ORIGINAL ARTICLE

Steroid-Free, Tacrolimus-Basiliximab Immunosuppression in Pediatric Liver Transplantation: Clinical and Pharmacoeconomic Study in 50 Children

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Corticosteroid-free immunosuppression (IS) may be potentially beneficial for transplanted patients, particularly children. The purpose of this study was to evaluate the efficacy and cost of such strategy in primary pediatric liver transplantation (LT). Fifty pediatric LT recipients were prospectively treated with a steroid-free, tacrolimus-basiliximab–based IS (group TB). A group of 34 children transplanted under a conventional tacrolimus-steroids regimen served as control series (group TS). Groups TB and TS were compared regarding patient and graft survival, rejection incidence, infectious complications, and growth, as well as cost of the transplant procedure. Patient and graft survivals at 3 years were 96% and 94% in group TB, versus 91% and 88% in group TS ($P = 0.380$ and $P = 0.370$, respectively). Rejection-free graft survival at 3 years was 72% in group TB, versus 41% in group TS ($P = 0.007$). Patients in group TB had significantly less viral infections than patients in group TS ($P = 0.045$). Height standard deviation score was significantly enhanced in children from group TB, when compared to group TS. Medical care costs were similar in both groups. Steroid avoidance together with basiliximab immunoprophylaxis was not harmful in terms of allograft acceptance, and even seemed to be beneficial in the long term. ***Liver Transpl 14:469-477, 2008.***

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Corticosteroids have invariably been part of induction and maintenance immunosuppression (IS) since the early days of clinical liver transplantation (LT).[1,2] Despite the potential benefits to be expected from early withdrawal or even avoidance of steroid administration, steroid therapy is still combined with cyclosporine or tacrolimus in the vast majority of adult and pediatric LT recipients worldwide.[3,4] The putative advantages of a steroid-free IS protocol are of particular interest in children, and include the reduction of infectious complications, decrease of arterial blood pressure and blood cholesterol with reduced atherogenesis in the long-term, better glucose tolerance, reduced risk of cataracts, reduced incidence of osteopenia, and improved linear growth.[3-5] Moreover, as suggested in rodent models, the administration of steroids may interfere with the process of hepatic regeneration as well as with the development of immunologic tolerance.[6,7] In 2003, we published a proof-of-concept study evaluating the safety of a steroid-free, tacrolimus-basiliximab–based IS protocol in 20 pediatric LT recipients.[8] The benefits of tacrolimus and basiliximab–based IS at 1-year follow-up have been confirmed in a randomized study.[9] The aim of the present work was to evaluate the long-term (3 years) benefit of this protocol with respect to patient and graft survivals, rejection and infection

Abbreviations: CU, cost unit; HDL, high-density lipoprotein; IL, interleukin; LT, liver transplantation; PTLD, posttransplantation lymphoproliferative disease; SD, standard deviation; SDS, standard deviation score; SG, subgroup.

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TABLE 1. Comparative Demographic and Transplant Data of 84 Pediatric Liver Transplant Recipients Treated with a Tacrolimus-Steroids (Group TS, n = 34) or Tacrolimus-Basiliximab (Group TB, n = 50) Immunoprophylaxis

	Group TS (n = 34)	Group TB (n = 50)	*P* Value
Interval	11/1999 to 5/2001	02/2001 to 6/2003	
Median age, years (range)	2.0 (0.4-14.0)	1.7 (0.4-14.0)	NS
Gender (M/F)	16/18	27/23	NS
Living-related donors/postmortem donors	19/15	26/24	NS
Indications			
1. Biliary atresia	13	26	NS
2. Other cholestatic diseases	7	2	
3. Hepatic malignancies	4	8	
4. Metabolic diseases	4	4	
5. Other	6	10	

Abbreviations: F, female; M, male; NS, not significant.

rates, growth, and metabolic follow-up in a series of 50 pediatric LT recipients, as compared to a control group treated with a steroid-based IS regimen. It was hypothesized that: (1) considering the current knowledge regarding the mechanisms of tolerance induction after allografting, the avoidance of steroids may result in an enhancement of graft acceptance in the long term as well as in a higher proportion of children becoming prope (nearly) tolerant[10] at 3 years post-LT; (2) the avoidance of steroids in children should also be associated with enhanced growth in stature, with a reduced incidence of infectious complications and arterial hypertension, as well as improvement of the lipid profile in the posttransplant period; and (3) considering the putative advantages from a clinical perspective, it was also hypothesized that the total costs in children treated with the steroid-free, basiliximab and tacrolimus–based protocol would be similar as in the steroid-treated group, if not lower.

