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ORIGINAL ARTICLE

The first one thousand liver transplants in Turin: a single-center experience in Italy

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Summary

The first Italian liver transplant center to reach the goal of 1000 procedures was Turin. The paper reports this single-center experience, highlighting the main changes that have occurred over time. From 1990 to 2002, 1000 consecutive liver transplants were performed in 910 patients, mainly cirrhotics. Surgical technique was based on the preservation of the retrohepatic vena cava of the recipient. The veno-venous bypass was used in 30 cases only and abandoned since 1997. Operating time, warm ischemia time and length of hospital stay significantly decreased over the years, while operating room extubation became routine. Immunosuppression pivoted on cyclosporine A. Management of retransplantations, marginal grafts, and of HCV-positive, HBV-positive and hepatocellular carcinoma recipients were optimized. Median follow-up of the patients was 41 months. Overall survival rates at 1, 5 and 10 years were 87%, 78% and 72% respectively. Survival rates obtained in the second half of the cases (1999–2002 period) were significantly better than those obtained in the first half (1990–1998 period) (90% vs. 83% at 1 year and 81% vs. 76% at 5 years respectively). Increasing experience in liver transplant surgery and post-operative care allowed standardization of the procedure and expansion of the activity, with parallel improvement of the results.

Introduction

Since its first human application by Starzl in 1963, liver transplantation (LT) has become the treatment of choice for chronic and acute end-stage liver failure as well as for selected cases of malignancies and metabolic disorders [1]. This paper reports the experience of the first 1000 consecutive LTs performed between 1990 and 2002 in a single Italian center dealing with a high rate of virus-related cirrhotic patients and fully exploiting the cadaveric donor pool, with particular focus on the changes in peri- and postoperative care that have occurred over time.

Patients and methods

Clinical series

From October 1990 to October 2002, 1000 LTs were carried out in 910 patients at the LT Center of the San Giovanni Battista Hospital in Turin. An informed consent was obtained from all candidates prior to LT. Median age of the recipients was 51 years (range 6 months to 68 years). Among these, 15 were children in a pediatric LT program started in October 1999 (median age 7 years, range 6 months to 14 years), and 71 were elderly (≥60 years). Survival data were collected on January 1, 2004, resulting in a median follow-up of the recipients of 41 months (range 0–155).

Indications

Cirrhosis was the first indication for LT in 742 patients. Among these, 656 had viral hepatitis [hepatitis C virus (HCV) present in 395 patients], 78 had alcoholic cirrhosis, and eight had autoimmune hepatitis. In 198 cases cirrhosis was associated with hepatocellular carcinoma (HCC). In addition, 74 patients were transplanted for cholestatic disease, 30 for acute liver failure, 23 for metabolic disorders, and 41 for other causes. The indications for LT are summarized in Table 1.

Retransplantations

Retransplantation (ReLT) was performed 90 times in 82 patients. ReLT was carried out twice in six patients and three times in one. Two retransplanted patients had undergone a first LT at another institution. Causes of ReLT are listed in Table 2.

Patient status

Patients were classified into three groups according to their clinical condition at the time of LT: 66% needed continuous medical therapy and clinical follow-up, 20% were hospitalized or recurrently hospitalized before transplant, and 14% were bound to an intensive care unit (ICU).

Table 1. Primary indications for LT in the 910 recipients of 1000 consecutive transplants in Turin.

Indication*	Number of patients	%
Viral cirrhosis	656	71
Alcoholic cirrhosis	78	9
Cholestatic diseases	74	8
Fulminant hepatitis	30	3
Metabolic disorders	23	3
Autoimmune cirrhosis	8	1
Other causes	41	5

*HCC present in 198 (22%) recipients.

Table 2. Causes of the 90 retransplantations.

