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# Rejection rates in a randomised trial of tacrolimus monotherapy versus triple therapy in liver transplant recipients with hepatitis C virus cirrhosis

## Key words:

randomised trial; tacrolimus monotherapy; hepatitis C; cirrhosis; liver transplantation

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**Abstract:** *Background.* Reducing immunosuppression not only reduces complications but also may lessen recurrent hepatitis C virus (HCV) infection after liver transplantation. *Patients/Methods.* HCV-infected cirrhotic patients randomised to tacrolimus monotherapy (MT) or triple therapy (TT) using tacrolimus 0.1 mg/kg/day, azathioprine 1 mg/kg/day, and prednisolone 20 mg/day, tapering over 3 months. *Results.* Twenty-seven patients (MT) and 29 (TT) – median follow up 661 days (range, 1–1603). Rejection episodes (protocol/further biopsies) within first 3 months and use of empirical treatment were evaluated. New rejection was diagnosed if repeat biopsy (5-day interval) did not show improvement. Treated rejection episodes: 20 MT (15 biopsy-proven) vs. 24 TT (21 biopsy-proven), with 19 (MT) vs. 24 (TT) methylprednisolone boluses. Overall: 35 episodes (MT) and 46 (TT). Fewer MT patients had histological rejection (70%) than TT patients (86%), with fewer episodes of rejection (18.5% vs. 10%), and more moderate rejection (22% vs. 41%). The MT group had higher early tacrolimus levels. Rates of renal dysfunction, retransplantation, and death were not significantly different. *Conclusion.* Tacrolimus monotherapy is a viable immunosuppressive strategy in HCV-infected liver transplant recipients.

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Optimal immunosuppression in orthotopic liver transplantation (OLT) should prevent rejection without serious complications. Liver allografts are considered “immunologically privileged,” because of an absence of hyperacute rejection despite a positive T-cell cross-match, a low incidence

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of graft loss from chronic rejection, and the capacity of hepatocytes to regenerate following tissue injury (1). Thus, there might be a greater possibility to minimise immunosuppression in liver transplant recipients.

A distinction is often made between biological rejection (histological changes of cellular rejection in absence of significant clinical or biochemical abnormalities) (2) and clinical rejection (histological changes accompanied by clinical signs of graft dysfunction). In liver transplantation clinically significant cellular rejection is reported in approximately 50% of patients, whereas histological abnormalities can be seen in up to 80% of protocol biopsies performed within the first week post OLT (3). Liver histology is the gold standard for the diagnosis of acute rejection (4, 5) and an international consensus on a common grading system has been achieved (6). In addition, eosinophilia in the graft is an independent diagnostic marker of cellular rejection as found in our centre (7).

The incidence of early clinical and biological cellular rejection is influenced by the type of immunosuppression (8). Severe rejection may affect graft and patient survival, possibly as a result of the use of more potent immunosuppression such as antilymphocytic preparations (9). Conversely, milder grades of rejection are associated with improved survival after liver transplantation, a situation different from renal transplantation (9). Moreover, early cellular rejection that responds promptly to treatment may be associated with a lower risk of immunological complications during follow up (8).

Complete steroid withdrawal is now common practice in many centres as patient and graft loss are not increased, and long-term steroid complications are reduced (10). However few studies have avoided, from immediately after transplant, the use of steroids in maintenance immunosuppression (11–14). When steroid use has been avoided, other agents have been substituted for the steroids so that 'total' immunosuppressive potency is not reduced. Our own experience without steroids, using only calcineurin inhibitor monotherapy (MT), showed that it was safe and effective (14).

Thus, we evaluated calcineurin inhibitor MT vs. triple therapy (TT) in a clinical setting, where less potent immunosuppressive therapy might be more beneficial. We chose recipients with hepatitis C virus (HCV) cirrhosis receiving a cadaveric liver transplant, because increased immunosuppression has been one of the factors leading to

HCV recurrence (15–18), and randomised them to groups receiving tacrolimus MT or TT consisting of tacrolimus, azathioprine, and prednisolone in a prospective trial. Tacrolimus is currently a first-line immunosuppressive agent with better outcomes than micro-emulsified cyclosporine in our patient group (19), and has reliable action and acceptable side-effect profile (20).