PATIENTS AND METHODS

Patients and Study Design

Fifty Caucasian pediatric (below 15 years old) recipients of a primary LT (Table 1), receiving transplantation at Saint-Luc University Clinics, Brussels, between February 2001 and March 2003, were prospectively included in the study, and treated with a steroid-free immunoprophylactic protocol combining tacrolimus and basiliximab (group TB). The safety and efficacy of this therapeutic approach were compared retrospectively to a historical control group of 34 primary Caucasian pediatric LT recipients, transplanted in the same institution between November 1999 and May 2001, and treated with tacrolimus and steroids (group TS). Demographic data of these children are summarized in Table 1. All 84 children were managed according to the standard transplant practices at our center.[11-13] Patients in group TB did not receive nutritional supplementation as compared to children in group TS during the whole duration of the study. All had a minimal posttransplantation follow-up of 3 years at the completion of the study and no patient was lost to follow-up. The steroid-free protocol was prospectively accepted by the Ethics Committee of the institution in January 2001, and all parents, and children when appropriate, in group TB gave their informed consent for inclusion in the steroid-free protocol group.

Immunosuppressive Protocols

Immunoprophylaxis consisted of tacrolimus as baseline immunosuppressant, combined to basiliximab induction in the pilot study group or chronic steroid therapy in the control group. Tacrolimus (Prograf, Fujisawa GmbH), a calcineurin inhibitor, was administered orally at an initial dosage of 0.1 mg/kg, and the dosage, administered twice daily, was subsequently titrated according to trough blood levels, aiming at 8-12 ng/mL during the first month posttransplantation and 5-8 ng/mL thereafter. In the steroid-free immunosuppressive protocol, basiliximab (Simulect, Novartis Pharma sa), a high-affinity mouse-human chimeric monoclonal antibody directed to the interleukin (IL)-2R (CD25) on activated T lymphocytes, was administered intravenously in a 2-dose regimen. The first dose was given within 8 hours following graft reperfusion, and the second on day 4 posttransplantation, the dosage depending on patient weight (below 35 kg body weight: 10 mg/dose; above 35 kg body weight: 20 mg/dose).[14,15] In group TS, steroids were administered according to a low-dose scheme, as used in a recent multicenter randomized trial in pediatric LT[16]: intravenous steroids were administered as an intraoperative methylprednisolone bolus (10 mg/kg) and subsequently between day 1 and day 6 post-LT (2 mg/kg/day); corticotherapy was then switched to oral prednisolone, and subsequently tapered gradually (days 7-13: 1 mg/kg/day; days 14-20: 0.75 mg/kg/day; days 21-28: 0.5 mg/kg/day; thereafter: 0.25 mg/kg/day, and switched to alternate day steroid therapy between months 3-6). Acute rejection episodes were diagnosed according to the conventional clinical, biochemical, and histological criteria,[13] and treated with 3 daily intravenous methylprednisolone boluses (group TB: 5 mg/kg/day; group TS: 10

mg/kg/day), followed by a 3-day scheme of recycling doses (group TB: 3.75, 2.5, and 1.25 mg/kg/day; group TS: 7.5, 5, 2.5 mg/kg/day).

Data Collected for the Study

Survival data reviewed included patient, graft, rejection-free, and steroid-resistant rejection-free survivals. Tacrolimus trough levels were recorded and compared at 7, 14, 21, 28 days posttransplantation as well as at 3, 6, 12, 24 and 36 months posttransplantation. Metabolic data collected included fasting glycemia, total plasmatic cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein-cholesterol, as well as triglycerides levels, at 6 months, 1 year, 2 years, and 3 years posttransplantation. The number of antihypertensive medications was recorded at the same time points. The total number of bacterial, viral, and fungal infections were reviewed and compared between groups. Sepsis was defined as a temperature >38°C, with a positive hemoculture or a positive culture of a catheter. Posttransplantation lymphoproliferative disease (PTLD) incidence and locations were studied, allowing the computation of a PTLD-free survival. Every episode of PTLD was included in the survival curve, even PTLD limited to the tonsils and spontaneously disappearing with IS minimization or withdrawal. We used the PTLD classification published by Schroff and Rees[17] in 2004.

