regression to calculate mutually adjusted hazard ratios with 95% confidence intervals. The proportionality assumption was assessed based on Schoenfeld residuals. The analysis assumed that missing data to have a random distribution and do not introduce bias.

Stata SE 15 (StataCorp, Texas, USA) was used for data management and statistical analyses.

3. RESULTS

3.1. STUDY POPULATION

During the study period, a total of 6,316 (Dallas – 2,761; Birmingham – 2,914; Barcelona – 641) adults patients received their first LT during the study period. Of which, a total of 3,682 (58.3%) patients, who survived at least 5 years following LT (long-term survivors) were included in the study. Of the total study population, 48.1% (n=1,771) were from Dallas, USA, 48.2% (n=1,774) were from Birmingham, UK and 3.7% (n=137) were from Barcelona, Spain.

The demographics and clinical characteristics of the total study population and individual centres are summarised in Table 1. Overall, median age at LT was 52 years (IQR 44 – 58) and 53.1% were males. The majority were Caucasians, whilst ethnic minorities represented 15.4% of the study population. Hepatitis C in Dallas (USA) cohort, autoimmune-related liver disease in Birmingham (UK) cohort and alcohol in Barcelona (Spain) cohort were the most common aetiologies of chronic liver disease, respectively. Decompensation was the commonest indication for LT in all 3 centres. Transplantation of liver from donors after brainstem death (DBD donation) comprised the main method of donation in all 3 centres.

3.2. OVERALL MORTALITY

Of the entire study cohort 49.4% (n=1,820) died during a follow-up period of 36,828 person-years (mean follow-up 10.0 years) – 80.2% (n=1,460) of all deaths were defined as premature deaths. The overall 10-, 15-, 20- and 25-year patient survival rates were 86.6%, 65.1%, 48.6% and 31.0%, respectively.

Age-standardised all-cause mortality (per 1000 person-years) of LT recipients were overall 3 times the respective general population (Dallas, US: males 49.2 Vs. 8.6 and females 61.8 Vs. 6.2; Birmingham, UK: males 34.7 Vs. 11.2 and females 31.9 Vs. 8.4; Barcelona, Span: males 15.1 Vs. 10.7 and females 26.6 Vs. 6.5) (table 2).

3.3. CAUSES OF DEATH

The three most common causes of death were *de novo* cancer (overall 17.5%), sepsis (overall 15.7%) and cardiovascular disease (overall 11.8%) among transplant recipients ≥5 years from LT. Deaths due to graft failure from diseases recurrence (overall 9.3%) and transplant-related complications (overall 6.7%) were common in this cohort of patients.

Over the study time-period, there was a gradual decline in death rates due to *de novo* cancer, cardiovascular disease, transplant-related complications and recurrence of primary disease. However, sepsis-related death rates rose with time. Death rates due to renal complications remained stable (Figure 1).

Overall, cancer-related mortality rates of LT recipients were two to five times the respective age-standardised general population with no difference among between males and females in the Dallas, US (males 7.7 Vs. 1.8 and females 5.3 Vs. 1.3) and Birmingham, UK (males 5.0 Vs. 2.2 and females 6.9 Vs. 1.5) cohorts (Table 2B). Further, *de novo* lung (15.6%), haematological (14.2%) and colon (8.5%) were the most common cancers that led to increased cancer-related deaths. Bacterial infections were the most common infective cause accounting for nearly two thirds of sepsis-related deaths (61.1%); fungal and viral infections were deemed responsible in a minority of cases (4.9% and 3.5%, respectively). Type of infection could not be established accurately in 30.5% of the cases due to lack of granularity.