Our current report focuses on acute cellular rejection rates in this ongoing trial during the first 3 months after liver transplantation, because during this period patients are at greatest risk of rejection. We assessed whether tacrolimus MT is a clinically viable option to prevent important cellular rejection.

## Patients and methods

### Inclusion–exclusion criteria and randomisation

From January 2000 to January 2004, we randomised consecutive transplant recipients with HCV cirrhosis (independently of whether they had concomitant alcoholic aetiology or hepatocellular carcinoma [HCC]) when they (a) were older than age 18 years, (b) gave informed written consent, (c) had a cadaveric liver transplant. Exclusion criteria were (a) multi-organ transplants, (b) retransplantation, (c) split or auxiliary transplants, (d) patients with contraindications to tacrolimus or azathioprine, and (e) refusal to participate. We evaluated all patients up to May 2004, i.e., had at least 3-month follow up.

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Hospital Ethics committee. Randomisation took place on arrival to the operating theatre. Sealed opaque envelopes were used, opened in numbered sequence containing the allocated treatment in a 1:1 proportion derived from a random number table using a blocked code.

### Patient–donor characteristics

We evaluated 56 consecutive patients (26 females, 30 males); 1 patient refused randomisation (given MT). Recipient and donor characteristics and surgical details are shown in Table 1. Typing of donor and recipient antigens was performed with a standard assay using lymphotoxic antibodies. For each locus (-A, -B, -DR, -DQ) the number of

**Patient characteristics in the two randomised treatment arms**

	Monotherapy	Triple therapy
Number of patients	27	29
Recipient age (years)		
Range	37–66	35–66
Median	48.5	53
HCC (pre-OLT/Incidental)	11 (10/1)	10 (9/1)
Concomitant alcohol aetiology	8	3
Ascites pre-OLT	14	23
Creatinine pre-OLT ( $\mu\text{mol/L}$ )		
Median	80	84
Range	60–116	64–231
Diabetes pre-OLT	10	9
Oral hypoglycemics/insulin	5/5	3/6
Diabetes post-OLT	7	8
Oral hypoglycemics/insulin	1/6	1/7
Patients with renal dysfunction		
Post-OLT ( $> 130 \mu\text{mol/L}$ )	18	17
Serum creatinine at 6 weeks		
Range (mmol/L)	67–233	66–241
Median	100.5	115.5
Serum creatinine/end follow up		
Range (mmol/L)	78–222	76–281
Median	110	111
Donor age (years)		
Range	21–67	11–73
Median	49	43
Significant HLA-DR mismatch (patients) <sup>1</sup>	6	7
Significant total mismatch <sup>2</sup> (patients)	8	7
Cold ischaemia time		
Range (min)	384–1043	274–929
Median	619.5	620
Warm ischaemia time		
Range (min)	28–67	31–100
Median	49	45
Values at first protocol biopsy/(reference range) (median, range)		
BIL ( $\mu\text{mol/L}$ ) (5–17)	72 (24–485)	88 (11–236)
GGT (U/L) (9–54)	277 (41–608)	310 (63–1866)
ALP (U/L) (42–128)	172 (41–385)	189 (54–342)
AST (U/L) (5–40)	59 (15–386)	59 (22–660)
ALT (U/L) (5–40)	183 (59–1263)	167 (56–1026)
ALB (g/L) (35–50)	27 (14–37)	26.5 (13–34)
INR (ratio) (0.9–1.20)	1.2 (1.1–3.2)	1.2 (1–2.1)
CRE ( $\mu\text{mol/L}$ ) (60–120)	119 (64–485)	112 (64–488)

	Monotherapy	Triple therapy
CMV viraemia <sup>3</sup> within 3 months	5	6
Valgancyclovir treatment	5	6
Antibiotics after day 5 from OLT	20	20
Follow up		
Range (days)	6–1582	1–1603
Median	636	685

<sup>1</sup>Significant HLA DR mismatch: both HLA DR loci were different in donor compared with recipient.  
<sup>2</sup>Significant total mismatch: a mismatch score 6 or more (from a maximum of 8) derived from HLA comparisons (2 HLA A locus, 2 HLA B locus, 2 HLA DR locus, and 2 HLA DQ locus) where no mismatch = 0, 1 mismatch = 1, 2 mismatches = 2.  
<sup>3</sup>Two consecutive CMV-DNA PCR with twice weekly sampling. Monotherapy, tacrolimus alone; triple therapy, tacrolimus/azathioprine/prednisolone therapy; OLT, orthotopic liver transplantation; HCC, hepatocellular carcinoma; HLA, human leukocyte antigens; DR, donor; BIL, bilirubin; GGT,  $\gamma$  glutamyl transferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine transaminase; ALB, albumin; INR, International normalised ratio; CRE, creatinine; CMV, cytomegalovirus.