Regarding growth assessment, height measurements were routinely performed and recorded at the time of LT (day 0), at days 90, 180, 365, 730, and 1080 post-LT. For statistical evaluation, the height data were expressed as standard deviation (SD) scores or z-scores, which were calculated according to the equation:

$$z\text{-}score = (X - mean\ height\ for\ normal)/SD\ for\ normal \quad (1)$$

where X is the actual measured height in centimeters, and SD is the standard deviation of the normal population for the same chronological age and gender. The z-scores indicate the patients' values in terms of the number of SDs above (positive value) or below (negative value) the mean (50th percentile) for the normal population.

Pharmacoeconomical Study

The total medical costs in children included in both study groups were analyzed during the first 3 months posttransplantation, as most of the complications and costs were taking place during this interval. The cost database came from the invoices recorded at Saint-Luc University Clinics financial department, relying on hospital receipts data. In order to eliminate the difference of period between both groups, hospitalization days prices and medical acts prices were valued at the Belgian Social Insurance prices of January 2003 and pharmaceutical products prices were indexed at January 2003. Consultations costs for 1999 were not available and these were thus estimated by the average cost of a consultation in January 2003 multiplied by the average number of consultations in 1999. One patient in group TB was excluded because of lack of available invoice. To preserve confidentiality, the financial data were expressed as an arbitrary cost unit (CU), its value being fixed between 500 and 1500 Euros. The same CU was used in both groups in order to allow comparison of pharmacoeconomic data.

Statistical Methods

Statistical analyses were performed using Statistical Packages for Social Sciences (software version 13.0; SPSS, Chicago, IL). Patient, graft, and rejection-free survival curves were estimated using the Kaplan-Meier product limit method, and compared using the log-rank sum test. Numerical variables were expressed as median and range, and compared between groups using *t* test for independent groups. Groups were compared using chi-square test for discrete variables. Regarding growth, day 0 values of z-score were compared between groups TB and TS using *t* test for unpaired variables; z-score values on days 90, 180, 365, 730, and 1080 post-LT were compared within the groups to day 0 values, using *t* test for paired variables). Differences with a *P* value of less than or equal to 0.05 were considered statistically significant.

RESULTS

Overall Results and Immunosuppressive Therapy

Two doses of basiliximab were given in all children of group TB, and no adverse event could be related to this administration. Three-year patient survival was 96% in group TB as compared to 91% in group TS ($P = 0.370$). The causes of death in group TB were hemorrhagic shock (first day post-LT) and multiple organ failure (day 5 post-LT) in 1 case each. The causes of death in group TS were ileal necrosis (day 2 post-LT), cerebral aspergillosis (day 83 post-LT), and tumor recurrence (day 159 post-LT) in 1 child each. Three-year graft survival was 94% in group TB and 88% in group TS ($P = 0.380$). The indication of the only case of retransplantation in group TB was hepatic artery thrombosis, whereas 2 grafts were lost due to patient death. Three grafts lost in group TS were due to patient death, whereas 1 child presented graft primary nonfunction and therefore received retransplantation. Three-year rejection-free survival was 72% in group TB and 41% in group TS ($P = 0.007$) (Fig. 1A). With 50 patients in group TB and 34 patients in group TS, we had a power of 89% with an alpha of 5% to detect a 31% difference in rejection-free survival. No child developed recurrent acute rejection in group TB, whereas recurring acute rejection, as defined as a second episode of clinically significant and histologically-proven rejection, was encountered in 3 cases in group TS requiring additional steroid administration and increase of tacrolimus levels. The difference between 3-year steroid-resistant rejection-free survivals in both

