Tacrolimus Monotherapy in Liver Transplantation

One-Year Results of a Prospective, Randomized, Double-Blind, Placebo-Controlled Study

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Background: Minimal immunosuppression (IS) is desirable in organ transplantation to reduce side effects and to promote the process of tolerance induction.

Material and Methods: Between February 2000 and September 2004, 156 adults (>15 years old) receiving a primary liver graft were enrolled in a prospective, randomized, double-blind, placebo-controlled, investigator-driven single-center study comparing tacrolimus (TAC)-placebo (PL) and TAC-low-dose, short-term (64 days) steroid (ST) IS. There were no exclusion criteria at moment of randomization. All patients had a 12-month follow-up (range, 12–84).

Results: Three- and 12-month patient survival rates were 93.6% and 87.2% in the TAC-PL group and 98.7% and 94.7% in TAC-ST group ($P = 0.096$ and $P = 0.093$, respectively). Three- and 12-month graft survival rates were 92.3% and 85.9% versus 97.4% and 92.3% ($P = 0.14$ and 0.13, respectively). By 3 and 12 months, rejection treatment had been given in 20.5% (16 pts) and 23% (18 pts) of TAC-PL patients and in 12.7% (10 pts) and 20.5% (16 pts) of TAC-ST patients ($P = 0.20$ and 0.54). Corticosteroid-resistant rejection (CRR) at 3 and 12 months was recorded in 12.8% (10 pts) of TAC-PL patients and 3.8% (3 pts) of TAC-ST patients ($P = 0.04$). When considering the 145 patients transplanted without artificial organ support (n = 145), CRR at 3 and 12 months was recorded in 8.8% (6/68 pts) of TAC-PL patients and in 3.9% (3/77 pts) of TAC-ST patients ($P = 0.22$). Vanishing bile duct syndrome was diagnosed in 1 (1.2%) TAC-PL patient and 4 (5.1%) TAC-ST patients ($P = 0.17$). By 1 year, 78.2% (61/78) of TAC-PL patients and 82% (64/78) of TAC-ST patients were on TAC monotherapy ($P = 0.54$). When considering 67 TAC-PL and 74 TAC-ST survivors, rates of monotherapy were 91% (61 pts) and 86.5% (64 pts) ($P = 0.39$). At 1 year, 62.5% (42 pts) of TAC-PL survivors and 64.9% (48 pts) of TAC-ST survivors were on low-dosage (<6 ng/mL) TAC monotherapy (P 0.79).

Conclusion: TAC monotherapy can be achieved safely without compromising graft nor patient survival in a primary, even unselected, adult liver transplant population. The higher incidence of early CRR in the TAC-PL group related to the significantly higher number of patients transplanted while being on artificial organ support. In such condition, this monodrug immunosuppressive strategy needs to be adapted. TAC monotherapy strategy should lay the basis for further large scale minimization studies in liver transplantation.

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The excellent results currently being achieved in liver transplantation (LT) mean that the medical community must look to long-term outcome with high quality of life for the recipient. This can only be achieved by designing immunosuppressive protocols using combinations of different drugs that reduce their respective toxicities, or protocols aimed at minimization, or withdrawal of immunosuppression (IS). Despite the fact that steroids interfere heavily with the quality of life of the recipient and that they may abrogate the active process of graft tolerance induction,[1–4] they are still considered to be the classic "mainstay" of both induction and maintenance IS in transplantation.[5–8] Both arguments are in favor of reducing the use of steroids and of minimization of IS protocols. This report examines the outcome of cal-

cineurin-inhibiting tacrolimus (TAC) monotherapy and steroid almost avoidance in adult LT.

MATERIALS AND METHODS

During the period February 2000 to September 2004, 156 adults (>15 years old) underwent primary LT. There were 97 men and 59 women, with a median age of 53.5 years (range, 21–70). To match the reality of current liver transplant practice, it was our intention to include all listed transplant patients, irrespective of their physical and immunologic condition. All consecutive patients undergoing primary LT during the study period, apart from 11, were enrolled in the study. Eight patients were not enrolled because of their primary, unfavorable oncological diagnosis, and 3 patients were included in the European FK778 study.

The patients were randomized, 1:1 into our previously used IS scheme consisting of TAC-low dose and short-term steroids (TAC-ST; n = 78) or into TAC-placebo (TAC-PL; n = 78). The randomization was done at the end of surgery using serially numbered, sealed, and opaque envelopes. Technical variants were not taken into account when randomizing the patients because in our center, results of elective living LT and split LT are similar to those obtained after whole LT. The need for artificial organ support also was not considered for randomization, as the placebo group would have a lower degree of IS anyway. The randomization was also independent of the presence of positive lymphocytotoxic cross-match and of viral HBV or HCV infection. The characteristics of the study population are summarized in Table 1. The groups differed significantly in relation to total ischemia time, frequency of living donor LT, need for artificial (renal, hepatic, and pulmonary) organ support, and mean creatinine values during the first 14 post-LT days.

In all patients, a vena cava preservation technique was applied without use of veno-venous bypass. The graft implantation was done using a large latero-lateral cavo-caval anastomosis.[9]

All patients had TAC-based IS with levels adjusted according to the clinical situation. The first TAC dose was given 12 hours after the end of surgery. The TAC dose was progressively increased to reach trough whole-blood levels (determined by monoclonal fluorescence assay) around 6 to 8 ng/mL. Low-dosage TAC level was defined as a level below the generally accepted level of 6 ng/mL. Steroids and placebos were administered in identical plastic containers containing a similar number of identical, opaque capsules. Their number, corresponding to a reducing dose that covered a post-LT period of 64 days, was prepared by an independent pharmacist. Intravenous hydrocortisone (400 mg) (Solucortef; Upjohn Pharmacia) was administered perioperatively, followed by 200 mg per day during the first 3 days. Methylprednisolone (MP) treatment (Medrol, Upjohn Pharmacia) was started at day 4 at a dose of 16 mg. Steroids or placebos were tapered from day 21 onwards. Every 14 days, steroids or placebos were reduced by 4 mg to be stopped in all patients at day 64, independently of any previously occurring immunologic event. The total equivalent dose of MP amounted up to 834 mg. Corticosteroid-sensitive rejection (CSR) was treated with 3 to 5 oral or IV boluses of 200-mg MP. Corticosteroid-resistant rejection (CRR), defined as graft loss due to hemorrhagic necrosis or nonresponse to 1-g MP, was treated with a 10-day IV course of muromonab Orthoclone OKT3 (Cilag-Jansen, NJ).

