Reduction fibrosis in recurrent HCV with tacrolimus, azathioprine and steroids versus tacrolimus: randomised trial long term outcomes

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ABSTRACT

Objective Early results of a randomised trial showed reduced fibrosis due to recurrent HCV hepatitis with tacrolimus triple therapy (TT) versus monotherapy (MT) following transplantation for HCV cirrhosis. We evaluated the clinical outcomes after a median 8 years of follow-up, including differences in fibrosis assessed by collagen proportionate area (CPA).

Design 103 consecutive liver transplant recipients with HCV cirrhosis receiving cadaveric grafts were randomised to tacrolimus MT (n=54) or TT (n=49) with daily tacrolimus (0.1 mg/kg divided dose), azathioprine (1 mg/kg) and prednisolone (20 mg), the last tailing off to tacrolimus alone.

Results No significant preoperative, peri-operative or postoperative differences between groups were found. During 96 months median follow-up, stage 4 fibrosis was reached in 19 MT/11 TT with slower fibrosis progression in TT (p=0.009). CPA at last biopsy was 12% in MT and 8% in TT patients (p=0.004). 14 MT/three TT patients reached HVPG≥10 mm Hg (p=0.002); 10 MT/three TT patients, decompensated. Multivariately, allocated MT (p=0.047, OR 3.23, 95% CI 1.01 to 10.3) was independently associated with decompensation: 14 MT/seven TT died, and five MT/four TT were retransplanted.

Conclusions Long term immunosuppression with tacrolimus, azathioprine and short term prednisolone in HCV cirrhosis recipients resulted in slower progression to severe fibrosis assessed by Ishak stage and CPA, less portal hypertension and decompensation, compared with tacrolimus alone.

ISRCTN94834276—Randomised study for immunosuppression regimen in liver transplantation.

INTRODUCTION

We published early results of a randomised trial in liver transplant recipients with HCV cirrhosis assessing tacrolimus monotherapy (MT) versus tacrolimus, azathioprine and prednisolone triple therapy (TT), which showed a slower onset of histological severe fibrosis and portal hypertension in the TT arm compared with tacrolimus alone, independent of other factors known to affect fibrosis.1 This was

SIGNIFICANCE OF THIS STUDY

What is already known on this subject?

▸ Immunosuppression worsens severity of recurrence of HCV after liver transplantation.
▸ There are very few studies and only one randomised study assessing immunosuppressive protocols on different severity of recurrence of chronic HCV hepatitis.

What are the new findings?

▸ Triple therapy with tacrolimus, azathioprine and tapering steroids resulted in less fibrosis progression compared with tacrolimus monotherapy, contrary to the initial hypothesis, that less immunosuppression should reduce progression of HCV recurrent disease.
▸ Triple therapy also resulted in less progression of clinically significant portal hypertension (hepatic venous pressure gradient ≥10 mm Hg) and less clinical decompensating events (ascites, bleeding, varices, encephalopathy).
▸ Discontinuation of azathioprine resulted in further fibrosis progression compared with continuation of azathioprine.
▸ The fibrosis progression with triple therapy is the lowest recorded in the literature.

How might it impact on clinical practice in the foreseeable future?

▸ This trial obliges a review of immunosuppressive protocols in patients transplanted with HCV cirrhosis. The use of azathioprine should be considered.
▸ Azathioprine and other immunosuppressive agents need to be tested in HCV replicon systems to assess if there is a direct antiviral effect and in cell systems for any potential immunological interaction with profibrotic and antifibrotic mechanisms.
contrary to the starting hypothesis that the lesser immunosup-
tency with MT should result in less fibrosis.

However, despite our initial trial results and other consistent
data with more patients,²⁻⁴ including a possible benefit with
azathioprine,⁵ the optimal immunosuppression for HCV transplant
recipients is still debated.⁶ Indeed, immunosuppression worsens the severity of HCV recurrence.⁶ Cyclosporine has no advan-
tage versus tacrolimus regarding stage progression⁷ despite
in vitro (but not in vivo) activity against HCV.⁸ Tacrolimus com-
pared with cyclosporine improves both patient and graft survival
including patients transplanted for hepatitis C cirrhosis⁶ and is the
preferred calcineurin inhibitor.