**Table 1**

mismatches was scored as 0, 1, or 2. Total human leukocyte antigen (HLA) mismatch score (range: 0–8) was defined as sum of the number of mismatches for the 4 loci – HLA-A, -B, -DR, or -DQ (21). Cold ischaemia time was defined as the interval from donor cross-clamp to removal from cold storage. Warm ischaemia time was defined as the interval between removal from cold storage and venous reperfusion. Twenty-one patients had known HCC, 11 had concomitant alcoholic liver injury, and 2 patients were co-infected with hepatitis B or D virus. Median follow-up was 661 days (range: 1–1603). Liver and renal biochemical parameters were documented at the first protocol biopsy. Renal dysfunction was defined (for the purpose of the study) as an increase in creatinine value  $\geq 130 \mu\text{mol/L}$  and was also evaluated at 6 weeks post OLT and at the end of follow up. No patient received HCV antiviral therapy within 3 months of transplantation.

**Immunosuppression regimens**

Tacrolimus (Prograf<sup>®</sup>, Fujisawa Ltd, Killorglin, County Kerry, Ireland) MT 0.1 mg/kg/day in 2 divided doses or TT of tacrolimus (same regimen), azathioprine – initially intravenously (IV) then orally – 1 mg/kg/day, and methylprednisolone (16 mg/day IV) until oral intake was established, when 20 mg/day prednisolone was used. The first doses of tacrolimus were administered initially via naso-

gastric tube starting within 6 h after the transplant and then orally. If poor renal and/or poor graft function was present, a suitable adjustment in initial and subsequent doses was made. Oral tacrolimus doses were adjusted according to the clinical progress and the occurrence of adverse effects, to maintain a whole blood level of 5–14 ng/mL (aiming for 5–10) by microparticle enzyme immunoassay (Imx Tacrolimus II, Abbot Laboratories, Illinois, USA). In patients randomised to TT, the tacrolimus adjustment was the same as for the MT group. Azathioprine was continued at the same dose unchanged unless neutropenia developed. Prednisolone was gradually tapered from 3 weeks and then stopped between 3 and 4 months.

### Liver biopsies-rejection episodes

Protocol liver biopsies were planned between the 5th and 7th days after transplantation, and thereafter if clinically indicated, performed transjugularly for those with contraindications to the percutaneous route (22). If rejection was treated (1 g/day for 3 days of methylprednisolone IV) a repeat biopsy was performed 5 days after the initial methylprednisolone bolus to assess histological response to treatment.

The liver biopsy samples were formalin fixed, paraffin embedded, and stained with haematoxylin and eosin. A separate set of 20 biopsies from transplanted livers showing different degrees of rejection or other post-transplantation pathology (e.g., cholangitis, ischaemia) were reviewed by all 3 histopathologists before the start of the study, using a multi-head microscope in order to develop “a similar attitude and threshold to each feature” as described before (7). All biopsies of this study were reviewed by 3 histopathologists (A.P., A.Q., and A.P.D.), using the grading of cellular rejection according to the Royal Free Hospital (RFH) scoring system as follows: mixed portal inflammation, endothelialitis, bile duct damage, eosinophils in the portal tract, giving a maximum score of 12 (score 4–6, mild rejection; score 7–9, moderate rejection; score 10–12, severe rejection) (7). Discordant cases were discussed using a multi-head microscope to achieve a final consensus. The criteria for grading into mild, moderate, or severe cellular rejection are similar, with the exception of eosinophils in the graft, to the international Banff criteria (6).

Acute cellular rejection was treated if the RFH score was  $\geq 7$  or if there was strong clinical suspicion of rejection

and consent was not given for biopsy (empirical treatment). Mycophenolate mofetil was used if there were more than 2 episodes of moderate/severe rejection not responding to methylprednisolone boluses or as a tacrolimus-sparing agent if there was chronic renal impairment. A new episode of rejection was considered as one diagnosed by biopsy 5 days or more after the histological diagnosis of the preceding episode only if it showed the same or worse grade than the initial one or if after 5 days from the start of empirical therapy there was moderate or severe rejection. Chronic ductopenic rejection was defined according to the criteria of the International Panel (23).