All patients received identical intraoperative and postoperative care. Systemic antimicrobials were administered

TABLE 1. Characteristics of 156 Primary, Isolated, Adult Liver Transplant Recipients

	TAC-Placebo (n = 78)	TAC-Steroid (n = 78)	*P*
Age (yrs)	49.0 ± 12.7	52.1 ± 13	0.14
Gender M/F	48/30	50/28	0.74
Diagnosis			
Parenchymatous	48	53	0.40
HCV cirrhosis	14	21	0.18
Cholestatic	8	10	0.62
Vascular	0	3	0.08
Metabolic	7	2	0.08
Tumor (benign/EHE)	5	4	0.73
HCCa	18	19	0.85
Fulminant failure	13	9	0.35
CMV donor+/recipient−	13	18	0.19
UNOS 1/2	11/10	9/13	0.92
UNOS 3/4	54/3	53/3	0.86
Child-Pugh A/B/C	16/21/21	24/18/20	0.32
MELD score	18 ± 11	17 ± 9	0.74
Technical variant	11	20	0.07
Whole graft domino	2	2	
Right liver living LT	0	9	0.01
Right lobe split liver	7	8	0.78
Reduced size liver	1	1	1
Reused	1	0	0.31
Splanchnic vein abnormality	12	8	0.33
Previous chemotherapy	10	12	0.64
Previous malignancy	3	0	0.64
Prev. upper abdominal surgery	7	6	0.77
Insulinodependent diabetes	10	10	1
Artificial organ support	10	1	0.004
Pulmonary	9	1	0.004
Renal	9	1	0.004
Liver	2	1	0.56
Cross-match positivity	8	6	0.58
HLA mismatch	4.65	4.43	0.22
Ischemia time (min)			
Total	682 ± 204	603 ± 231	0.03
Warm	41 ± 17	55 ± 63	0.08
Mean eosinophilia			
Day 1–7	361 ± 298	330 ± 277	0.07
Mean creatinine (mg/dL)			
Day 1–7	1.40 ± 0.81	1.23 ± 0.47	0.09
Day 1–14	1.53 ± 0.98	1.26 ± 0.48	0.01
Mean tacrolimus level (ng/mL)			
Day 1–7	5.82 ± 1.55	6.16 ± 2.06	0.53
Day 1–14	6.8 ± 1.9	6.6 ± 0.8	0.73
Post-LT biliary problems	13	23	0.06

for 4 days: low-risk patients (elective and nonhemorrhagic LT) received oxacillin and temocillin, whereas high-risk patients (fulminant hepatic failure, prolonged pretransplant hospital and/or ICU stay, recent history of infection, urgent and/or hemorrhagic LT) received ceftazidim and vancomycin. Low-dose (10 mg) amphotericin B was given IV for 10 days. Oral and topical selective bowel decontamination using a mixture of tobramycin, polymyxin, and amphotericin B was given throughout the entire first hospital stay. Cytomegalovirus (CMV) prophylaxis using gancyclovir IV (Cymevene, Roche, CH) adapted to renal function was used for 3 weeks in CMV +/− donor-recipient pairs. Oral acyclovir (Zovirax, Glaxo-Wellcome, UK) (800 mg for 4 months) was given as herpes simplex prophylaxis. Trimethoprim sulfamethoxazole (Bactrim-Roche, CH) was given 3 times a week as antiprotozoal prophylaxis until TAC monotherapy was achieved.[10] These medications were given only from the eighth post-LT day onwards.

All patients received careful clinical, biochemical, and histologic follow-up. Bilirubin, aminotransferase (ALT), γ-glutamyltransferase (GGT), creatinine, uric acid and cholesterol levels (values expressed as mean ± SD), incidence of de novo bacterial, viral and fungal infections, arterial hypertension, insulin (in)dependent diabetes mellitus, malignancy, osteomuscular (pain, osteonecrosis, and/or fractures), cutaneous, and ophthalmologic complications were recorded at 3, 6, and 12 months. Liver test values up to 1.5× normal were considered normal. Hypercholesterolemia was defined as fasting serum cholesterol level ≥220 mg/dL, hyperuricemia as uric acid level ≥9 mg/dL, and renal insufficiency as creatinine level ≥1.5 mg/dL. Arterial hypertension was defined according the European Society of Hypertension as systolic and diastolic pressures ≥140 and 100 mm Hg.

Diagnosis of bacterial and fungal infections was based on the presence of positive cultures or on the presence of significant clinical infection in the absence of positive culture. Viral infection was diagnosed on the basis of positive tissue culture, viral excretion (detection of pp65 positive cells), and/or seroconversion. CMV disease was defined as a combination of clinical syndrome, thrombopenia, lymphopenia, and tissue invasion.

Biochemical and histologic scores were calculated to diagnose rejection and its need for treatment more precisely. Scores were calculated at post-LT day 7, the time at which the incidence of rejection is highest.[5] Progressive rise in total bilirubin and peripheral blood eosinophilia, absolute eosinophilia count above 600 mm^3, and progressive lowering of platelets during days 5 to 7 post-LT were each scored 0 to 1.[11–15] To avoid interference with these variables, no blood products and medications, except those mentioned above, were administered within the first post-LT week. A biochemical score of >2 was considered significant. Biopsies, carried out at day 7, 180, and 365 and when clinically indicated, were read blindly by 3 experienced transplant pathologists. The day 7 biopsy was graded according to the Banff score.[16] Moderate or severe histologic rejection was recorded if the score was ≥6 or ≥8. Treatment of early cellular rejection was considered only if biochemical (>2) and histologic (≥6) scores were present simultaneously. Chronic rejection or vanishing bile duct syndrome (VBDS) was defined as the absence of bile ducts in more than 50% of portal tracts on a representative biopsy (>10 portal tracts).[17] HCV reinfection was scored according to Ludwig.[18]

Primary end point of the study was graft and patient survival at 3 and 12 months. Secondary endpoints were incidence of rejection at 3 and 12 months, incidences of TAC monotherapy and of low-dosage TAC monotherapy, renal insufficiency, diabetes mellitus, hypercholesterolemia, hyperuricemia, arterial hypertension, infectious, and tumor complications and performance status defined on the basis of the Karnofsky index at 1 year after transplantation. Survival rates were analyzed separately for the whole patient group (n = 156) and for the patient group without artificial organ support (WOS-patients) at moment of LT (n = 145). Results were analyzed on an intention-to-treat basis, including all early graft and patient losses. Follow-up of 12 months from date of LT was complete for all patients (range, 12-84). There were no dropouts or withdrawals in either intervention group. Following the practice of the European Liver Transplant Registry, early and late events were divided into those occurring before and after the third post-LT month.