Therefore, we evaluated outcomes in our trial, after a median
of 8 years of follow-up, including the original end points as well as
differences in hepatic venous pressure gradient (HVPG) and
collagen proportionate area (CPA) as a quantitative measure of
fibrosis and clinical decompensation.

PATIENTS AND METHODS
Inclusion—exclusion criteria, randomisation and endpoints
From January 2000 to June 2007, at the Royal Free Hospital,
consecutive transplant recipients were randomised if they had
cirrhosis, were HCV RNA positive in serum and previous hist-
ology was compatible with HCV liver disease. Randomisation at
the Royal Edinburgh Infirmary and St Vincent’s University
Hospital was between 12/2003 and 5/2006. Inclusion and exclu-
sion criteria were published previously.¹ The study protocol was
approved by the Hospital Ethics committees at each site.

Follow-up stopped at death, retransplantation or 1/2013. We
analysed the original primary endpoints—progression to Ishak
stage 4 and graft failure either resulting in retransplantation or
patient’s death. We also evaluated patients’ survival, acute cellular
rejection episodes, chronic rejection, recurrence of HCV (defined
by Ishak inflammation score ≥4), HVPG progression to 10 mm Hg,
CPA, fibrosis progression assessed by CPA and Ishak stage (for
comparison with other published studies), and time to first episode
of clinical decompensation defined as whichever occurred first,
of ascites/hydrothorax, variceal bleeding or encephalopathy.

For each patient, the following were evaluated: demographic
and clinical data (including concomitant hepatocellular carcino-
ma (HCC) and/or alcoholic aetiology), donor age/gender, cold/
warm ischaemia time, initial immunosuppression and subse-
quent changes, occurrence and treatment of rejection episodes,
cytomegalovirus or other infections, histological episodes of de
novo hepatitis, genotype, viral load pre-LT and 1 year post-
transplantation (LT), antiviral treatment and sustained viro-
logical response (SVR), diabetes mellitus pre-transplant and post-
transplantation (LT), human leucocyte antigen and blood group compati-
bility of donor/recipient, sex match/mismatch and tacrolimus
levels at 5, 15 and 30 days post-LT.

Immunosuppression regimens
The MT group received tacrolimus 0.1 mg/kg/day (two divided
doses). The TT group received the same tacrolimus dosing,
together with azathioprine—initially intravenously then orally—
1 mg/kg/day, and methylprednisolone (16 mg/day intravenous)
until oral intake was established (20 mg/day prednisolone).
Tacrolimus trough concentrations were evaluated on alternate
week days. The doses were adjusted to maintain a whole blood
level within 5–14 ng/mL (aiming for 5–10). Azathioprine was
continued at the same starting dose unless neutropenia devel-
oped: 16 patients discontinued azathioprine between 10 and
37 months (median 14 months) post-LT. Prednisolone was grad-
ually tapered from 3 weeks onwards and then stopped between
3 and 6 months, according to each centre’s practice. Protocol
biopsies were undertaken to diagnose acute cellular rejection
between days 5 and 10. If moderate or severe rejection was
diagnosed, daily intravenous 1 gm methylprednisolone was
given for 3 days.⁹

Virological assays
Determination of HCV genotype was performed by reverse
transcription PCR and reverse hybridisation assay of the ampli-
ﬁed sequence (InnoLipa HCV II, Innogenetics, Zwijnaarde,
Belgium).¹⁰
Qualitative tests were performed by a reverse transcription
PCR assay (Amplicor HCV Roche Diagnostic Systems Inc,
Branchburg, New Jersey, USA—later Bayer Thermomechanical
Analyzer Component system, Berkeley, California, USA).¹¹

Liver biopsies
Protocol liver biopsies were performed at yearly intervals and
also if clinically indicated. All liver biopsies were reviewed by
liver trained histopathologists in each centre, in a blinded
fashion.