Cause	Number of cases	%
Vascular complications	28	31
Primary graft nonfunction	18	20
Early graft nonfunction	18	20
Biliary complications	12	14
Recurrent disease	8	9
Rejection	4	4
Bleeding	1	1
De novo hepatitis B	1	1

Surgical features

The types of transplanted liver grafts and the graft survival rates at 1 year are shown in Table 3. The living-related liver transplantation (LRLT) program was started in May 2001.

Caval reconstruction was obtained preserving the recipient inferior vena cava [2] in 947 cases (piggy-back technique: $n = 847$; side-to-side cavo-caval anastomosis: $n = 100$), while the classical technique with recipient retrohepatic vena cava removal was used in the other 53 cases. Veno-venous by-pass (VVB) was employed in 30 LTs only (nine piggy-back, 21 classical technique). Since 1997 the VVB has not been used at all.

Graft arterial revascularization was achieved through an end-to-end anastomosis between the recipient and donor hepatic artery in 854 cases (with iliac patch interposition in 10 of those); in the other 146 LTs arterial reconstruction was provided by an infrarenal iliac arterial conduit. Because of donor arterial anomalies, back-table graft arterial anastomosis was necessary in 73 cases.

Biliary tract reconstruction was performed with an end-to-end anastomosis whenever technically feasible (799 cases). T-tube stenting was almost universal in this population (96%). Roux-en-Y hepatico-jejunal anastomosis was carried out in 156 transplants. In the other 45 cases the common bile duct of the graft was cannulated and a temporary external biliary fistula was obtained: 38 of them underwent proper biliary reconstruction 2–6 days after transplant.

Perioperative care

In the first 4-year period all recipients were transferred to the ICU for at least 24 h after surgery. Since 1994 an operating room (OR) extubation program started, because of the increasing number of procedures, limited ICU resources and deeper experience with perioperative management. Such an attitude allowed a direct middle care unit destination after surgery without ICU needed.

Table 3. Types of liver graft implanted in the 1000 transplants and graft survival rates at 1 year.

Graft	Number of cases	Graft survival at 1 year (%)
Whole liver*	950	80
Right split liver	18	74
Left split liver	14	54
Right liver LRLT	10	60
Reduced-size graft	4	25
Domino LT	3	67
Auxiliary LT	1	–

*Combined with kidney transplantation in six cases.

Immunosuppression

Prophylactic immunosuppressive therapy usually consist of a triple induction regimen, including cyclosporine A, azathioprine and steroids. Azathioprine was stopped 12 months after LT, while steroids were progressively tapered and discontinued after 6 months.

Diagnosis of rejection episodes was based on clinical, biochemical and histological findings. Steroid boluses (total dose of 1.5 g up to 3.5 g of methylprednisolone) were usually administered starting immediately after diagnosis. Cortico-resistant rejection was treated by switching from cyclosporine A to tacrolimus. In case of resistance to this switch, a 10-day course of monoclonal anti-CD3 antibodies was given. Other instances of switch from cyclosporine A to tacrolimus were mostly due to specific cyclosporine A toxicity (mainly neurological).

Statistical analysis

Statistical analysis employed the SPSS statistical software program (SPSS Inc., Chicago, IL, USA). Continuous variables were analyzed using Kolmogorov–Smirnov test in order to verify normal distribution data; continuous data not approximated by a Gaussian distribution were described adopting median, and differences were assessed by nonparametric tests (Mann–Whitney, Kruskal–Wallis). Comparisons of normal continuous data were analyzed using mean ± SD and differences were pointed out by *t*-test or ANOVA and Bonferroni test. Chi-square test (Pearson) was used for comparing categorical variables between groups. Survival was estimated according to the Kaplan–Meier method; log-rank and Breslow tests were used to compare groups. Significant level for all tests was set at $P < 0.05$.