This study was developed to show equivalence between TAC-PL and TAC-ST. The method by Blackwelder was used to compute sample size. Sample size was based on an 80% 1-year rejection-free survival in both groups. Type I and type II errors were set at $P = 0.05$ and $P = 0.20$, respectively According to these assumptions, the required number of patients on each treatment was 69, ie, a total of 138 patients. To analyze outcomes in patients without artificial organ support at the time of transplantation (about 90% of all patients), it was decided to increase the sample size to 152. Finally, to correct for the possibility, although unlikely, of a few dropouts (0%–5%), the final sample size was set at 78 patients on each treatment, ie, a total of 156. Binomial estimates of odds ratios have been made for graft and patient survival. Because there were no dropouts, special types of intention-to-treat outcome models were not necessary. Statistical analysis was performed using SPSS for Windows version 12.0 (SPSS Inc, Chicago, IL). Normality of variables was tested using Skewness and Kurtosis measurements. Comparisons for graft and patient survival and for rejection-free survival were made using Kaplan-Meier product limit method and log rank sum test. *t*-tests were done for parametric continuous variables, χ^2 for scale categorical variables, and Mann-Whitney *U* test for nonparametric variables. A *P* value <0.05 was considered significant.

The institutional review board approved the study. Informed written consent was obtained from all patients or their next of kin before transplantation. All patients, health care providers, and outcome assessor teams were blinded until the 12-month analysis was complete. This investigator-driven study was designed, initiated, and managed by the senior author (J.L.). The authors adhered to the guidelines of the Consolidated Standards on Reporting Trials (CONSORT).

RESULTS

Patient Survival

Three and 12-month patient survival rates were 93.6% and 87.2% in the TAC-PL group and 98.7% and 94.7% in TAC-ST group ($P = 0.096$ and 0.093, respectively). Three and 5-year actuarial patient survival rates were 80.8%, and 78.2% in the TAC-PL group, and 87.2%, and 83.3% in the TAC-ST group ($P = 0.27$ and 0.41, respectively).

When considering the 145 patients without artificial organ support (WOS) at moment of LT, 3 and 12-month patient survival rates were 95.6% and 89.7% in the TAC-PL group (68 pts) and 98.7% and 94.8% in the TAC-ST group (77 pts) ($P = 0.25$ and 0.24, respectively). Three and 5-year actuarial survival rates of WOS patients were 82.4%, and 79.4% in the TAC-PL group and 87%, and 83.1% in the TAC-ST group ($P = 0.43$ and 0.56, respectively).

Ten (12.8%) TAC-PL patients died during the first year; of whom 3 were on artificial organ support at moment of LT. Five TAC-PL patients died early. Two died at days 11 and 14 from multiorgan failure caused by hemorrhagic allograft necrosis of ABO-identical liver graft, in the absence of vascular thrombosis. The first patient, who was transplanted for fulminant hepatitis B, had a 100% positive cross-match; the second one was the only Asiatic patient in the series. Their Banff scores on day-7 biopsy were respectively 9 and 7. The third patient presented with sepsis after steroid treatment of a severe rejection; he died at day 32 due to multiorgan failure. One patient, transplanted because of an acute liver failure, died of allograft failure at day 44 due to hepatic artery and portal vein thrombosis, which followed early rejection treatment of a small-for-size graft (liver of 50 kg donor in 120 kg recipient). This graft was accepted in a period of extreme organ shortage. She could not be regrafted in time. One patient died at day 80 because of prolonged cholestasis complicated with sepsis.

Five TAC-PL patients died between post-LT months 4 to 12. One patient committed suicide at day 93. One patient, transplanted in an advanced chronic disease stage with hepatorenal syndrome, died of sepsis at day 153. One patient, transplanted because of a fulminant liver failure, died at day 236 because of CMV pneumonia. One patient died at day 233 of sepsis induced by a split liver graft dysfunction due to severe peritonitis caused by a spontaneous jejunal perforation; at death liver tests were normal in the absence of any IS. One patient died at day 302 due to a chronic rejection, which followed TAC withdrawal necessary because of severe epileptic crises and blindness.

Four (5.1%) TAC-ST patients died during the first year. One patient died due to a false deglutition at day 53 after retransplantation (re-LT) done because of graft nonfunction. Three patients died between post-LT months 4 to 12. One patient had a difficult LT after multiple biliary interventions after laparoscopic bile duct injury. At the time of LT, segment I of the liver was necrotic and infected; she died at day 289 due to sepsis. Two patients died at 5 and 7 months due to recurrent cholestatic HCV.

Graft Survival

Three and 12-month graft survival rates were 92.3% and 85.9% in the TAC-PL group and 97.4% and 92.3% in the TAC-ST group ($P = 0.14$ and 0.13, respectively). Three and 5-year actuarial graft survival rates were 76.9%, and 74.4% in the TAC-PL group and 83.3%, and 78.2% in the TAC-ST group, respectively ($P = 0.42$ and 0.57).

When considering the 145 WOS patients, 3 and 12-month graft survival rates were 94.1% and 85.3% in the TAC-PL group (68 pts) and 97.4% and 93% in the TAC-ST group (77 pts) ($P = 0.55$ and 0.59, respectively). Three and 5-year actuarial graft survival rates were 80.9%, and 77.9% in the TAC-PL group and 83.1%, and 77.9% in the TAC-ST group, respectively ($P = 0.88$ and 0.82, respectively).

Besides patient mortality, 2 other grafts were lost in the TAC-PL group. One patient transplanted because of acute liver failure developed an intrahepatic lymphoproliferative disease (PTLD); he had successful re-LT at day 117. One patient transplanted because of acute Wilson disease had a successful re-LT at day 31 because of a chronic rejection developed under mycophenolate mofetil (MMF) and steroid treatment, instaured after withdrawal of TAC. This modification of IS was necessary because of severe neurotoxicity and hemolytic uremic syndrome necessitating plasmapheresis. She had re-LT under cyclosporine (Cy), MMF, and steroid IS. Despite this reinforced IS, OKT3 treatment became necessary 2.5-months post-re-LT because of CRR. At 37-months post-re-LT, she is clinically and biochemically doing well under TAC-rapamycin IS; histology, however, still shows a VBDS.

Besides the patient mortality, 2 other grafts were lost in the TAC-ST group. One living-related right liver graft was lost at 7 months in a cystic fibrosis patient because of intrahepatic biliary problems. He had a successful re-LT under TAC monotherapy at day 210. One patient had a successful re-LT at day 7 because of hemorrhagic allograft necrosis of ABO-identical liver graft, with no evidence of vascular thrombosis.