Liver biopsy samples were formalin ﬁxed, parafﬁn embedded
and stained with H&E, Gordon and Sweet staining was used
for reticulin and Sirius red and Van Gieson stains were used in
Edinburgh. All biopsies at the Royal Free Hospital were
retained with picroSirius Red to ensure comparable staining
for collagen quantification and determination of CPA by digital
image analysis. The stage of disease (ﬁbrosis 0–6) and the grade
of necroinﬂammatory activity were evaluated according to Ishak
et al.¹²

The number of liver cores, length of biopsy (lengths of each
core summed), and portal tract number per core and in total
were recorded.¹³ Liver biopsies<12 mm long were excluded (29
of 310). Complete portal tracts were deﬁned according to
Crawford and colleagues.¹⁴ The equipment used and CPA mea-
surement were performed as previously described.¹⁵

Acute cellular rejection was graded using the Royal Free
Hospital score.¹⁶ Histologico novohepatitis (HDNH) C
termed ‘histological acute hepatitis C’ previously¹ ² was deﬁned
as before as an increase in alanine aminotransferase levels (>2
upper normal limit), together with histological changes consist-
ent with hepatitis without diagnostic features of cellular rejec-
tion, duct loss or other cause of liver injury, including
alloimmune hepatitis (autoantibodies negative).²

Fibrosis progression in each patient was calculated by percent-
age change of ﬁbrosis according to CPA (CPA in latest biopsy
subtracted from CPA at 1 year post-LT, divided by time in years
between the two biopsies: CPA%/year). Ishak stage progression
was calculated as in latest biopsy subtracted from stage in the
biopsy at 1 year, divided by time in years—stage ‘units’/year
between the two biopsies.

Statistical analysis
Results are expressed as median and ranges. Categorical vari-
ables were compared using the χ² or Fisher’s exact tests.
Continuous variables were compared by Student t test, or if not
normally distributed by the Mann–Whitney test. We compared
the two randomised groups for histological, virological and clin-
ic outcome in a preplanned evaluation.

Statistical comparison was made by life tables (Kaplan–Meier)
and log rank testing. All the following variables associated with
HCV recurrence in the literature (table 1)—except IL28B—were
evaluated in the Cox regression analysis: donor age/gender, cold/
warm ischaemia time, initial and changes in immunosuppression

ocurrence and treatment of rejection episodes, cytomegalovirus or other infection, HDNH episodes, genotype, viral load pre-LT and 1 year post-LT, antiviral treatment and SVR, diabetes mellitus pre-LT and post-LT, human leucocyte antigen and blood group compatibility of donor/recipient, sex match/mismatch and tacrolimus levels at 5/15/30 days post-LT.

A p value<0.05 was considered statistically significant. SPSS V2.0.0 was used.

Patients were censored at the time of SVR, when analysing progression to HVPG≥10 mm Hg, to CPA cut-offs of 6% and 7.2%, to first clinic decompensation event, and survival. As reported previously, sample size was calculated to be 110 patients: S4 was estimated to occur in 35% by 3 years with a projected decrease to 10% with tacrolimus MT, and this required a sample size of 99. Considering a dropout rate of 10% (including early deaths), we concluded that 110 patients would be needed with a two-tailed test with significance at 5% and a power of 80% (1-0.05).17

RESULTS
Patients’ demographic and transplant data
A total of 103 patients (89 Royal Free Hospital, eight Royal Edinburgh Infirmary and six St Vincent’s Hospital) were randomised as previously documented:1 29 women, 74 men; 30 had HCC and 22 concomitant alcoholic liver injury. In the last follow-up, there were only two alcohol relapers, one in each randomised arm. Two patients were coinfected with hepatitis B or D virus (remained HBV–DNA negative in blood throughout the study).

By randomisation, 54 received MT and 49 TT. The groups were well matched at randomisation with no significant differences in preoperative, peri-operative or immediately post-operative variables: four were retransplanted early post-LT, two for hepatic artery thrombosis and one for primary non-function in the MT group; and one TT for hepatic artery thrombosis. Two MT patients were withdrawn within the first month post-LT because of side effects of treatment.

We analysed the remaining 97: 49 MT and 48 TT. Median follow-up was 96 months (range 1–146); 91.6 months MT and 98.7 months TT; 15/97 (15.5%) died within 3 years from randomisation. In all, 78/97 (80%) had a follow-up of more than 3 years, fulfilling the sample size calculation based on Log rank testing.