The following factors were independently and inversely associated with long-term survival after 5 years of transplantation (Table 3): increasing recipient age at LT, increasing donor age, and history of pre-LT cardiovascular disease and malignancy and post-LT renal replacement therapy (Table 3). Requirement of dialysis post-LT (HR 2.4; 95% CI 1.6–3.7) and recipient age >60 at the time of LT (HR 2.1; 95% CI 1.4–3 .0) had the most significant negative impact on long-term survival beyond the first

322 5 years. Recipient sex, aetiology of liver disease, severity of liver disease at the time of LT, donor type,
323 and the choice of long-term immunosuppression had no significant adverse impact on long-term
324 survival (Table 3).

4. DISCUSSION

To the best of our knowledge this is the largest study to date to investigate the outcomes of long-term survivors of any solid organ transplantation. The study includes more than 3,800 LT recipients from high volume quaternary LT centres across 2 continents. Our study demonstrates that LT recipients are disadvantaged from a survival perspective compared to age-matched general population even after a prolonged period following transplantation. LT recipients were 3 to 10 times more likely to die than their age-matched general population counterparts even after surviving the first 5 years post-transplantation.

The advances in surgical techniques, optimisation of perioperative management, organ preservation and immunosuppressive therapy have markedly improved the short-term and medium-term outcomes [4,5]. The longer-term outcomes beyond 5-years of LT less well studied and reported. The existing data on the long-term outcome after LT includes patients from the time of transplant [11], or those who have survived the first year post-transplant [9]. The causes of death during short- and medium-term (less than 5 years from LT) are historically due to transplant-related complications and recurrence of primary disease such as recurrence of HCC or fibrosing cholestatic hepatitis from hepatitis C recurrence [12]. Therefore, the above reported long-term survival data from these studies [9,11] must be interpreted with caution. To mitigate the short- and medium-term causes of death on the interpretation of long-term survival, the current study has only included patients who have survived first five years post-LT. As expected, this is reflected in the observed difference in the causes of death in the current study and previously published ones (e.g., transplantation-related causes only accounted for 6.7% of all deaths in the current study compared to 15.9% in the Nordic cohort followed from 1-year post-transplant [9]).

In the current study, we compared the age-standardised mortality rates of LT recipients to the respective general population. The all-cause age-standardised mortality rates of LT recipients were 3

to 10 times higher compared the respective general population in all 3 centres, but the relative increase in the mortality rates were different between the centres. The exact reason(s) for this was unclear and is beyond the scope of this study. Considering specifically the individual causes of death, the leading causes of death of the entire cohort were malignancy, sepsis and cardiovascular disease. Previous large series have also reported malignancy as one of the leading causes of death among LT recipients [7,12,13], compelling the question as to whether more vigilant cancer surveillance measures should be implemented as part of standard of post-LT care for long-term survivors, especially with the advances in curative cancer treatments worldwide. Simple and non-invasive screening measures such as annual faecal immunochemical test (FIT) for early detection of colon cancer [14], and annual chest -X-ray or low-dose computerised tomography (CT) for early detection of lung cancer in this high-risk group [15] could be considered as a preventative follow-up strategy [16]. It is not known how the more recent national cancer screening programs such as the U.S. Preventive Services Task Force's (USPSTF) lung cancer screening recommendations (first issued in 2013), which targets adults aged 50 to 80 years who are current and recent smokers, would impact organ transplant recipients. Other proposed strategies include (in appropriate patients) immunosuppression minimization strategies that may reduce de novo malignancy formation rates [17] and also reduce risk of sepsis.

Post-LT deaths due to sepsis from bacterial, fungal and viral infections have been well-documented. However, a large proportion of these deaths occur in the immediate transplant period up to one year post-LT [13,18]. In Europe, 78.9% of all the deaths due to sepsis occurred within the first year of post-LT period [7], while it was 80.2% in a US series [13]. However, our study demonstrates that sepsis remains a leading cause of death even after 5 years of LT. Although the underlying reason was not obvious, one plausible reason could be over-immunosuppression. Active tapering of immunosuppressant dose in long-term survivors should be encouraged to reduce the mortality due to sepsis.