Rejection

Day 7 biopsies showed almost identical Banff scores in both groups (5.4 ± 1.8 in TAC-PL group and 5.4 ± 1.7 in TAC-ST group ($P = 0.89$) and similar incidences of moder-

TABLE 2. Results of Day 7 Protocol Biopsy in 156 Adult Liver Recipients

BANFF Score	TAC-Placebo (n = 78)		TAC-Steroid (n = 78)		*P*
No or borderline	7		10		
		50%		51.2%	0.75
Mild	32		30		
Moderate	30		31		
		50%		48.7%	0.75
Severe	9		7		
Mean score at day 7	5.4 ± 1.8		5.4 ± 1.7		0.89
Mean TAC level day 1–7	5.82 ± 1.55		6.16 ± 2.06		0.53
Infratherapeutic TAC	37/78 (48.7%)		42/78 (53.8%)		0.42

TABLE 3. Three-Month Immunological Results

In 156 Adult Liver Recipients

Rejection Treatment	TAC-Placebo (n = 78)	TAC-Steroid (n = 78)	*P*
Corticosteroid-sensitive	6 (7.6%) (d 9, 13, 27*, 42, **42**†, 48)	7 (8.9%) (d 14, *26‡, 28, 51, 67, 69, 88*)	0.77
Corticosteroid-resistant§	10 (12.8%) (d **7**¶, 7, 7, 10**,‖, 13**, **17**, 21, **23**, 27, 42)	3 (3.8%) (d 7††, 9, *88*)	0.04
Total	16 (20.5%)	10 (12.7%)	0.20

In 145 Adult Liver Recipients Without Artificial Organ Support (WOS) at Moment of LT

Rejection Treatment	TAC-Placebo (n = 68)	TAC-Steroid (n = 77)	*P*
Corticosteroid-sensitive	5 (7.4%)	7 (9%)	0.70
Corticosteroid-resistant§	6 (8.8%)	3 (3.9%)	0.22
Total	11 (16.6%)	10 (13%)	0.50

The time points in italic signify rejection during period of steroid withdrawal, those indicated in bold correspond to patients transplanted being on artificial organ support at moment of LT.

*Hepatic artery and portal vein thrombosis in the context of rejection of a small-for-size LT.

†Rejection after IS withdrawal due to post-transplant allograft lymphoma, ‡cerebrovascular accident, and ¶severe neurotoxicity necessitating reduction of TAC.

§Corticosteroid-resistant rejection (CRR) means no response to 5 boluses of 200-mg MP or graft loss due to hemorrhagic necrosis.

††Lethal hemorrhagic allograft necrosis, one patient**‖ had 100% T-cell positive cross-match.

ate to severe rejection scores (≥6) [39 TAC-PL patients and 38 TAC-ST patients ($P = 0.75$)] (Table 2).

The TAC-PL group had slightly lower mean TAC levels during the first post-LT week, which can be explained by the changes in the TAC dose because of the significantly higher mean creatinine level during the first 2 weeks and need for organ support at the time of LT. At day 7, 37 (48.7%) of TAC-PL patients and 42 (53.8%) of TAC-ST patients were on infratherapeutic TAC monotherapy (<6 ng/mL) ($P = 0.42$). Sixteen TAC-PL patients (20.5%) required treatment for rejection during the first 3 months; 5 of them had artificial organ support at moment of LT (Table 3). Six (7.6%) patients had CSR and 10 (12.8%) had CRR. Three of the latter patients needed OKT3 therapy and 1 needed both OKT3 and ATG therapy. Two patients presented with lethal hemorrhagic graft necrosis at days 10 and 13. One, CMV-negative, patient needed re-LT because of development of VBDS after important reduction of IS due to severe neurologic problems and hemolytic uremic syndrome. After re-LT she needed triple IS and OKT3 treatment because of refractory rejection (Table 4). One patient developed chronic rejection after TAC withdrawal imposed by the development of a severe neurologic syndrome. One patient developed vascular thrombosis in the context of a small-for-size graft presenting a rejection not responding to steroids; unfortunately, she died before re-LT could be done. After the third post-LT month, 2 more patients needed steroid boluses at day 95 and 210, bringing the total number of patients treated for rejection in the TAC-ST group during the first post-LT year to 18 (23%).

Ten TAC-ST patients (12.8%) required rejection treatment during the first 3 months (Table 3). Seven (8.9%) had CSR. One patient developed rejection after IS reduction because of an early cerebrovascular accident. One patient developed VBDS at 2 months, which was treated with addition of MMF. Three (3.8%) patients developed a CRR. Two patients required OKT3 therapy; one of them had to undergo reduction of IS because of severe neurotoxicity. One patient had successful re-LT at day 7 because of hemorrhagic allograft necrosis. After the third post-LT month, 6 patients were treated bringing the number of patients treated for rejection in the TAC-ST group during the first post-LT year to 16 (20.5%) ($P = 0.54$) (Table 4).

TABLE 4. One-Year Immunological Results in 156 Adult Liver Recipients

	TAC-Placebo (n = 78)	TAC-Steroid (n = 78)	*P*
Rejection			
Treatment	23% (18)	20.5% (16)	0.54
Vanishing bile duct syndrome	1.2% (1)	5.1% (4)	0.17
Tacrolimus monotherapy			
Intention-to-treat	78.2% (61/78)	82% (64/78)	0.54
One-year survivors	91% (61/67)	86.5% (64/74)	0.39
Tacrolimus low-dosage monotherapy (<6 ng/mL)			
Intention-to-treat	53.8% (42/78)	61.5% (48/78)	0.33
One-year survivors	62.7% (42/67)	64.9% (48/74)	0.79

Four patients had VBDS at the 6-month biopsies. After addition of Azathioprine (AZA) or MMF; 2 patients had normalized and 2 had improved their liver biopsy after 1 year. Their bilirubin levels were all normal; GGT was raised from 5 to 36 times normal. Three had donor+/recipient-CMV pairing, and 1 of them had CMV infection.