Clinical characteristics were well balanced and HCV genotypes were similarly distributed. In tacrolimus MT: 20 patients genotype 1 (41%), seven genotype 2 (14%), 16 genotype 3 (33%) and six genotype 4 (12%). In TT group, the distribution was 20 patients genotype 1 (41%), one genotype 2 (2%), 19 genotype 3 (40%), seven genotype 4 (15%) and genotype 5 (2%).

Median HCV RNA levels were not significantly different, pre-LT and 3 m post-LT. Genotype 1/1b, Antiviral treatment SVR DM

Table 1 Variables evaluated in the univariate analysis for the different endpoints examined

<table>
<thead>
<tr>
<th>Variable</th>
<th>MT</th>
<th>TT</th>
<th>p Value</th>
<th>CPA≥6%</th>
<th>CPA≥7.2%</th>
<th>HVPG≥10 mm Hg</th>
<th>Decompression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold/warm ischaemia time (min)</td>
<td>680/46</td>
<td>688/41</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment allocation (evaluated)</td>
<td>54 (49)</td>
<td>49 (48)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stopped azathioprine</td>
<td>NA</td>
<td>16 (33%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td>0.013</td>
</tr>
<tr>
<td>Tacrolimus trough concentrations (ng/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 days</td>
<td>7.7</td>
<td>5</td>
<td>0.002</td>
<td>0.002</td>
<td>0.01</td>
<td>0.017</td>
<td>0.01</td>
</tr>
<tr>
<td>15 days*</td>
<td>8.5</td>
<td>6.1</td>
<td>0.003</td>
<td>0.001</td>
<td>0.01</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>30 days*</td>
<td>7.9</td>
<td>6.7</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Conc. HCC</td>
<td>17</td>
<td>13</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
</tr>
<tr>
<td>Conc. ALD</td>
<td>10</td>
<td>12</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>48.9 years</td>
<td>50 years</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Donor age</td>
<td>48.5 years</td>
<td>44 years</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Gender mismatch</td>
<td>17</td>
<td>17</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Rejection episodes</td>
<td>42</td>
<td>64</td>
<td>0.002</td>
<td>0.0033</td>
<td>0.04</td>
<td>0.033</td>
<td>NS</td>
</tr>
<tr>
<td>Rejection treatment (courses)</td>
<td>21</td>
<td>30</td>
<td>0.002</td>
<td>0.01</td>
<td>0.03</td>
<td>0.038</td>
<td>NS</td>
</tr>
<tr>
<td>HDNH</td>
<td>17</td>
<td>8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CMV viraemia treated</td>
<td>7</td>
<td>10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Viral load logIU/mL (median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-LT</td>
<td>5.29</td>
<td>5.36</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>3 m post-LT</td>
<td>6.6</td>
<td>6.39</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Genotype 1/1b</td>
<td>41%</td>
<td>41%</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Antiviral treatment</td>
<td>19</td>
<td>11</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>SVR</td>
<td>3</td>
<td>3</td>
<td>NS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-LT (last follow-up)</td>
<td>13</td>
<td>13</td>
<td>0.013</td>
<td>0.038</td>
<td>0.044</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Median trough levels derived from all measurements up to days 15 and 30 post-LT.

ALD, alcoholic liver disease; CMV, cytomegalovirus; CPA, collagen proportionate area; DM, diabetes mellitus; HCC, hepatocellular carcinoma; HDNH, histological de novo hepatitis; HVPG, hepatic venous pressure gradient; LT, liver transplantation; MT, monotherapy; NA, non-applicable; NS, non-significant; SVR, sustained virological response; TT, triple therapy.
800 mg daily. The HDNH episodes were only diagnosed within 6 months post-LT, and occurred in 17 MT and eight TT patients.