Results

Survival

Overall patient survival rates at 1, 5 and 10 years after LT were 87%, 78% and 72%, respectively, while graft survival rates at 1, 5 and 10 years were 79%, 70% and 64%. Survival rates at 1, 5 and 10 years for children (93%, 93% and 93%), adults (87%, 79% and 72%) and elderly (80%, 70% and 63%) were not significantly different. If the clinical experience was split into two time intervals including equally numerous cases, patient survival rates obtained in the 1999–2002 period (503 cases performed) were significantly better than those obtained in the 1990–1998 period (497 cases performed) (90% vs. 83% at 1 year and 81% vs. 76% at 5 years) ($P = 0.044$) (Fig. 1). Graft survival rates had similar increases in the second time interval in comparison with the first (83% vs. 76% at 1 year and 73% vs. 68% at 5 years).

Causes of death

Of the 910 recipients 200 died during the follow-up. The median survival of the deceased patients was 6 months (range 0–154). Peroperative death occurred in three transplanted patients only. Early mortality (122 cases within 1 year) was essentially because of infections (38%) and multi-organ failure (22%), while late mortality (75 cases after 1 year) was mainly due to disease recurrence (36%) and cancer (*de novo* 21%, recurrent 12%).

Technical aspects and complications

Mean (±SD) operating time was 358 ± 123 min. Mean total ischemia time was 569 ± 179 min, with a mean warm ischemia time of 31 ± 14 min. Table 4 shows highly significant improvements that occurred over the years as to operating and warm ischemia times. In particular, since 1997 the mean operating time was steady, <6 h, and since 1998 the mean warm ischemia time remained <27 min.

In 1994, for the first time, two LTs were performed on the same day. From 1997 on, this happened on an average of 15 times per year. In 2001, for the first time, three LTs were performed on the same day, and this happened three times over the 2001–2002 period.

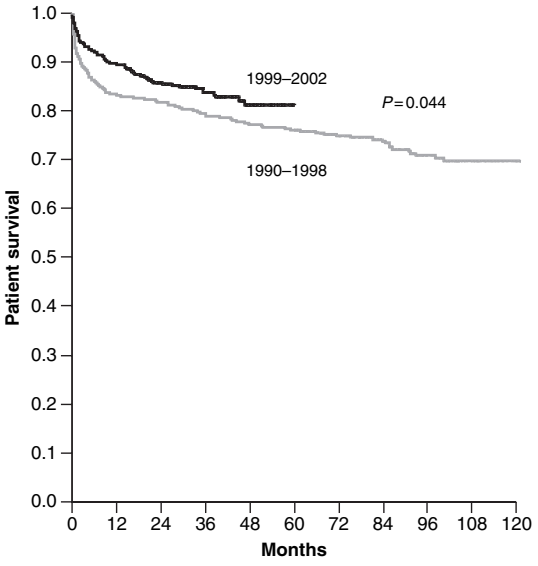

Figure 1 Comparison of patient survival curves obtained by splitting the clinical experience in two time intervals including equally numerous cases (1990–1998, $n = 497$; 1999–2002, $n = 503$).

Table 4. Intra- and postoperative features that showed remarkable improvements over the 12-year period.

Time interval (number of cases)	Operating time* (min) (mean ± SD)	Warm ischemia time* (min) (mean ± SD)	Hospital stay* (days) (median, range)
1990–1994 ($n = 126$)	491 ± 145	51 ± 21	26 (12–99)
1995–1998 ($n = 371$)	361 ± 131	32 ± 11	17 (7–71)
1999–2002 ($n = 503$)	321 ± 97	25 ± 7	12 (5–48)

*$P < 0.001$ between groups.

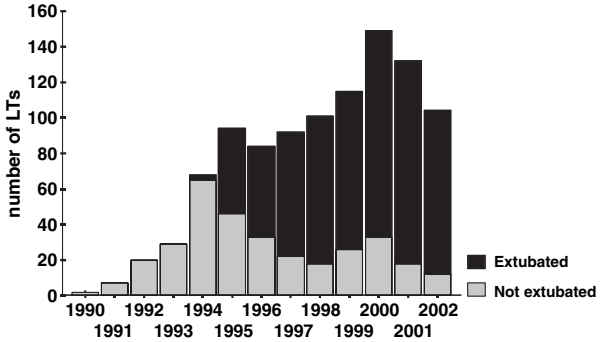

Figure 2 Year-by-year evolution of the operating room extubation rate in the 1000 transplants performed over the 12-year period.