Freedom-from-rejection comparisons were similar between the 2 groups (Table 3). Three and 12 month rejection-

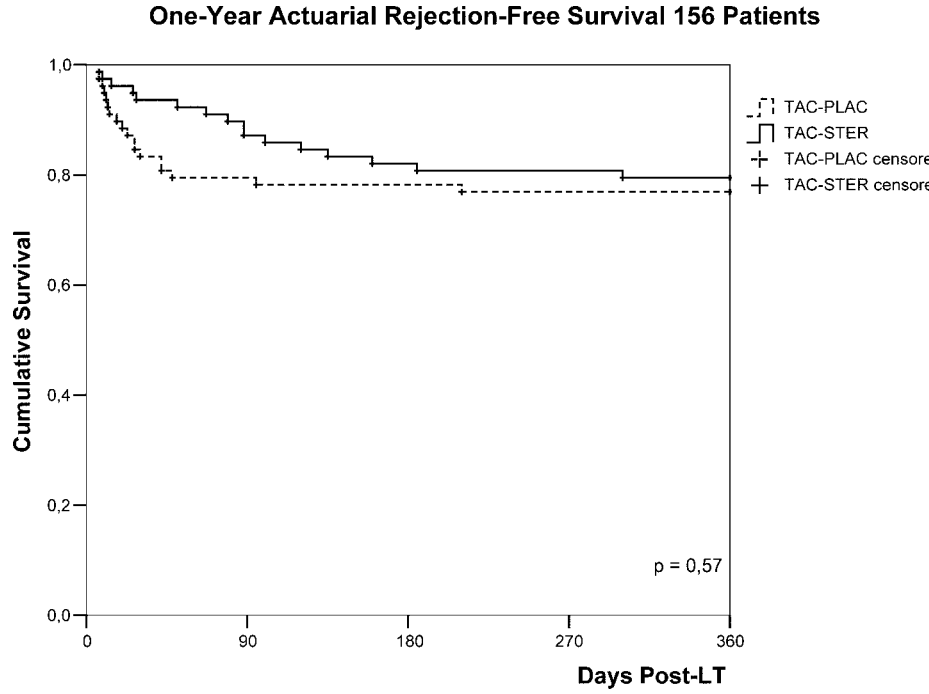

FIGURE 1. Rejection-free survival in 156 adult liver recipients.

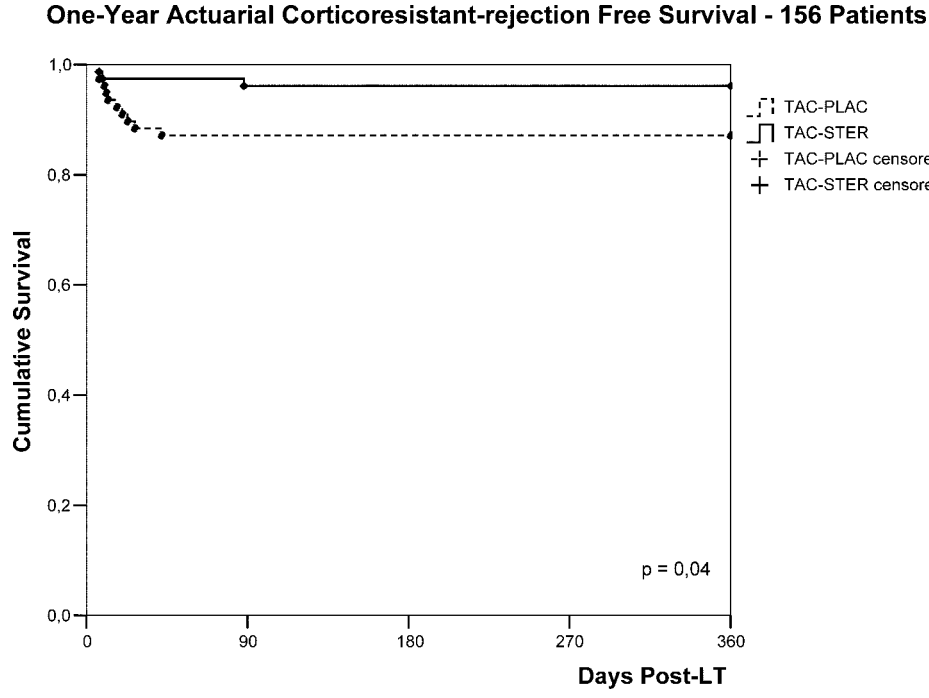

FIGURE 2. Corticosteroid-resistant rejection-free survival during the first post-transplant year for the whole patient group (n = 156).

free survival rates were 79.5% and 76.9% in TAC-PL group versus 87.2% and 79.5% in TAC-ST group ($P = 0.19$ and $P = 0.69$, respectively) (Table 3 and Fig. 1). When considering the 145 WOS patients, 3 and 12 month rejection-free survival rates were 83.8% and 80.9%-in TAC-PL group versus 87% and 79.2% in TAC-ST group ($P = 0.58$ and $P = 0.80$, respectively). CRR free survival at 3 (and 12) months was significantly lower in the TAC-PL group (86.9% vs. 96% in TAC-ST group; $P = 0.04$) (Fig. 2). When considering the 145 WOS patients, these differences became nonsignificant; 90.9% versus 96% (6/68 TAC-PL pts–8.8% vs. 3/77 TAC-ST pts–3.9%; $P = 0.22$) (Table 3 and Fig. 3).

Twelve months post-LT, 78.2% (61/78) of TAC-PL patients and 82% (64/78) of TAC-ST patients were on TAC monotherapy ($P = 0.54$). Taking only the surviving patients into account, these incidences were 91% (61/67 pts) and

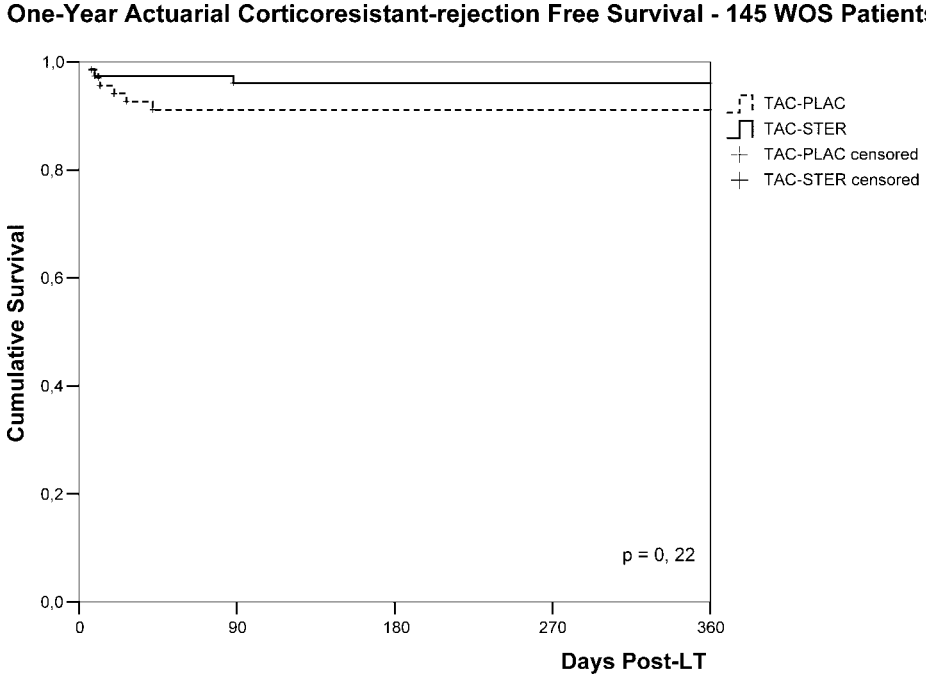

FIGURE 3. Corticosteroid-resistant rejection-free survival during the first post-transplant year for the WOS patient group (n = 145).