### Virological assays

Serum samples before transplant and then at 1 and 3 months post transplant, were collected, stored at  $-70^{\circ}\text{C}$ , and analysed for quantitation of serum HCV-RNA by a second-generation branched DNA assay (bDNA 2.0, Quantiplex, Chiron, Emeryville, California, USA). Determination of HCV genotype was performed by reverse transcription polymerase chain reaction (PCR) and reverse hybridisation assay of the amplified sequence (InnoLipa HCV II, Innogenetics, Zwijnaarde, Belgium). Cytomegalovirus (CMV) viraemia was screened for by PCR assay twice weekly (24). Two consecutive positive CMV DNA samples were an indication to treat with ganciclovir or valganciclovir for 14 days.

### Statistical analysis

Fisher exact test was used for frequency tables and Mann–Whitney rank-sum test was used for means. Bio-Medical Data Processing (BMDP Dynamic version 7, University of California, Los Angeles, California, USA) was used for all calculations. A *P* value less than 0.05 was considered statistically significant.

## Results

By randomisation 27 patients received tacrolimus MT and 29 TT. The groups were well matched at randomisation with no significant differences except for ascites, which was more frequent in TT ( $P = 0.02$ ).

### Rejection in the first protocol biopsy

From the 56 patients, 51 had 1 biopsy or more (median 3, range: 1–7). Of the 5 without biopsy, 4 (3 MT, 1 TT) were clinically very sick and died within 1–20 days, and 1 (MT) refused consent. No histological rejection was diagnosed in 14 (MT: 8–30%; TT: 6–21%), mild in 25 (MT: 11–41%; TT: 14–50%), moderate in 11 (MT: 3–11%; TT: 8–28%), and severe rejection in 2 (MT: 2–7.5%; TT: 0–0%) patients. Median time to protocol biopsy was 6 days (MT) and 7 days (TT). Following the first biopsy, 43% TT and 46% MT were treated with methylprednisolone boluses.

### Rejection during 3 months after transplant

During the first 3 months, following and including the first biopsy, 19 MT patients (70%) had 35 rejection episodes and received 19 courses of methylprednisolone (5 empirical therapy, in 5 patients). In comparison, 25 TT patients (86%) had 46 rejection episodes and received 24 courses of methylprednisolone (3 empirical therapy, in 3 patients). Thus, over the course of 3 months, 15 MT patients (10 biopsy-proven episodes) and 17 TT patients (21 biopsy-proven episodes) were treated for rejection. Details of rejection episodes are shown in Table 2. The TT group had more rejection episodes and more moderate rejection episodes than the MT group, but neither difference was statistically significant ( $P = 0.5$  and  $0.1$ ).

### Patients without rejection or mild rejection in the first protocol biopsy

The TT patients, who did not have rejection in their first protocol biopsy, did not develop any episode of severe rejection thereafter and only 2 subsequently had moderate rejection. However, if mild rejection was present, moderate or severe rejection subsequently occurred (Fig. 1). One patient had mild rejection on first biopsy and subsequently moderate rejection and then (responding fully to treatment) developed chronic ductopenic rejection, and was re-grafted 6 months after the first transplant.

In the MT group, of those who had no rejection in the first protocol biopsy, only 1 developed moderate rejection subsequently; some who had histological rejection did subsequently develop severe rejection (Fig. 2). No patient developed chronic rejection.

**Frequency of rejection episodes in the two randomised treatment arms using protocol biopsies during the first 3 months from transplantation**

Variable	Mono-therapy	Triple therapy
Total rejection episodes	35	46
Patients with any biopsy-proven rejection episode	19 (70%)	25 (86%)
Patients without any biopsy-proven rejection episode	5 (18.5%)	3 (10%)
Patients with rejection untreated	7 (26%)	8 (27.5%)
Patients with at least 1 rejection episode	19 (70%)	26 (90%)
Patients with only 1 rejection episode	9 (33%)	11 (38%)
Patients with only 2 rejection episodes	5 (18.5%)	9 (31%)
Patients with only 3 rejection episodes	4 (15%)	3 (10%)
Patients with only 4 rejection episodes	1 (3.5%)	2 (7%)
Patients with no more than mild rejection	11 (41%)	11 (37%)
Patients with no more than moderate rejection	6 (22%)	12 (41%)
Patients with severe rejection	2 (7%)	2 (7%)
Patients receiving methylprednisolone bolus at any stage	15	17
Number of courses of methylprednisolone boluses	19	24

Monotherapy, tacrolimus alone, triple therapy, tacrolimus/azathioprine/prednisolone therapy.