Given that, a third of our recipients had hypertension, 28% were obese (BMI ≥30) and 17% had diabetes at LT, it is not surprising that cardiovascular disease was a major cause of death in the current study. The presence of pre-transplant metabolic syndrome seems to have a significant effect on the development of major cardiovascular events than de novo post-LT metabolic syndrome [19]. In the period of the current study, non-alcoholic fatty liver disease (NAFLD) as a cause of chronic liver disease may have been largely under-recognised or wrongly coded. In the US and in Europe, NALFD has been the fastest growing indication for LT in the last 20 years [20,21]. As such, the pre-LT metabolic syndrome in patients with NAFLD could have a significant impact on the long-term survival of these patients. The study period also predates the use of direct-acting antiviral therapy for chronic hepatitis C and the number of patients with hepatitis C requiring LT is likely to decrease with the use of direct-acting antiviral (DAA) therapy.

Multiple donor and recipients factors were independently associated with long-term survival including recipient age [11,18,22,23], donor age and the presence of pre-LT metabolic syndrome. Patient sex, the aetiology of liver disease, donor type, and the choice of long-term immunosuppression did not impact long-term survival in this study. For example, LT recipients who underwent transplantation for HCC and are alive at 5 years, have a similar long-term survival compared those transplanted for other indications.

This study has its own strengths and limitations. The retrospective nature of the study was a major limitation as evidenced by the lack of detailed clinical characteristics in a proportion of recipients in the Birmingham, UK cohort (those transplanted before 2005) due to part of the patient information being only available on paper records and not accessible due to remote archiving, lack of data on smoking status in the Dallas, USA cohort due to not being collected routinely, blood test results at the time of LT in the Barcelona, Spain cohort and the lack of data on post-LT tobacco use in all three centers due to not being collected routinely. These collective missing data should be acknowledged

as a limitation of the quality of data and possibly would have impacted the results. In addition, the data on hepatitis C virus (HCV) post-LT recurrence was not available for all patients who underwent LT for HCV-related indications, and therefore HCV recurrence could not be used as a variable in the analysis to predict long-term outcome. However, the introduction of DAAs, that happened towards the end of the study revolutionised the HCV treatment landscape and thereby making the data on HCV recurrence from pre-DAA era (which includes the study period), virtually futile for predicting long-term outcomes in the era of DAAs. Thus, it is somewhat reassuring to know that the lack of HCV recurrence data of this study would not have impacted the recommendation of this study. Further, statistical comparison to compare centres was deliberately not undertaken due to the inherent differences in the patient populations, selection processes, post-transplant management strategies and the number of patients included from each centre. Due to the retrospective nature of the study, it was not possible to verify the cause of death individually. These have been obtained from a variety of sources such as primary care physicians, secondary and tertiary hospitals and their accuracy remains a limitation.

In conclusion, this study demonstrates an increased mortality rate in LT recipients even after 5 years of transplantation compared to respective general population. The increased mortality was primarily due to *de novo* cancer, sepsis and cardiovascular disease. It is likely that implementation of simple strategies such as non-invasive cancer screening measures as detailed above, minimisation of immunosuppression, intensive primary and secondary cardiovascular prevention such as addressing obesity, optimal control of diabetes and hypertension and programs directed towards smoking cessation could potentially further improve survival of organ transplant recipients. This requires further evaluation in prospective studies. Formal recommendations from national transplant governing bodies and international transplant societies are essential to inform a change of practice towards intense screening.

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6. TABLE LEGEND

Table 1: Demographics and clinical characteristics of all patients at liver transplantation

Table 2A: Summary of causes of death

Table 2B: Age-standardised mortality rates (per 1000 person-years) by centre

Table 3: Association between mortality and demographic, clinical and donor factors†

7. FIGURE LEGEND

Figure 1: Trend of common causes of death 5 years following liver transplantation

Figure illustrates the trend of common causes of death in liver transplant recipients 5 years after the transplantation.