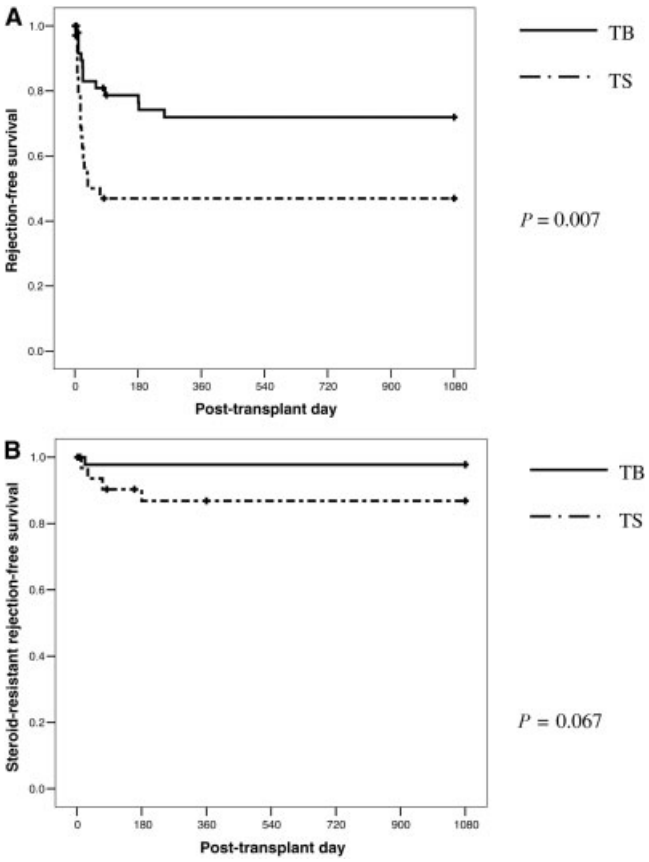

Figure 1. Three years rejection-free (A) and steroid-resistant rejection-free (B) survival curves of 84 pediatric liver transplant recipients, treated with a tacrolimus-steroids (group TS, n = 34, dotted line) or tacrolimus-basiliximab (group TB, n = 50, continuous line) immunoprophylaxis.

groups did not reach the statistical significance (Group TB: 98%, Group TS: 87%; $P = 0.067$) (Fig. 1B). Median interval between LT and the first rejection episode were not significantly different in both groups, 23 days in group TB versus 16 days in group TS ($P = 0.172$).

Tacrolimus Trough Levels

No significant differences were observed regarding mean tacrolimus trough levels between groups during the first year posttransplantation. Mean (±SD) tacrolimus trough levels (ng/mL) in group TB and TS were 9.9 ± 3.1 versus 11.8 ± 5.2 at day 7 posttransplantation ($P = 0.222$), 10.2 ± 4.1 versus 11.3 ± 4 at day 14 ($P = 0.201$), 9.5 ± 2.9 versus 9.8 ± 3.7 at day 21 ($P = 0.570$), 9.0 ± 2.6 versus 8.8 ± 5.1 at day 28 ($P = 0.159$), 6.9 ± 2.8 versus 7.7 ± 2.1 at month 3 ($P = 0.185$), 6 ± 2.4 versus 6.3 ± 2.5 at 6 months ($P = 0.797$), and 5.9 ± 3.8 versus 6.3 ± 2.3 at 1 year ($P = 0.179$), respectively. Thereafter, patients in group TB had significantly lower mean (±SD) tacrolimus trough levels: 4.0 ± 2.0 versus 5.9 ± 2.2 at 2 years ($P = 0.002$) and 3.6 ± 2.0 versus 4.8 ± 1.5 at 3 years ($P = 0.027$). Eleven children in group TB had reached a state of *prope* tolerance (alive with their first graft, without any ongoing rejection episode, and under tacrolimus monotherapy with trough levels below 3 ng/mL) at 3 years, versus no children in group TS ($P = 0.002$).

Metabolic Assessment and Antihypertensive Medications

No significant differences were observed between groups regarding the lipidic profile of these patients, except lower HDL-cholesterol in patients of group TB at 6 months and 1 year posttransplantation (Table 2). No significant differences could also be observed regarding fasting glycemia (data not shown). Patients in group TB needed significantly less antihypertensive medications than children in group TS at 6 months, and 1 and 2 years posttransplantation: at 6 months, the mean (±SD) number of antihypertensive medications per patient was 0.21 ± 0.52 in group TB versus 0.69 ± 0.47 in group TS ($P < 0.001$), the corresponding figures being 0.07 ± 0.26 versus 0.48 ± 0.5 at 1 year ($P = 0.001$), and 0.05 ± 0.22 versus 0.28 ± 0.46 at 2 years ($P = 0.012$). No patient in both groups was treated for arterial hypertension at 3 years posttransplantation.

Infectious Complications and Posttransplantation Lymphoproliferative Disorders

The mean number of infectious episodes (bacterial, viral, and fungal) per patient was reviewed and compared between groups. A mean of 1.1 bacterial infections per patient was encountered in group TB, as compared to 1.3 bacterial infection per patient in group TS ($P = 0.711$). Seven sepsis episodes were encountered in patients of group TB, versus 12 in group TS ($P = 0.033$). A mean of 0.2 fungal infections per patient was documented in group TB, versus 0.2 in group TS ($P = 0.862$). One of the fungal infections in group TS was a severe cerebral aspergillosis who finally resulted in patient death. Although no statistical difference was found between groups in terms of proportions of bacterial and fungal infections, the number of viral infections was lower in group TB, with 0.5 viral infections per patient as compared with 0.7 in group TS ($P = 0.045$).