**Table 2**

### Other immunosuppression and virology evaluation

In 3 cases (2 MT, 1 TT) rejection was treated without methylprednisolone boluses but with an increase in tacrolimus dosage, and in 2 patients (1 MT at 3rd week post-OLT; 1 TT at 2nd week) mycophenolate mofetil was used to potentiate the immunosuppressive regimen. Overall mycophenolate mofetil was used in 10 patients in the MT group (8 for renal dysfunction, 1 for additional immunosuppression, 1 after tacrolimus toxicity) commenced within the first month of OLT (days 4–25), and in 7 patients in TT group (3 for renal dysfunction, 3 for toxicity, 1 for ongoing rejection despite adequate tacrolimus levels) commenced within the first month of OLT (days 4–19). No antilymphocytic preparations were used.

HCV genotypes were similarly distributed: genotype 1/1b MT 41% vs. TT 41%, genotype 2/3 MT 36% vs. TT 38%, genotype 4 MT 7% vs. TT 13%. Median HCV RNA levels pre-transplant were not significantly different 150,000 IU/mL (MT) vs. 252,000 IU/mL (TT) ( $P = 0.7$ ), and nor at

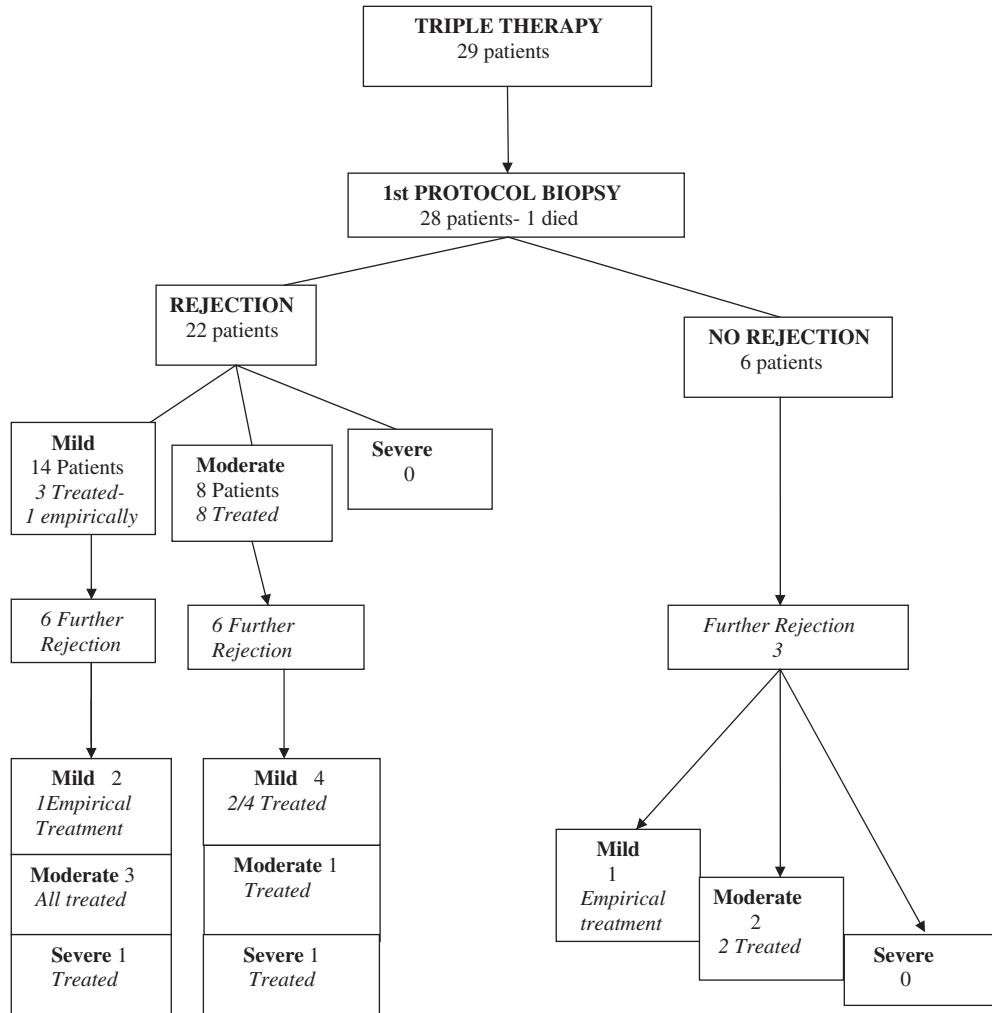


Fig. 1. Patients with tacrolimus/azathioprine/prednisolone triple therapy: rejection episodes.