86.5% (64/74 pts) ($P = 0.39$). The 5 TAC-PL patients and 3 TAC-ST patients who died after 3 months were on TAC monotherapy; 1 completely weaned off TAC-PL patient died with a normal liver function. Low-dosage TAC monotherapy (<6 ng/mL) was obtained after 1 year in 62.7% (42/67) of TAC-PL survivors and 64.9% (48/74) of TAC-ST survivors ($P = 0.79$) (Table 4).

One-year post-LT, 7 (10.4%) of 67 TAC-PL patients were on double IS. One patient each had TAC-AZA IS due to arthritis manifested in the context of a primary biliary cirrhosis (PBC) (1pat) and autoimmune cirrhosis (1pat), to recurrent primary biliary cirrhosis (1pat) and to previous CRR (1pat). One patient each had TAC-MMF IS due to autoimmune hepatitis and to previous CRR. One patient was on combined TAC-rapamycin due to CRR after re-LT done because also CRR. Eleven (14.9%) of 74 TAC-ST patients were on double IS (5xMMF and 6xAZA) due to autoimmune hepatitis (1pat), arthritis in a PBC patient (1pat), VBDS (3pts), TAC neurotoxicity (3pts) and nephrotoxicity (1pat), and status after CRR (2pts). Three TAC-PL patients needed steroid therapy because of omeprazole nephrotoxicity, pleuro-pericarditis, and suspicion of pulmonary granulomatosis during a period of 8, 3, and 1.5 months, respectively. One TAC-ST patient needed steroid therapy due to arthritis.

Biochemistry

Normal bilirubin (55/67 TAC-PL pts–82.1% vs. 63/74 TAC-ST pts–85.1%; $P = 0.63$), ALT (61/69 TAC-PL pts–9% vs. 62/74 TAC-ST pts–16%; $P = 0.19$), and GGT (51/67 TAC-PL pts–76.1% vs. 59/74 TAC-ST pts–79.7%; $P = 0.60$) values at 12 months were similar in both groups. The somewhat higher ALT at 12 months in the TAC-ST group can be partly explained by their higher incidence of biliary problems (23 vs.13 pts in TAC-PL group; $P = 0.06$).

Histology

Besides the protocol biopsies, 73 and 52 more biopsies were done in the TAC-PL and TAC-ST groups; 17 and 12 biopsies respectively showed moderate or severe rejection. Those needing treatment for rejection have already been mentioned above. All other biopsies showed modifications essentially related to allograft viral reinfection or biliary problems. At 1 year, the results of biopsies were similar. The evolution of HCV allograft reinfection was significantly more aggressive (with evolution towards cirrhosis) in the TAC-ST group ($P = 0.03$) (results not shown).

TABLE 5. One-Year Medical and Metabolic Results in 141 Adult Liver Recipients

	TAC-Placebo (n = 67)		TAC-Steroid (n = 74)		*P*
PTLD	2	(3%)	1	(1.4%)	0.50
Renal insufficiency (creatinine >1.5 mg/dL)	4	(6%)	8	(11%)	0.44
Insulin-dependent diabetes	18	(26.8%)	14	(19%)	0.26
De novo	5	(7.5%)	6	(8.1%)	0.88
Hyperuricemia (uric acid >9 mg/dL)	5	(7.5%)	3	(4.%)	0.38
Hypercholesterolemia (fasting >220 mg/dL)	12	(17.9%)	10	(13.5%)	0.47
Arterial hypertension	6	(8.1%)	10	(13.5%)	0.39
De novo	2	(3%)	0	(0%)	0.13
Osseo-muscular pain/fractures	6	(9%)	13	(17.6%)	0.13
Cataract	4	(6%)	4	(5.4%)	0.88
Mean Karnofsky index	95.9		92.5		0.38

TABLE 6. One-Year Infectious Results in 156 Adult Liver Recipients

	TAC-Placebo (n = 78)		TAC-Steroid (n = 78)		
De Novo Infection	**<3 mo**	**>3 mo**	**<3 mo**	**>3 mo**	***P***
Viral	27 (34.6%)	24 (30.8%)	9 (11.5%)	11 (14.1%)	0.61/0.63
HSV, HZV, CMV	(25 episodes)	(25 episodes)	(8 episodes)	(8 episodes)	
CMV disease	2 (2.5%)	1 (1.3%)	2 (2.5%)	—	0.65/0.15
Fungal	9 (11.5%)	7 (9%)	5 (6.4%)	4 (5.1%)	0.59/0.73
Oral/esophageal	6 (7.7%)	6 (7.7%)	3 (3.8%)	1 (1.3%)	1/0.31
Bacterial*	43 (55.1%)	44 (56.4%)	13 (16.6%)	22 (28.2%)	0.87/0.08
	(71 episodes)	(78 episodes)	(23 episodes)	(38 episodes)	
Pneumonia	10 (12.8%)	4 (5.1%)	3 (3.8%)	1 (1.3%)	0.09/0.31

*All infections included, eg, asymptomatic urinary tract infection.

Medical, metabolic and infectious results, and performance status, expressed using the Karnofsky index, were similar in both groups (Tables 5 and 6).

DISCUSSION

The excellent results being achieved currently in transplantation are shifting the interest of transplant physicians towards quality of life and tolerogenic strategies.[1,19] Well-balanced use of drug combinations minimizing toxicity and reduction or even avoidance of steroids and/or calcineurin inhibitors in induction and/or maintenance IS protocols are options for achieving these aims. The first step to be taken to improve quality of life and to achieve minimal IS in LT is to design steroid-free IS.[5–8] This can be achieved in many different ways using low-dose steroids from the start of transplantation, early (within days or weeks) or late (within months) steroid withdrawal (STWD), alternating doses of steroids and finally, partial or complete avoidance of steroids in induction and/or maintenance therapy, and/or in the treatment of rejection.[8]

Steroid-free IS still remains controversial, mainly due to the lack of evidence-based selection criteria and of well-conducted clinical trials and due to the fear of chronic rejection.[7,8] The fear on the adverse effect of STWD on allograft survival relates to the results reported in renal transplantation by the single available Canadian multicenter randomized study showing that the adverse effect on allograft survival became only clear after 5 years (73% rejection rate in placebo group vs. 85% in low dose steroid group; $P < 0.03$).[20] Based on experimental and clinical evidence, the liver allograft is the most appropriate organ for IS protocols avoiding the use of steroids.[1,21] Since the first report by the Birmingham group in 1993, 16 more studies have been reported by the end of June 2007 in adult LT.[8,22–28] Eight were prospective randomized controlled trials; only 2 were done in a double-blind, placebo-controlled fashion.[22,28] In 13 studies, IS was Cy-based and in 4 TAC-based. With follow-up ranging from 3 to 60 months, the success of STWD varied from 68% to 100% and the incidences of acute and chronic rejection varied from 4.5% to 48% and from 0% to 6.9%. In 7 studies, Cy monotherapy was achieved in 21% to 90% of patients, and in 2 studies, TAC monotherapy was achieved in 21 and 62%. Similar results were reported in pediatric LT.[29] All these studies showed excellent, long-term, patient and graft survival, and significant metabolic benefits.