Tacrolimus trough levels were a median of 7.7 ng/mL (MT) and 5 ng/mL (TT) (p = 0.03) at 5 days; 8.5 ng/mL (MT) and 6.1 ng/mL (TT) (p = 0.017) at 15 days post-LT; and 7.9 ng/mL (MT) and 6.7 ng/mL (TT) (p = 0.031) at 30 days post-LT. The difference in median tacrolimus levels was maintained up to 6 months post-LT: 7 ng/mL in MT group versus 5.8 ng/mL in TT arm (p = 0.044). There was no difference at 1 year post-LT. The median tacrolimus levels at 1 year post-LT were not significantly different between those who discontinued azathioprine before 1 year post-LT (7.7 ng/mL) versus those who were still on azathioprine at that point (6.7 ng/mL).

There were no new rejection episodes compared with those previously published. During the first 3 months, 22 MT had 42 rejection episodes and 16 received 21 courses of methylprednisolone. In comparison, 31 TT had 64 rejection episodes and 24 received 30 courses of methylprednisolone. It should be noted that after the last bolus, steroid dosage (if in TT arm) reverted to 20 mg/day (ie, no high to low tail off of oral steroids) or none in MT arm.

The proportion of patients whose serum creatine rose to ≥130 μmol/L (1.5 mg/dL) during the first 3 months post-transplant was similar: 23% MT versus 20% TT. At the last follow-up, abnormal serum creatine (>1.5 mg/dL) was noted in nine out of 37 (24%) patients alive in the MT and five out of 41 (12%) patients alive in TT group. No patient has ended up on dialysis. Incidence of diabetes post-LT was 33% MT and 31% TT.

There was no difference in numbers of biopsies and average number of biopsies/patient performed between groups. Patients' characteristics are shown in table 2. The number of patients with suitable biopsies evaluated for Ishak staging was 90/97 (45 MT/45 TT) and for CPA was 81/89 Royal Free patients (37 MT/44 TT).

Factors associated with reaching Ishak stage 4 or greater
At least stage 4 was reached by 19 MT patients at a median of 32 months: eight reached stage 4, eight stage 5 and three stage 6. Correspondingly, 11 TT patients reached stage 4 or greater at a median of 49 months (six reached stage 5, none stage 6). Patients receiving antiviral treatment or achieving SVR were not censored as treatment in all patients was initiated after reaching Ishak stage 4 or greater. In univariate and multivariate analyses, three factors were associated with fibrosis stage ≥4: randomisation to allocated MT treatment (p = 0.001, OR 2.94, 95% CI 1.3 to 6.7), HDNH (p = 0.015, OR 2.67, 95% CI 1.23 to 5.8) and discontinuation of azathioprine (p = 0.036, OR 2.24, 95% CI 1.3 to 3.9). Patients who died without reaching stage 4 (four MT, one TT) were censored at the last biopsy with its Ishak stage. Hazard curves of MT/TT allocation (p = 0.005 Mantel–Cox) and use/discontinuation of azathioprine (p = 0.003 Mantel–Cox) are shown in figure 1.

Factors associated with CPA cut-offs
As previously published, CPA of 6% at 1 year biopsy post-LT was highly predictive of clinical outcome in a larger recurrent HCV population after liver transplantation and CPA of 7.2% was independently associated with portal hypertension, that is, HVPG ≥6 mm Hg. We used the cut-off of 6% of CPA and the time to first reach CPA ≥6% to compare the allocated treatments, including a multivariate analysis: 20 MT patients reached CPA ≥6% (20/37 or 54%) at a median of 41 months, while 13 TT reached CPA ≥6% (13/44, 30%) at a median of 49 months. In the multivariate analysis, allocated treatment with MT (p = 0.007, OR 2.67, 95% CI 1.31 to 5.46) and HDNH (p = 0.001, OR 3.27, 95% CI 1.61 to 6.6) were independently associated with CPA ≥6%. For CPA ≥7.2%, allocated MT treatment (p = 0.004, OR 2.85, 95% CI 1.38 to 5.86), HDNH (p = 0.006, OR 2.69, 95% CI 1.32 to 5.48) and discontinuation of azathioprine (p = 0.011, OR 2.1, 95% CI 1.1 to 2.8) were independent factors. Hazard curves are shown in figure 2 for CPA cut-offs of 6% and 7.2% (p = 0.002 and p = 0.001 Mantel–Cox respectively).