The OR extubation rate increased with time from 4% in 1994 to 88% in 2002 (Fig. 2) and it was always >75% after 1997. In the 1997–2002 period more than half of the patients (55%) were immediately discharged to a middle care unit after surgery, with no ICU needed. The overall reintubation rate within 48 h of the OR extubated patients was only 3% (in 90% of the cases because of urgent surgical reoperation).

Surgical reinterventions after transplantation were performed 739 times in 371 LTs (median 2 reinterventions; range 1–13). The most frequent causes of reoperation were bleeding ($n = 121$, in 85 transplants) and biliary tree complications ($n = 158$).

Table 5 summarizes the type and the management of the biliary complications that required surgical treatment and of the vascular complications in the 1000 LTs. In particular, the hepatic artery complication rate was kept below 5% and the venous complication rate was globally below 2%.

Table 5. Type and management of the biliary tree complications requiring surgery and of the vascular complications in the 1000 transplants.

Complication	Management
Biliary tree ($n = 158$)	
Biliary collection ($n = 81$)	
Anastomotic stricture ($n = 56$)	New biliary anastomosis ($n = 113$)
Sludge ($n = 11$)	ReLT ($n = 12$)
T-tube related ($n = 9$)	Other surgical therapy ($n = 33$)
Kinking ($n = 1$)	
Hepatic artery ($n = 49$)	
Thrombosis ($n = 37$)	ReLT ($n = 22$)
Anastomotic stricture ($n = 5$)	Endothrombectomy + anastomosis redo ($n = 21$)
Splenic artery steal ($n = 4$)	Splenic artery ligature ($n = 4$)
Kinking ($n = 2$)	Radiological stenting ($n = 1$)
Pseudoaneurism ($n = 1$)	Death w/o treatment ($n = 1$)
Caval vein ($n = 23$)	
Stenosis ($n = 13$)	Surgical revision ($n = 15$)
Bleeding ($n = 10$)	ReLT ($n = 3$)
	Radiological management ($n = 3$)
	Death w/o treatment ($n = 2$)
Portal vein ($n = 11$)	
Thrombosis ($n = 9$)	Endothrombectomy + anastomosis redo ($n = 3$)
Anastomotic stricture ($n = 1$)	ReLT ($n = 3$)
Kinking ($n = 1$)	Radiological stenting ($n = 1$)
	Death w/o treatment ($n = 4$)

Hospital stay

Among the patients discharged home after transplant surgery, the median hospital stay gradually and significantly decreased over the years, from 28 days in 1991 (range 20–31) to 12 days in 2002 (range 6–48) (Table 4).

Retransplantation

Survival rates at 1, 5 and 10 years of retransplanted patients were significantly lower than those of single-grafted patients (65%, 56% and 46% vs. 89%, 81% and 75% respectively) ($P < 0.001$). Among the 41 deceased patients after ReLT, the most frequent causes of death were infection ($n = 15$) and multiorgan failure ($n = 7$). Considering early versus late ReLT (cut-off set at 3 months after first LT), no differences in survival rates were found. However, mortality within 3 months of ReLT (26/41) was more frequent in the early ReLT group (14/20) than in the late ReLT group (12/21), but the difference was not statistically significant.

Marginal grafts

The influence of steatosis and other donor and recipient variables on the outcome of LT was evaluated in this series, in order to define 'high-risk' grafts. Donor

variables considered were: age, hepatic enzymes, bilirubin, total and warm ischemia times, macrovesicular and microvesicular steatosis. Recipient variables considered were: age, clinical status and indication for LT. Macrovesicular steatosis affecting 15% or more of the hepatocytes was the only donor variable independently associated with shorter patient and graft survival ($P = 0.001$ and $P = 0.003$ respectively). In this group of grafts ($n = 69$, 7% of the 1000 LTs), a significant worse prognosis was to be expected if total ischemia time >10 h ($P = 0.048$) or donor age >65 years ($P = 0.016$) or HCV recipient positivity ($P = 0.001$) were associated.