A PTLD related to Epstein-Barr virus primary infection was diagnosed in 8 children in group TB and 6 in group TS. All of these PTLDs were of type 1, and this condition could be controlled by reducing or temporarily interrupting tacrolimus administration in these children. One patient (group TB) had his IS withdrawn on day 39 post-LT, and was still therapy-free at the last follow-up, with liver function tests and histology within normal ranges at 5-years follow-up.

Growth Follow-Up

Height standard deviation score (SDS) in children from group TB showed a growth catch-up already at 3 months posttransplantation ($P = 0.012$, as compared to height SDS at the day of the transplantation), as well as at 6 months ($P < 0.001$), 1 year ($P < 0.001$), 2

TABLE 2. Comparative Lipidic Profile of 84 Pediatric Liver Transplant Recipients Treated with a Tacrolimus-Steroids (Group TS, n = 34) or Tacrolimus-Basiliximab (Group TB, n = 50) Immunoprophylaxis

	Group TS (n = 34)	Group TB (n = 50)	*P* Value
Total cholesterol (mg/dL)			
At 6 months	119 ± 28	119 ± 26	0.225
At 1 year	123 ± 22	117 ± 26	0.257
At 2 years	139 ± 49	123 ± 27	0.269
At 3 years	123 ± 24	129 ± 25	0.602
LDL-cholesterol (mg/dL)			
At 6 months	59 ± 23	59 ± 24	0.861
At 1 year	58 ± 16	54 ± 21	0.257
At 2 years	73 ± 44	61 ± 20	0.661
At 3 years	60 ± 18	70 ± 18	0.182
HDL-cholesterol (mg/dL)			
At 6 months	51 ± 12	43 ± 13	0.014
At 1 year	52 ± 14	43 ± 12	0.003
At 2 years	52 ± 11	47 ± 12	0.131
At 3 years	49 ± 13	45 ± 12	0.258
Triglycerides (mg/dL)			
At 6 months	114 ± 71	87 ± 37	0.137
At 1 year	79 ± 44	101 ± 50	0.065
At 2 years	67 ± 27	75 ± 44	0.992
At 3 years	75 ± 56	71 ± 37	0.774

NOTE: Total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides are expressed as means ± SD.

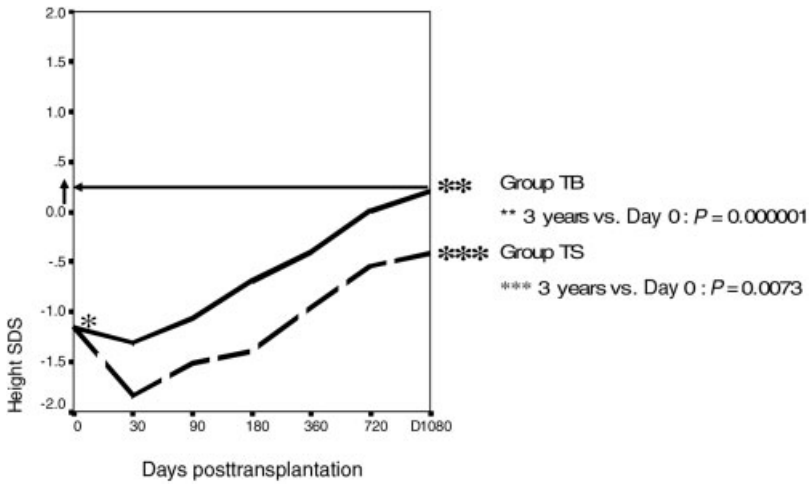

Figure 2. Linear growth of 84 pediatric liver transplant recipients, treated with a tacrolimus-steroids (group TS, n = 34, dotted line) or tacrolimus-basiliximab (group TB, n = 50, continuous line) immunoprophylaxis. *NS between groups at day 0.

years ($P < 0.001$), and 3 years posttransplantation ($P < 0.001$). At 3 years, patients in group TB had a mean height SDS superior to 0 meaning that their height reached the average of children of the same age and gender not receiving transplantation (Fig. 2; Table 3). In contrast, patients in group TS had only significant growth improvement at 2 years ($P = 0.032$) and 3 years posttransplantation ($P = 0.007$). At 3 years posttransplantation, mean height SDS of patients in group TS was −0.42, below the average of children of the same age and gender not receiving transplantation (Fig. 2; Table 3).