Factors associated with portal hypertension
HVPG measurement was performed combined with transjugular biopsies in 33 MT and 31 TT patients. These represent 78% MT and 72% TT patients surviving at 1 year at the Royal Free Hospital as HVPG was not measured in Edinburgh or Dublin. There were 154 HVPG measurements post-LT: 64 at a median of 17 months, 41 at a median of 31 m, 30 at a median of 48 m, 15 at a median of 63 m and four at a median of 86 m. HVPG ≥10 mm Hg (clinically significant portal hypertension) was reached in 11 MT compared with four TT patients. Cox regression analysis revealed HDNH (p = 0.015, OR 3.68, 95% CI 1.26 to 10.69) and allocated MT treatment (p = 0.017, OR 4.13, 95% CI 1.28 to 13.29) to be independently associated with HVPG ≥10 mm Hg: hazard curves are shown in figure 3 (p = 0.027 Mantel–Cox and 0.019 Breslow).

Table 2 Characteristics of patients transplanted for HCV cirrhosis randomised to either tacrolimus monotherapy or triple therapy (tacrolimus, prednisolone and azathioprine)

<table>
<thead>
<tr>
<th>Follow-up (median)</th>
<th>Monotherapy</th>
<th>Triple therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 (1–146) m</td>
<td>91.6</td>
<td>98.7</td>
</tr>
<tr>
<td>Biopsies performed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Years 1/2</td>
<td>34/27</td>
<td>40/30</td>
</tr>
<tr>
<td>Years 3/4</td>
<td>26/17</td>
<td>27/19</td>
</tr>
<tr>
<td>Years 5/6</td>
<td>18/14</td>
<td>16/13</td>
</tr>
<tr>
<td>Years 7/8</td>
<td>11/9</td>
<td>10/9</td>
</tr>
<tr>
<td>Years 9/10</td>
<td>2/5</td>
<td>2/2</td>
</tr>
<tr>
<td>Biopsy index*</td>
<td>3.31</td>
<td>3.48</td>
</tr>
<tr>
<td>Reaching Ishak stage 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Median</td>
<td>32 m</td>
<td>49 m</td>
</tr>
<tr>
<td>Reaching CPA ≥6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20/33 (61%)</td>
<td>13/39 (33%)</td>
</tr>
<tr>
<td>Median</td>
<td>41 m</td>
<td>49 m</td>
</tr>
<tr>
<td>Reaching CPA ≥7.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>21/33</td>
<td>14/39</td>
</tr>
<tr>
<td>Median</td>
<td>42 m</td>
<td>51 m</td>
</tr>
<tr>
<td>Reaching HVPG ≥10 mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>11/33</td>
<td>4/31</td>
</tr>
<tr>
<td>Decompensated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (months)</td>
<td>9 (70 months)</td>
<td>4 (91 months)</td>
</tr>
<tr>
<td>Deaths</td>
<td>14</td>
<td>7</td>
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</table>

*Biopsy index per patient (months of follow-up divided by the number of biopsies from 1 year onwards). CPA, collagen proportionate area; HVPG, hepatic venous pressure gradient.
Factors associated with clinical decompensation

Decomposition occurred in 13 patients (all reached stage 5/6 at the time of decompensation) at a mean of 82 months: nine MT patients (seven ascites, one variceal bleeding, one encephalopathy) at a mean of 70 m and four TT patients (three ascites, one encephalopathy) at a mean time of 91 m. Hazard curves are shown in figure 3 (p=0.015 by Mantel–Cox, 0.037 Breslow).

In the Cox regression analysis, allocated MT treatment was the only statistically significant factor associated with clinical decompensation (p=0.047, OR 3.23, 95% CI 1.01 to 10.3).