Hepatitis C virus

The HCV-related cirrhosis was present in 395 patients (43% of transplanted patients, median age 54 years, range 17–67). According to the adopted immunosuppressive protocol, 85% of HCV-positive recipients received cyclosporine A as main immunosuppressive drug, while only 15% of them received tacrolimus. Survival rates at 1, 5 and 10 years in HCV-positive patients were significantly worse than in HCV-negative recipients (82%, 72% and 64% vs. 90%, 83% and 77%, respectively) ($P < 0.001$). The outcome of HCV-positive transplanted patients had no significant change with time (1990–1998 period: survival at 1 year = 80%, at 5 years = 71%; 1999–2002 period: survival at 1 year = 85%, at 5 years = 73%).

Considering data from 348 patients with a minimum follow-up of 3 months, viral genotypes were distributed as follows: genotype 1 in 226 (65%), genotype 2 in 65 (19%) and genotypes 3 or 4 in 57 (16%) recipients. Biopsy-proven recurrent hepatitis was observed in 272 patients (78%); disease recurred within 24 months in 251 cases (92%) (median 5 months, range 1–24). Recurrent hepatitis was mild in 49 cases (18%), moderate in 145 (53%) and severe in 78 (29%). Development of cirrhosis was evidenced in 51 grafts (15%). While mild hepatitis never progressed to cirrhosis, 10 cases of moderate hepatitis and 41 cases of severe hepatitis evolved to cirrhosis. Among the patients diagnosed with severe hepatitis, 5-year survival was significantly worse in those with cirrhotic evolution (49% vs. 87%) ($P < 0.001$). Since 1996 antiviral therapy (interferon and ribavirin) was started for recurrent hepatitis. Among the 108 patients who had a complete treatment (6 or 12 months) [3], sustained virologic response (12-month post-therapy negative HCV-RNA) was obtained in 20% of genotype 1 and 50% of genotype 2 patients. However, side effects (anemia, leukopenia, iron overload) were still a major drawback that mandated reduction of the therapy in 51% of the cases and suspension in 4%.

HCV-positive donors

From July 1998 to October 2002, 14 HCV-positive patients were transplanted with livers from HCV-positive donors. All HCV-positive grafts were submitted to heart-beating biopsy during procurement to evaluate the Ishak score [4]: the grading for inflammation was >2/18 in three cases, while only one graft presented a staging for fibrosis >2/6. In only two instances different HCV genotypes were matched. In those cases the donor strain took over the recipient strain. In one patient donor genotyping 2a-2c took over recipient genotyping 1b; 9 months after LT recurrent hepatitis was documented, and antiviral therapy (interferon + ribavirin) cleared HCV; sustained virologic response was confirmed 3 years after therapy discontinuation. In the other patient subtype 1b became the predominant strain over donor genotyping 4; mild recurrent hepatitis (Ishak score: grading 4/18, staging 2/6) was documented 12 months after LT. In this group of HCV-positive donors only one ReLT was performed: portal hypertension developed 2 months after LT in the only graft with Ishak score staging >2/6. Cumulative 1-year patient and graft survival rates in HCV-positive recipients did not significantly differ in the HCV-negative donor group from the HCV-positive donor group.