Influence of Steroids as a Treatment for Rejection

To better assess the role of steroids administration and its relation to side effects, we have performed a subgroup (SG) analysis on basis on the IS protocol and the treatment for rejection. This generated 4 SGs: patients under prophylactic steroid administration (group TS) who did not developed any rejection episode (SG1: n = 16); patients under prophylactic steroid administration (group TS) who underwent an acute rejection episode and were subsequently treated by intravenous steroid

TABLE 3. Linear Growth of 84 Pediatric Liver Transplant Recipients Treated with a Tacrolimus-Steroids (Group TS, n = 34) or Tacrolimus-Basiliximab (Group TB, n = 50) Immunoprophylaxis

	Group TS (n = 34)	*P* Value (Compared to Day 0)	Group TB (n = 50)	*P* Value (Compared to Day 0)
Day 0	−1.15 ± 2.22		−1.16 ± 1.67	
At 30 days posttransplantation	−1.84 ± 2.40	0.137	−1.31 ± 1.77	0.65
At 90 days posttransplantation	−1.52 ± 1.8	0.336	−1.07 ± 1.64	0.001
At 6 months posttransplantation	−1.39 ± 1.62	0.815	−0.69 ± 1.55	<0.001
At 1 year posttransplantation	−0.95 ± 1.65	0.201	−0.40 ± 1.65	<0.001
At 2 years posttransplantation	−0.54 ± 1.72	0.032	0.01 ± 1.61	<0.001
At 3 years posttransplantation	−0.42 ± 1.84	0.007	0.205 ± 1.29	<0.001

NOTE: Results are expressed as mean height z-score ± SD. *P* values were obtained with the *t* test for paired data.

TABLE 4. Comparative Pharmacoeconomic Data of 84 Pediatric Liver Transplant Recipients Treated with a Tacrolimus-Steroids (Group TS, n = 34) or Tacrolimus-Basiliximab (Group TB, n = 50) Immunoprophylaxis

	Group TS	Group TB	*P* Value
Total duration of hospital stays within the first 3 months (days)	37 (2-90)	29 (2-71)	0.199
Duration of first hospital stay (days)	23 (2-90)	25 (2-69)	0.919
Duration of intensive care stay (days)	4 (0-78)	4 (0-40)	0.996
Duration of subsequent stays (days)	17 (1-49)	5 (1-42)	0.042
Number of outpatient visits	8 (0-14)	10 (0-19)	0.011

NOTE: Data are expressed as means ± SD. Numbers in parentheses are ranges.

boluses (SG2: n = 18); patients treated with the steroid-free, tacrolimus-basiliximab based IS protocol (group TB) who did not present a rejection episode and thus remained totally steroid-free during the 3-year study interval (SG3: n = 37); and finally patients within group TB who developed a rejection episode and were treated with intravenous steroid boluses and subsequently with oral steroids (SG4: n = 13). Three-year patient survival was not significantly different between SGs, with 81.3% for SG1, 100% for SG2, 94,6% for SG3, and 92.3% for SG4 ($P = 0.188$). however, the lowest graft survival was encountered in SG1 (patients from group TS without a rejection episode), with a graft survival of 75%, as compared to 100% in SG2, 92% in SG3, and 100% in SG4 ($P = 0.037$). Interestingly, the lowest PTLD-free survival was encountered in SG2 (patients from group TS with rejection episode and administration of steroid boluses), with a PTLD-free rate of 78%, as compared to 87% in SG1, 82% in SG3, and 83% in SG4, but it did not reach statistical significance ($P = 0.987$). Regarding growth analysis, only patients in SG3 (patients from group TB without rejection, thus totally steroid-free) showed a significant height SDS increase at day 90 ($P = 0.002$), day 180 ($P < 0.001$), 1 year ($P = 0.001$), 2 years ($P = 0.001$), and 3 years posttransplantation ($P < 0.001$). Concerning the metabolic follow-up, no significant differences could be found between SGs except that patient in SG3 had the lower levels of HDL-cholesterol at 6 months and 1 year posttransplantation (data not shown). Mean tacrolimus trough levels were not statistically significant between SGs (data not shown).