Based on this clinical experience, steroid avoidance protocols were developed. Such approach has 4 major theoretical advantages: (*a*) absence of steroid dependence, as the patient has not been exposed to steroids; (*b*) elimination of all potential side effects from the moment of transplantation onwards; (*c*) absence of taper with its inherent risk of breakthrough rejection; and (*d*) absence of interaction with the active process of tolerance induction. Indeed, the steroid-adapted immune system may behave differently from one that has not been exposed to steroids.[3,4,7,8,30–32] Steroid avoidance IS implies that no steroids at all were used or that steroids were administered only during the first 3 post-LT days ("almost steroid avoidance"). The feasibility of steroid-avoidance IS, using various drug combinations, has been investigated during the period from 1999 to June 2007, in 22 adult and 2 pediatric studies.[8,33–47] Twelve studies were prospective, randomized, controlled ones; only 1 study was done in a double-blind, placebo-controlled fashion.[39] These studies were merely done on TAC based (20 studies), and severely reinforced IS using antilymphocytic sera, anti-IL 2 monoclonal antibodies, or m-TOR inhibitor (17studies); only 3 times IS consisted of tacrolimus monotherapy.

It is difficult to draw conclusions from these experiences due to different study designs and variable (3–44 months) follow-up. Incidences of acute and chronic rejection varied from 6% to 86%, and from 0% to 5%, steroids had to be introduced in 21% to 60% of patients, and TAC monotherapy was obtained in 37% to 96.9% of patients. One-year graft survival was around 90% and metabolic benefits were clear.[8,33–47] Although done mostly at the price of a heavier IS, all these steroid avoidance studies have opened the way to a minimal IS strategy. Our double-blind, placebo-controlled study combining "almost steroid avoidance" and calcineurin inhibition monotherapy using TAC including primary adult liver recipients, irrespective of their medical and immunologic status at the time of grafting, was set up to test the concept of steroid avoidance without initial reinforcement of the IS scheme. The results do not show significant differences in patient and graft outcomes nor in infectious, tumor and metabolic complications, and performance status between the TAC-placebo and the TAC, short-term steroid patient groups.

The immunologic results are important as Banff histologic scores on day-7 protocol biopsies were identical in both groups, an observation that was made already previously in an open-labeled study conducted at the Royal Free Hospital in London.[46] Moreover, rejection-free survival rates at 3 and 12 months were similar. The results in the TAC-PL group were obtained despite the tendency to lower tacrolimus dosage (related to a higher number of patients presenting in worse clinical condition and renal function at time of LT), the progressive lowering of TAC dosing during follow-up and the fact that almost half of the patients had a low-dosage TAC level during the first post-LT week. The only significant difference related to the incidence of CRR during the first 3 post-LT months. This difference can be explained by the very restrictive definition of CRR in our center (ie, a nonresponse to 1 g of MP only), the strict algorithm of rejection treatment, closely correlating histologic and biochemical evolution, and by the significantly higher incidence of artificial organ support in the TAC-PL patient group. Indeed when analyzing separately, the 145 patients, which did not have artificial organ support at moment of LT, the differences in incidence of CRR became nonsignificant. It is easy to understand that the perioperative handling, especially of a TAC monodrug IS, is much more difficult and hazardous in the presence of especially severe renal insufficiency. Lowering of TAC levels to spare renal function may easily result in more severe rejection episodes, such as observed in the study. It should be stressed in this context that patients with even mild renal insufficiency are usually excluded from enrollment in prospective (randomized) liver trials dealing with immunosuppression.

Our study indicates that an individually tailored steroid-free TAC induction and maintenance IS protocol in LT is indicated in clinical or immunologic high-risk patients (due to high MELD-score, pretransplant renal insufficiency and severe encephalopathy, need for artificial organ support at moment of LT or positive cross-match) and in patients presenting a difficult early post-transplant course (due to ie, allograft dysfunction, hemorrhagic surgery, renal insufficiency, etc). The refinement of TAC monodrug IS by combining its use with other nonneurotoxic or nonnephrotoxic drug regimens adjusted to the evolution of the recipient may be more appropriate to overcome a possibly more complex post-transplant course in such patients. Feasibility and benefits of such approaches using MMF, single high-dose rabbit antithymocyte globulin or humanized anti-CD52 monoclonal antibody (alemtuzumab) as induction therapy in conjunction with TAC have been shown by the New Orleans, Pittsburgh, and Miami groups.[1,35,48]

The 2 TAC-PL patients who lost their grafts early due to hemorrhagic allograft necrosis might be of concern. This exceptional cause of early graft loss is not strictly related to a, possibly too weak, induction IS as we experienced also 1 case in the TAC-ST group and 4 other such cases at our center (from 700 previous adult LT) despite much stronger induction IS in all of them. Hemorrhagic necrosis is an insufficiently understood early cause of immunologic graft loss, which has been observed using all the different forms of induction IS.[49,50]

VBDS was seen somewhat more in TAC-ST patients than in TAC-PL patients. Although high-risk CMV pairing and CMV infection possibly played a role,[17,51] VBDS could also be explained by the development of rejection while tapering steroids and by the fact that steroids may influence the immunologic response to the allograft.[2,3,4] Long-term follow-up of these patients will allow us to answer this question.