Fibrosis progression rate

We evaluated 310 biopsies for Ishak stage and 289 biopsies for CPA due to inadequate material for restaining. The number of biopsies performed every year were: 74 in year 1 (34 MT/40 TT), 57 in year 2 (27 MT/30 TT), 53 in year 3 (26 MT/27 TT), 36 in year 4 (17 MT/19 TT), 34 in year 5, (18 MT/16 TT), 27 in year 6 (14 MT/13 TT), 21 in year 7 (11 MT/10 TT), 18 in year 8 (9 MT/9 TT), 4 in year 9 (2 MT/2 TT) and 5 in year 10 (3 MT/2 TT). Fibrosis progression according to CPA and stage progression over time are shown in figure 4. Median CPA fibrosis progression rate was 0.7%/year (0.74%/year MT, 0.67%/year TT). Mean stages per year (given for comparison with published studies) were: at 1 year (1.45), 2 years (2.1), 3 years (2.38), 4 years (2.86), 5 years (3), 6 years (3.2), 7 years (3.2) 8 years (3.47), 9 years (4) and 10 years (5). Mean stages for MT group were: 1.58, 2.37, 2.77, 3.18, 3.28, 3.66, 3.22, 4, 5 and 3, and for TT group were: 1.3, 1.9, 2.57, 2.6, 2.77, 3, 3.87, 3 and 3 for years 1–10 post-LT respectively. Mean Ishak stage progression rate was 0.2 stage U/year (0.3 stage U/year in MT, 0.2 stage U/year TT).

Fibrosis progression rate was not significantly different between patients requiring steroids boluses for acute cellular rejection or not. Median CPA fibrosis progression rate was 0.72%/year in those requiring steroids boluses and 0.69%/year in those not treated for acute cellular rejection. Mean Ishak stage progression rate was 0.2 stage U/year in both groups.

Factors associated with early survival overall survival and graft survival

In the TT group three patients died within 3 months: one patient after retransplant (sepsis—2 days) and two at 1 month from sepsis and multiple organ failure. There were another four deaths: one due to non-liver related causes at 6 months, two from recurrent HCV disease (30 and 42 months) and one at 95 months from recurrent HCC.

In the MT group, 14 patients died: five from sepsis and multiple organ failure (0.3, 0.3, 0.6, 1 and 4 months), one from graft failure (0.5 months), one from pulmonary hypertension (0.2 months), one from ductopenic rejection (18 months) and one from recurrent HCV disease (30 months). Six more patients died in the current update: three from recurrent HCV disease (36, 37 and 72 months post-LT), one from embolic stroke (56 months) and one from lung adenocarcinoma (96 months).

Overall, mortality in the randomised 103 patients favoured TT patients (p=0.036) as well as the 97 currently evaluated (p=0.025). However, there was no statistically significant factor associated with mortality in the multivariate analysis, and no significant difference in deaths due to HCV recurrence.

Similarly, graft survival favoured the TT arm (p=0.019): 15 MT and seven TT patients died, and five MT/4 TT were retransplanted, but this difference was not due to recurrent disease.
**DISCUSSION**

In this final evaluation of our randomised study,\(^1\) the median follow-up was just over 8 years, the longest published to date following liver transplantation in a randomised trial concerning HCV cirrhosis,\(^6\)\(^\text{18}\) and given the numbers who either died within 3 years or survived more than 3 years (used for sample size calculation), this allowed a robust evaluation of clinical outcomes of recurrent HCV disease. Our outcomes included first clinical decompensation event (evaluated for the first time), HVPG and fibrosis progression defined by CPA and Ishak stage.

Fibrosis progression after liver transplantation for hepatitis C related cirrhosis has been studied several times\(^{19-24}\) mainly by assessing changes in Ishak/Metavir scores. Fibrosis progression is well recognised to have prognostic significance before\(^25\) and after transplantation.\(^4\) The overall rate of increase of disease stage described by changes in Ishak staging in our total trial population is similar or lower than fibrosis rates previously described.\(^{22-24}\) The mean fibrosis Ishak stage was 1.45 and 2.1 in years 1 and 2 post-LT in our whole cohort, and 1.3 and 1.9 at years 1 and 2 post-LT respectively for the TT group, which are as low as those recently published in patients receiving sirolimus (which may have antifibrotic properties) compared with historical controls (0.62 and 1.15 metavir in year 1 and 2 biopsies, respectively).\(^26\)

### Table 1

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<tr>
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<td>20</td>
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### Table 2

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<th>CPA 7.2%</th>
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</tbody>
</table>