Hepatitis B virus

LT was performed in 276 hepatitis B virus (HBV)-positive patients (30% of transplanted patients, median age 49 years, range 14–66) because of chronic ($n = 249$) or acute ($n = 27$) liver failure. Co-infection by hepatitis D virus was present in 98 patients, by HCV in 30. Until 1994, only patients who were HBV-DNA negative by non-amplified assays were transplanted. After 1994, patients with a spontaneous viral load $\leq 10^4$ copies/ml by polymerase chain reaction (PCR) assay (COBAS Amplicore Roche, Roche Diagnostics, Basel, Switzerland, sensitivity of 200 copies/ml) were considered 'low-risk' patients and, after LT, received only anti-HBs immunoprophylaxis, as patients with negative HBV-DNA. Viremic patients (HBV-DNA $>10^4$ copies/ml) were preemptively treated with antivirals (lamivudine) before LT, while combined prophylaxis with lamivudine and anti-HBs antibodies was continued after LT [5]. Considering data from 246 patients with a mean follow-up of 44 months, hepatitis B recurrence was observed in 16 cases (7%). Recurrence was never observed in patients with spontaneous or antiviral-induced PCR-negative serum HBV-DNA level at the time of LT. Residual risk of HBV recurrence after LT was 8% in patients transplanted with a viral load <100 000 copies/ml and 50% in patients with higher viral loads.

Hepatocellular carcinoma

Hepatocellular carcinoma was present in 198 patients (median age 54 years, range 12–67): it was the LT indication in 181 cases (91%), while it was an incidental finding in the explanted liver in the other 18 (9%). Viral infection was the etiology of the cirrhosis in 185 patients (93%). Cases with infiltrating forms of HCC, with lesions outside the liver, or with macroscopic vascular invasion were systematically excluded from LT. Preference was given to patients with small (<3 cm), mono-oligofocal (<3 nodules), monolobar tumors (80% of the cases). Candidates with more advanced lesions (20% of the cases) were selectively admitted to LT after examination of each individual case by the multidisciplinary team and evaluation of a presumable short time on the waiting list. Recurrent HCC was diagnosed in 10 patients (5%) at a time ranging from 6 to 51 months after LT (median 29 months). Seven patients died of neoplastic spread. The overall survival rates were 70% at 5 years and 66% at 10 years, while the recurrence freedom rate was 90% both at 5 and 10 years.

Discussion

In the 1980s, the clinical introduction of cyclosporine A marked an impressive progress in LT, with a rapid widening of the indications to many acute and chronic liver diseases [1]. Today, LT represents an example of standardized care, with consistently good patient and graft survival rates [6,7]. The review of this largest Italian single-center experience aimed to highlight the major changes in peri- and postoperative care that have occurred in Turin in the 1990s and the beginning of the 2000s, period during which LT had a real boom and the first 1000 consecutive procedures were performed.

Some critical points constituted the basis for the expansion of the LT activity in Turin. The first and most remarkable one was the routine preservation of the inferior vena cava of the recipient. This surgical technique allows avoidance of VVB use (which is responsible for specific complications such as embolism, hypothermia, and vascular and neurological lesions), reduction in blood-product requirements and faster graft revascularization [1,2,8,9]. In this series, the so-called 'piggy-back' was safely performed without VVB in nearly all recipients and permitted short surgical and warm ischemia times, which favored good graft function. In particular, the duration of warm ischemia (period during which the vascular anastomoses are constructed and the graft warms up) has been identified to be the most predictive factor of the quality of initial graft function after LT [10,11]. This may be due to the fact that, as suggested by experimental observations, the amount of damage to the hepatocytes depends on the length of warm ischemia [12] rather than on the length of cold ischemia. Among the 1000 procedures, the rates of technical complications requiring surgical treatment (venous anastomoses <2%; arterial reconstruction <5%; biliary tree anastomosis <16%) were similar to those reported in the literature [13–15].

The second major evolution was the immediate OR extubation after LT. Such strategy has some advantages compared with conventional postoperative mechanical ventilation: avoidance of prolonged sedation, improved comfort of the patient, reduced ICU resources utilization and potentially better graft perfusion [16,17]. With increasing confidence by the team with surgical technique and perioperative management, OR extubation after LT became a routine, being safe and allowing direct middle care unit discharge in most of the cases.