Pharmacoeconomic Study

Hospitalization and Outpatient Visits Analysis

The number of days spent in the intensive care unit during the first hospital stay was not significantly different between both groups. Hospital stays were slightly longer for group TS than for group TB, this difference resulting from longer duration of subsequent hospitalizations (Table 4). Conversely, children in group TB came more frequently to the outpatient transplant clinic than those in group TS (Table 4).

Public Health Costs Analysis

Concerning the duration of hospital stays, median total cost was slightly lower for the children of group TB. For this group, pharmaceutical products costs tended to be a little higher, whereas laboratory tests costs were similar and daily hospitalization costs as well as medical imaging costs were slightly lower but these differences were not statistically significant (data not shown). With regard to the consultations, pharmaceutical products costs, medical imaging costs, or laboratory tests costs were a little higher for the group under basiliximab, without reaching statistical significance (data not shown). Overall, it should be mentioned that the cost of basiliximab did not increase the total median cost in

group TB, as the total median (range) cost per patient for the first 3 months post-LT in group TB was 32.0 CU (16.8-55.9 CU), as compared to 32.9 CU (7.7-81.4 CU) in group TS ($P = 0.790$).

DISCUSSION

Although corticosteroids have been invariably part of virtually all IS regimen since the early days of solid organ transplantation, steroid avoidance could be particularly helpful in pediatric recipients. From a theoretical viewpoint, steroid-free IS could reduce the incidence of complications such as infections, PTLD, de novo diabetes mellitus, hyperlipidemia, arterial hypertension, and improved growth in stature. Secondary steroid withdrawal and steroid-free IS have been shown as valid alternatives to long-term steroid therapy in adult and pediatric LT.[3,5,18,19] In the present series, the option of combining tacrolimus with anti-IL-2R blockade for steroid substitution was based on the 2 following arguments: (1) a recent European multicenter randomized study in a total of 181 pediatric LT recipients has shown that tacrolimus provides a lower incidence of acute rejection when compared to cyclosporine microemulsion formulation, both study groups being treated with steroids in this work;[16] and (2) experimental data suggest a key role for the IL-2/IL-2R pathway blockade in various models of transplantation tolerance in animals.[20-23] This study included 50 patients transplanted under steroid-free IS, which is, to the best of our knowledge, the largest monocentric pediatric LT series to be treated with a steroid-free protocol, including a 3-year follow-up and the in-depth study of metabolic data. The pharmacoeconomic impact of the steroid-free, tacrolimus and basiliximab IS-based protocol was also analyzed and compared to a control group treated with tacrolimus and steroids.

Patient and graft survival data were very satisfactory in both groups, with, however, 2 children in the control, steroid-treated group who died from complications potentially related to over-IS, 1 from tumor recurrence and 1 from complications secondary to cerebral aspergillosis.

Graft acceptance without steroid therapy was already studied in a previously published pilot study at our center, which did not suggest any deleterious effect of steroid avoidance.[8] This observation could be confirmed and extended in this study, with a 3-year acute rejection-free survival significantly superior in group TB. It was also shown that administration of basiliximab did not significantly delay the time to first rejection episode, as previously suspected. The reduced rejection rate under tacrolimus and basiliximab therapy may also suggest a putative beneficial impact of avoiding steroid-related nonspecific IS, in addition to the simultaneous blockade the IL-2/IL-2R pathway, both effects contributing possibly to enhanced graft acceptance.[21,22] Although not significant, there was also a trend toward lower steroid-resistant acute rejection-free survival in children treated with tacrolimus and basiliximab. No chronic rejection was observed in this presented series. It should be mentioned that the better rejection-free survival observed in our study group could not be related to higher tacrolimus trough levels as, in our work, the study of mean tacrolimus trough levels at various time points showed similar, and even lower tacrolimus levels in group TB. Interestingly, IS could be completely withdrawn in 1 child of group TB after an early PTLD. Data regarding this patient support that the association of tacrolimus with basiliximab could shift the immune response toward clinical tolerance in the context of Epstein-Barr virus infection. Analysis of peripheral cytokines in this child revealed a dramatic post-LT decrease of T1 helper cytokines interferon-γ and tumor necrosis factor-α, therefore suggesting the role of T1 helper cytokine down-regulation in graft acceptance. Four years after his transplant this patient is doing fine and is completely off IS.[24]