**Figure 2** Hazard curves of collagen proportionate area (CPA) 6% and 7.2%. Hazard curves of reaching CPA 6% and 7.2% with those achieving sustained virological response censored (18 m MT, 36 m TT, 36 m MT, 38 m TT, 40 m TT, 52 m MT) in the two treatment arms (p=0.002 for CPA 6% and p=0.001 for CPA 7.2% by Mantel–Cox). Sixteen patients discontinued azathioprine between 10 and 37 months post-LT. MT, monotherapy; TT, triple therapy.
The relatively low overall rate of increase of disease stage in our trial population most likely reflects use of combined therapy of tacrolimus with azathioprine and steroids and/or the generally lower trough levels of tacrolimus achieved. Since our first publication, we have shown that CPA assessment in HCV post-LT patients correlates with both Ishak stage scores and HVPG, with greater percentage changes in CPA, compared with HVPG in early portal hypertension. Moreover, CPA at 1 year biopsy post-LT is highly predictive of clinical outcome in HCV transplanted patients and better than Ishak stage or HVPG. In the current update, MT and HDNH were independently associated with reaching CPA cut-offs of 6% and 7.2%, the latter corresponding to HVPG ≥ 6 mm Hg, reinforcing both the value of CPA and validating these cut-offs as endpoints.

As in our first publication, outcomes favouring the TT group are supported by HVPG measurements. There was more clinically significant portal hypertension (associated with advent of complications and worse survival) in the MT group (11 patients reached HVPG≥10 mm Hg, compared with four TT) corroborating the more severe fibrosis found in MT patients. The significance of HDNH is again confirmed by us and others as this was independently associated with Ishak stage 4, but also CPA cut-offs for decompensation and HVPG.

A limitation in this study could be the lack of evaluation of recipient IL28B polymorphism, which was also missing in the recent sirolimus study. Some studies suggest a higher recurrence rate of HCV hepatitis with the poor interferon response allele of IL28B in the recipient. However, randomisation will most likely have balanced out the more favourable polymorphism, which in any case is under represented in liver transplant populations. Moreover, IL28B was not significantly associated with either overall or liver related mortality in one study, and there has been a selection bias in most studies, as only patients with biopsies were evaluated, that is, not every patient with recurrent hepatitis C was biopsied. Randomisation will most likely have balanced out the more favourable polymorphism, which in any case is under represented in liver transplant populations. Moreover, IL28B was not significantly associated with either overall or liver related mortality in one study, and there has been a selection bias in most studies, as only patients with biopsies were evaluated, that is, not every patient with recurrent hepatitis C was biopsied. Randomisation will most likely have balanced out the more favourable polymorphism, which in any case is under represented in liver transplant populations. Moreover, IL28B was not significantly associated with either overall or liver related mortality in one study, and there has been a selection bias in most studies, as only patients with biopsies were evaluated, that is, not every patient with recurrent hepatitis C was biopsied.

Our TT group received azathioprine, which many centres have substituted with mycophenolate which however may worsen fibrosis progression compared with azathioprine. In addition, low dose maintenance steroids may also favour less severe recurrence and improve graft survival. However, the use of steroids in transplantation for HCV cirrhosis remains an unresolved issue. Interestingly, although steroids boluses greatly increase HCV RNA levels, our TT group had more rejection episodes treated with high dose steroids, so it may be that the long term benefit of immune modulation with steroids is more important than the risk of HCV activation following steroid boluses for rejection. Indeed, a recent study has shown no distinct advantage of steroid avoidance and another group have shown that steroid-free immunosuppression leads to a higher rate of chronic rejection.

In conclusion, long term maintenance immunosuppression with tacrolimus, azathioprine and shorter term prednisolone in HCV cirrhosis recipients resulted in a slower onset of histological severe fibrosis confirmed by Ishak stage and CPA, less portal hypertension, and less clinical decompensation compared with tacrolimus alone. Our results support and extend our initial report. Given that our TT arm has the lowest fibrosis progression rate published so far—lower than sirolimus—and that there is some evidence that azathioprine is better than...
mycophenolate, our TT regimen could be considered as a first choice for patients transplanted for HCV cirrhosis, until other evidence proves otherwise.

**Competing interests** AKB and APD have an unrestricted educational grant from Pfizer.

**Ethics approval** The hospital ethics committees at each site.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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**REFERENCES**