1 month (MT)  $1.5 \times 10^6$  IU/mL vs. 860,000 IU/mL (TT) ( $P = 0.98$ ), nor at 3 months (MT)  $4 \times 10^6$  IU/mL vs.  $1.5 \times 10^6$  IU/mL (TT) ( $P = 0.52$ ) in keeping with the previous study from our group (25). CMV viraemia was not different in the 2 groups (5 MT, 6 TT).

### Tacrolimus concentrations

Tacrolimus levels just before or on the day of first protocol biopsy were significantly higher in the MT group: median 7.3 (range: 3.5–26.2 ng/mL) vs. TT median 5.1 (range: 2.8–12.2 ng/mL) ( $P = 0.015$ ), as also on day 3 (MT: 10 ng/mL, 2.9–28.2 vs. TT: 6 ng/mL, 2.9–14) ( $P = 0.011$ ), day 5 (MT: 9 ng/mL, 2.9–22.7 vs. TT: 6.3 ng/mL, 2.7–11.8) ( $P = 0.045$ ), and day 21 (MT: 9.6 ng/mL, 3.3–17.7 vs. TT: 6.1 ng/mL,

3–20) ( $P = 0.006$ ). Median levels at 1 month (MT: 8.5 vs. TT: 8.5 ng/mL), 2 months (MT: 7 vs. 7.5 ng/mL), and 3 months (MT: 7 vs. 6.7 ng/mL) were not significantly different ( $P > 0.05$ ). Thus, within 3 months of transplantation among the 22 patients with moderate or severe rejection, more had subtherapeutic concentrations of tacrolimus (12 patients – 4 MT and 8 TT), compared to those with mild rejection (5–2 MT and 3 TT of 22 patients), or no rejection (1 MT of 13 patients).

### Deaths and retransplantation

The median follow up was 661 days (1–1603). In both groups, 3 received a second transplant: 2 MT patients for hepatic artery thrombosis (8–12 days after first OLT) and