Tacrolimus is currently accepted as the superior immunosuppressive drug in LT.[52,53] This study not only confirms that TAC monotherapy is a feasible, safe induction, and maintenance IS in adult LT but also that such a strategy can be applied in an unselected adult LT population without compromising patient and graft survival. Our study goes some steps further than the recently reported prospective, randomized, double blind, placebo-controlled trial of the Mainz group comparing TAC monotherapy and TAC-steroid taper after a period of 14 days in a cohort of 110 adults.[28] These authors concluded that TAC monotherapy is indeed feasible in LT. It should, however, be noted that there are important differences between the studies. In the Mainz study, patient inclusion was selective, incidence of rejection was almost double despite higher TAC and 3.5 times higher steroid dosages, treatment of rejection was more aggressive, 10% of patients were secondarily excluded because of recurrent rejection, one third of patients were withdrawn from the trial for various reasons including rejection, and primary endpoints looked at incidence of metabolic and infectious complications.[28] Our study also indicates that (subtherapeutic) tacrolimus monotherapy and not the use of several, "tolerizing drug cocktails,"[54,55] might be a logical and easier way to optimize interaction between donor and recipient immune systems allowing minimal IS and eventually complete IS withdrawal.[1,2] Early minimal IS is a prerequisite to avoid erosion of the seminal tolerance mechanism of clonal exhaustion-deletion, allowing thereby a more frequent IS withdrawal in well selected cases.[1,55] Confirmation of the results presented here in larger prospective, placebo-controlled, and blinded studies is warranted to allow practical application of our findings on a broader, routine, clinical scale.

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Discussions

J. BAULIEUX: Recently another prospective randomized, "double blind" study from Mainz, (Moench et al. *Am. J. Transpl.* 2007), reported a not so different experience, but the complete follow-up was achieved in only 65% of patients. Twenty percent of them were excluded from the study because of recurrent rejection.

This work has the benefit of not having excluded any patient from the study, not even those with the most severe disease. However, I noticed that the 2 groups are not exactly comparable. The group "TAC + placebo" concerns the most severe patients who needed respiratory and kidney assistance more often (10 vs. 1; $P = 0.004$), an ischemia time and significantly higher creatinine level. Despite these differences, the results are not different between the 2 groups except concerning the corticosteroid resistant rejections, (10 vs. 3, $P = 0.04$). It seems that, in high-risk patients with a very high MELD score, it seems possible to provide a less aggressive monotherapy with Tacrolimus.

I have 2 questions: What exactly is your definition of corticosteroid-resistant rejection? Have you observed an influence from these 2 different immunosuppression regimens for the evolution of HCV allograft reinfection?

J. LERUT: As I stated, we have not used steroids at all in our center for about 15 years. We started to use steroids again to do the study, but we did not change our policy regarding the definition of cortico-resistant rejection. Thus, cortico-resistant rejection means that a patient does not respond to 5 boluses of 200 mg of methylprednisolone. If you look in the literature, rejections are usually treated with 1, 2, 3, or even up to 5 g of methylprednisolone followed by a recycling that gives each patient about 6 g steroids. If we applied this to the study, there would have been no difference at all, but it was the design of the study, so we had to stay with the protocol.

In relation to the hepatitis C reinfection, this matter deserves our special attention as 25% of the patients we transplant nowadays have hepatitis C. As you know, there is much discussion, which is also mostly industry-driven, on how to treat these patients. There is a tendency now to give some steroid based immunosuppression to these patients.

I think you can only draw conclusions in this matter based on a blinded-study of biopsies. All patients received a prospective and blinded histology review. The patients were classified after the Mayo-Ludwig classification and Metavir score. I used the Mayo classification because it is a little bit more detailed. There is a highly significant difference between the patients that took a placebo and those who received steroids. I think we now have a valid answer because these results are based on a blinded study. This is of great importance because the problem for hepatitis C patients is that the biopsies often show features that are almost the same as in rejection. We will, of course, publish these results separately.

K. HOCKERSTEDT: I will go directly to my question, but first I would like to say that there are a few other centers such as Dr Tisone's center in Rome, which follows a similar policy. As you know, they do not have as good a randomized study as yours. The more we learn about the drug the less we use annually, and now you say at 1 year after transplantation, you see about half of the patients in both groups with a tacrolimus trough level less than 6. Now that is the optimistic side, the pessimistic side is to say that half of the patients reach a level that is higher. Could you explain to us why the other half receives higher doses of tacrolimus? That is my main question, and, of course, when we reduce the doses we want to eliminate the side effects, but we see them only after many years.

J. LERUT: In fact, we do lower immunosuppression all the time. That is our philosophy because there are still too many late complications, so we start low and go even lower. When I say that patients have less than 6 ng/mL of tacrolimus, I must say that we usually cannot even detect any more tacrolimus in the blood. We also have several patients who are on spaced monotherapy in which we do not even perform any more tacrolimus dosages. In some patients, we keep the tacrolimus levels low and stable. This policy is important, in my opinion, for our largest patient group, the hepatitis C patients. If hepatitis C patients get low doses of around 6 ng and if they are stable, we keep them like this. Many times it is not wise to change or to lower their immunosuppression because you can provoke a viral reactivation. This is the reason why in one half of the patients, we still have a higher amount of tacrolimus but usually this level is then around 6 to 8 ng.

P-A. CLAVIEN: I have 1 question regarding patients with renal failure. Your recipient population was very sick, possibly with a high incidence of concomitant renal failure. Most of us would either use an induction therapy introducing a calcineurin inhibitor, such as tacrolimus or cyclosporine, at a later stage, or minimize the use of a calcineurin inhibitor by adding, for example, mycophenolate mofetil (MMF), or replacing the calcineurin inhibitor by rapamycin or an analogue drug. Thus, I was wondering with regard to your series, particularly in the second part of the study, whether you adjusted the immunosuppression for patients with pre-existing or postoperative renal failure, especially those on dialysis. Did you "unblind" those patients to switch them to a less nephrotoxic immunosuppressive regimen or did you just persist in giving them tacrolimus?

J. LERUT: We did not unblind 1 single patient and I think this is very important. If a patient has renal failure, we never give the dose the company proposes to give. We start 10 to 12 hours after the transplantation with very low doses; in fact we give half of half of the recommended dose, and then we climb with the immunosuppression to reach what the patient tolerates. The reason we do not use MMF, especially in studies like, is that we place a lot of importance on the "pure biopsy" of day 7 and correlate this with the "pure patient treatment." Thus, none of our patients receives any medication or any transfusion between day 0 and 7, otherwise you cannot judge the evolution of thrombocytes and eosinophils. We do this because there is a large experience from thoracic transplantation that showed that these parameters are of interest. Thoracic transplantation is a very good model to teach liver people how to work. There are very good studies done in cardiac transplantation about the sequence and the dynamics of, for instance, thrombocytes and thrombocytopenia. I think when thrombocytes decline, that there is a problem on the endothelium. Of course, if you give a patient MMF or acyclovir or similar medications, you induce thrombopenia. This is the reason we start only with oral prophylactic antiviral therapy and other therapies, if we need them, at day 8 when the biopsy is done. Then we judge what to do. You can see that 80 patients of the 156 experienced a severe or very severe rejection but we only treated 8 patients from day 7 to 10.