Regarding the metabolic follow-up, the steroid-free protocol could not be associated with any beneficial effects in terms of blood cholesterol and triglycerides. The signification of lower HDL-cholesterol in children of group TB, at 6 months and 1 year posttransplantation, remains uncertain. The incidence of arterial hypertension seemed to be lowered at 6 months, 1 year, and 2 years in group TB. Two children in group TB were transplanted for cystic fibrosis, a condition carrying the risk of developing of insulin-dependent diabetes mellitus in steroid-treated patients[25]: these 2 patients showed normal glucose metabolism and remained insulin-free during their 3-year posttransplantation follow-up. The present study did not show any statistically significant advantage in group TB regarding overall bacterial and fungal infections. However, there were also significantly less sepsis episodes and less viral infections in group TB, the latter mainly because no cytomegalovirus hepatitis were diagnosed in group TB as compared to 5 in group TS.

The data presented in Fig. 2 support the initial hypothesis that steroid avoidance in children, by eliminating steroid-related growth impairment, may allow catch-up growth to start within the first weeks following transplantation, whereas no significant growth was recorded in group TS in the early posttransplantation phase. It should be mentioned that from experimental and clinical experience, patients who have been previously exposed to steroids who present clinical acute rejection need a higher dose of steroids to fully respond to that treatment, and for that reason children in group TS with acute rejection received a reinforced steroid treatment as compared to patients in group TB who presented acute rejection episodes. This could be a potential confounder to the overall effect regarding growth improvement in children in group TB. However, it seems unlikely that differences in acute rejection treatment are responsible for the difference in growth between groups during the first month posttransplantation, especially since growth analysis in SGs of patients did not reveal any statistically significant differences during that period. In children with steroids, growth catch-up was shown to be delayed until alternate-day steroid therapy, and, beyond, after steroid withdraw-

al.[3,4] Moreover, the presented data show that at 3 years posttransplantation, only the children in the steroid-free group have a height SDS superior to 0, meaning that these children have the same height as the normal population of equivalent age and gender. Children undergoing transplantation and treatment with steroids, despite significant growth catch-up, remained with a mean height SDS below 0 at 3 years follow-up. The impact of steroid-free IS on subsequent growth during adolescence would be particularly interesting to study in children of group TB.

The incidence of PTLD was an important endpoint of this study. A first observation was the significant number of PTLD in all children analyzed, an apparently higher incidence when compared to the various incidences reported in the literature by other centers performing pediatric LT. This finding may be explained by 2 factors: (1) as the PTLD is clearly a time-dependent variable, it was decided to express the results as a PTLD-free survival; the figures obtained at 3 years in terms of PTLD-free survival are higher than if expressed as an incidence, because some patients are censored for this criteria (due to death or retransplantation), thus limiting the number of patients at risk; and (2) in this study, we reported every single case of PTLD, even of type 1, consisting in mononucleosis type symptomatology or tonsillitis, that resolves spontaneously following IS reduction. Most of the PTLD cases encountered in both groups were of type 1, and no patient died or was retransplanted as a consequence of PTLD at 3 years follow-up. It is important to note that induction with basiliximab did not increase the number of PTLDs as compared to the control group.

An increasing proportion of pediatric LT patients are now transplanted for hepatic malignancy, mainly hepatoblastoma, unresectable by partial hepatectomy, within protocols combining pretransplant chemotherapy, total hepatectomy with LT, and posttransplantation chemotherapy with low dose IS. The putative benefit of avoiding steroids post-LT in such strategies could not be addressed in this limited series, except that one child died due to tumor recurrence after receiving transplantation under steroid and tacrolimus IS. The question regarding the ideal so-called "low" IS scheme, aiming to preserve antitumor immunity and to reduce the incidence of tumor recurrence, will need specific evaluation in the context of multicenter studies.

Finally, data regarding the pharmacoeconomic impact of both therapy regimens showed a similar profile in the patients treated with tacrolimus and basiliximab as compared to the classical tacrolimus and steroids protocol.

CONCLUSION

In conclusion, the data presented in this retrospective study support that steroid-free, tacrolimus-basiliximab–based IS following pediatric LT was safe and beneficial at 3 years follow-up in terms of graft acceptance, growth, arterial hypertension, and viral infections as compared to children undergoing transplantation under a classical protocol containing steroids, with similar total cost in the first 3 months posttransplantation. A future randomized, double-blind, placebo-controlled trial analyzing the long-term outcomes could definitely establish steroid-free, tacrolimus and basiliximab IS as a fully validated IS protocol following pediatric LT.

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