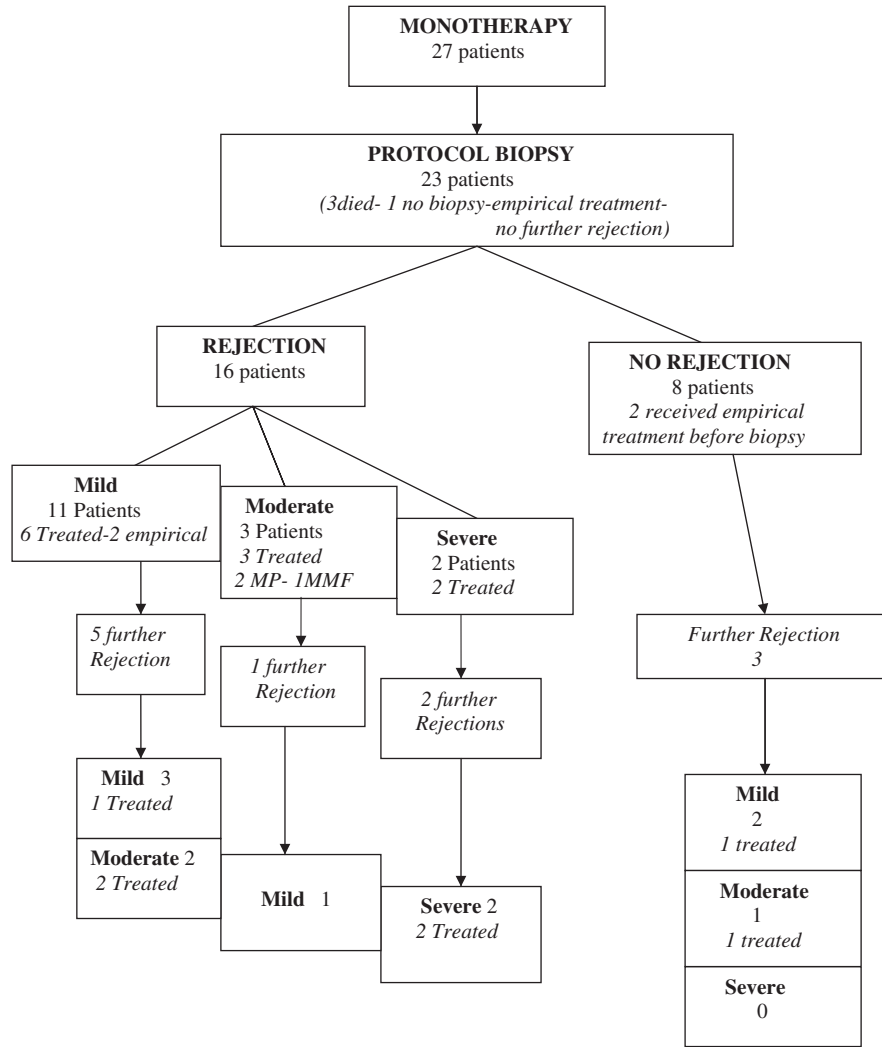


Fig. 2. Patients with tacrolimus monotherapy: rejection episodes. MP, methylprednisolone boluses; MMF, mycophenolate mofetil.

1 for primary non-function (at 2 days), while in the 3 TT patients: 1 for hepatic artery thrombosis (42 days), 1 for chronic rejection (6 months), and 1 for recurrent HCV cirrhosis (31 months). In the MT group 6 patients died; 4 from sepsis-multiple organ failure (8, 8, 20, and 123 days), 1 from graft failure (14 days), and 1 from pulmonary hypertension (6 days). In the TT group 1 patient died after re-transplant (sepsis – 2 days) and 1 at 44 days from sepsis and multiple organ failure.

**Side effects and renal function**

During the first 3 months tacrolimus was discontinued in 6 MT patients (5 because of severe renal impairment with multiple organ failure shortly before death, and in

1 because of neurotoxicity – converted to cyclosporine), and in 3 TT patients all because of neurotoxicity (2 converted to cyclosporine and 1 to mycophenolate mofetil substituting azathioprine and continuing steroids). The proportion of patients whose serum creatinine was  $\geq 130 \mu\text{mol/L}$  during the first 3 months was similar in the 2 groups, 63% vs. 62%. At 6 weeks, median creatinine was  $100.5 \mu\text{mol/L}$  in the MT group vs.  $115.5 \mu\text{mol/L}$  in the TT group (not significant), and at 3 months both groups had same median creatinine value ( $110 \mu\text{mol/L}$ , range MT: 78–222 and TT: 76–281  $\mu\text{mol/L}$ ). Antibiotics given after the first 5 days were similar, 20 in MT and 20 in TT group patients. No significant difference was found in the incidence of diabetes pre- and post-OLT according to treatment regimen.

## Discussion

We evaluated 2 different immunosuppressive regimens in liver transplant recipients with HCV cirrhosis, with respect to the frequency and severity of cellular rejection, using a schedule of protocol liver biopsies, which is the gold standard for the diagnosis of cellular rejection (26). The first 3 months after transplant, rather than just the first 6 weeks were evaluated, because this longer period usually encompasses most acute rejection episodes (9, 27) and during this time there should be far less histological confusion with recurrent HCV (28). Protocol biopsies of HCV-infected recipients are particularly useful to minimise empirical treatment of rejection, which might otherwise be given if liver dysfunction occurs, but it is not clear if some biopsies are not performed because of poor clotting or other reasons, and what severity of rejection warrants treatment.