Thirdly, in an effort to overcome the organ shortage, marginal donors were fully evaluated in order to optimize graft allocation and outcome [18]. From this clinical experience, obvious macrovesicular steatosis emerged as a feature identifying marginal livers. The lesson learned was that such grafts can be used, but that the transplantation team should be aware of the major risk when total ischemia time >10 h, or donor age >65 years, or HCV-positive recipient are associated. The combination of these factors should be avoided whenever possible, and especially total ischemia time should be maintained within 6–8 h. If early graft nonfunction develops, early retransplantation must be performed.

In the same struggle against organ scarcity, an experience with HCV-positive grafts transplanted in HCV-positive recipients was carried out. It showed that livers from HCV-positive donors can be safely used in LT for HCV cirrhosis. Hepatic biopsy must always be performed before using such organs, because the outcome of LT seems to be influenced by the histological features of the donor liver. The takeover of one viral strain by another is possible, and it may really change the prognosis of the patient if the predominant strain is more sensitive to antiviral therapy.

Many other issues are still a matter of debate in the field of LT. In this paper, a focus was made on ReLTs, viral liver diseases and HCC.

The rates of ReLT (9%) and of long-term survival after ReLT (46% in 10 years) were similar to those reported by other large experiences [19,20]. These data confirm the effectiveness of such a therapy for patients with graft failure, but underline that ReLT should be used as efficiently as possible. The thesis that heavy immunosuppression contributes to reduce survival after ReLT is supported by the high rate of lethal infections that occurred, as reported by other centers [20,21]. Further

analysis of donor/recipient traits, degree of immunosuppression and use of antimicrobials will be helpful in improving results of ReLT.

The HCV-related cirrhosis is the current most frequent indication for LT [22] (43% in this series). As reported in other papers [23,24], survival after LT for HCV-positive patients was significantly worse than for HCV-negative patients. If a combination therapy of interferon with ribavirin proved to be effective for the treatment of recurrent HCV hepatitis in a proportion of liver grafts [3], the clinical impact of pegylated interferon is currently being evaluated. Regarding immunosuppression, some studies recently documented an inhibition of HCV replication exerted by cyclosporine A in cultured hepatocytes [25,26], and a beneficial effect of a combined cyclosporine-interferon therapy in the treatment of chronic hepatitis C in nontransplanted patients [27]. These reports suggest that the extensive use of cyclosporine A in the present clinical experience could have contributed to prevent the deterioration of the outcome of HCV-positive recipients reported by other authors in recent years [28], despite the general aging of the organ donor population [24].

As for HBV-related cirrhosis, the policy to admit to LT patients with negative or low level (spontaneous or induced) serum HBV-DNA only resulted in an overall HBV recurrence rate after LT below 10% [5]. In fact, in a recent study by our team, the spontaneous or antiviral-induced HBV-DNA viral load at the time of surgery was identified as the key factor capable of classifying the risk of HBV recurrence after LT and indicating the best prophylaxis strategy [29].

In this series, the so-called 'Milan criteria' were mostly adopted to select candidates with HCC for LT [30]; adherence to these criteria kept the neoplastic recurrence rate as low as 5%, with excellent long-term survival rates. The ongoing wave to expand the limits of candidature to LT for HCC [31] finds a counterbalance in the persistent lack of adjuvant therapies capable of effectively preventing recurrence after LT. Data from our team shows that identification of HCC patients at high risk for recurrence after LT can be obtained through the immunohistochemical detection of neoplastic microscopic vascular invasion [32]. These patients should be the target of innovative therapeutic strategies after LT.

In conclusion, LT is today a well-standardized therapy for liver diseases. Unfortunately, organ shortage persists. The use of marginal grafts together with the implementation of advanced transplant programs (such as split liver and living donor) represent the way by which limited donor pool can be expanded. It is the experienced LT centers' responsibility to thoroughly explore these paths and provide reliable data in the next years.

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