We arbitrarily defined episodes of rejection as separate, if a protocol biopsy 5 days after histological diagnosis showed the same degree or worse rejection, and if a biopsy 5 days after the start of empirical therapy for rejection showed moderate or severe rejection. This evaluation accounts for the apparent high rate of cellular rejection over the 3-month period. If solely the first protocol biopsy data are evaluated (common to many reports on rejection rates in liver transplantation) then only 18.5% of the MT group and 27.5% of the TT group had moderate or severe rejection. Overall 56% of our cohort (4/5 of these following the first protocol biopsy) received treatment for rejection which approximates to the rate of 42% in a recent study of HCV patients with transplants, in which protocol biopsies were not performed (29).

Currently, management of patients with HCV cirrhosis who are transplanted is a major challenge. These patients constitute the leading indication for transplantation, but HCV recurrence reduces survival (30). Early management, including modification of immunosuppression, may influence severity of recurrent HCV infection (15, 17, 27, 31). Reducing immunosuppression has become more common (32, 33) and, in addition, avoidance of steroids in HCV recipients is increasingly popular using substitution with interleukin-2 receptor blockers. Methylprednisolone boluses and antilymphocytic preparations are associated with a worse evolution of recurrent HCV infection (17, 18, 34–36), with few exceptions (11). In one study (37), rejection was significantly more common among HCV recipients (genotype 1b)

who subsequently developed cirrhosis post OLT compared with those without, but another study (27) showed that cumulative frequency of acute cellular rejection was not significantly different in HCV and non-HCV recipients within the first 6 weeks post OLT. A further study reported no difference in the incidence and timing of acute cellular rejection in HCV vs. a control group (38). In contrast, a recent study (without protocol biopsies) showed that HCV aetiology was strongly associated with acute rejection (49% within 6 months and 42% receiving treatment) (29). It was unclear whether this finding was the result of either an immunologic environment associated with HCV infection, or because rejection episodes were over-diagnosed in the setting of recurrent HCV (29). The question could not be answered, as protocol biopsies were not used. The interactions between HCV, allograft rejection, and immunosuppression are complex and the diagnostic dilemma between recurrent HCV and cellular rejection can only be minimised with histological evaluation (28).

In our randomised trial, tacrolimus MT was not associated with an increased number or severity of cellular rejection episodes. The trend for more moderate rejection episodes in the TT group was probably associated with lower tacrolimus levels similar to another cohort of HCV-infected transplant recipients (29). Less likely, but also possible, is that ascites is associated with increased rejection (9), and there was more ascites in the TT group. Lower tacrolimus levels in either group were associated with moderate or severe rejection in a similar fashion so the added azathioprine, prednisolone, and mycophenolate mofetil as a renal-sparing agent (39) or to potentiate immunosuppression (used similarly in both groups) did not “compensate” for the lower tacrolimus levels. Neither immunosuppressive regimen resulted in unusual rates of retransplantation (11% in TT and 10% in MT) or of chronic rejection (1/56 – 1.8%). Interestingly, in our cohort, patients in either trial group who did not have any rejection in the first protocol biopsy did not have severe rejection during follow up (Figs. 1 and 2).

In conclusion, MT with tacrolimus is comparable to TT with tacrolimus, azathioprine, and prednisolone in terms of the frequency and severity of cellular rejection and its treatment, in HCV recipients transplanted with cadaveric livers, so that this trial continues. Whether the absence of maintenance azathioprine or steroids will be beneficial in terms of recurrence of HCV can only be evaluated with pro-

longed follow up. Our preliminary data, mainly with cyclosporine as MT (*ab initio*), was associated with less severe fibrosis (21). In a randomised study of microemulsified cyclosporine (C2 monitoring) vs. tacrolimus (40), there was no difference in cellular rejection rates diagnosed by non-protocol biopsies, but graft and patient survival was significantly better in liver transplant patients with HCV cirrhosis given cyclosporine. This is in contrast to another randomised study (41) in which graft and patient survival was no different between the 2 drugs, but HCV RNA concentrations rose far more in the cyclosporine group. On the other hand, azathioprine-containing regimens have been documented as reducing histological recurrence and progression of HCV (16, 42), and while an antiviral effect against HCV has been proposed (43), we found no substantial difference in viral loads between trial groups before or after transplantation. In addition, maintenance steroids in HCV-infected recipients have been recently associated with favourable histological outcomes (16, 44–46). The long-term histological outcomes in this randomised study will help determine whether minimising initial immunosuppression and avoiding maintenance azathioprine and steroids is of benefit or not in minimising the severity of recurrence of HCV.

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