EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Immunosuppression and HCV recurrence after liver transplantation

Dimitrios N. Samonakis, Giacomo Germani, Andrew K. Burroughs*

The Royal Free Sheila Sherlock Liver Centre and University Department of Surgery, Royal Free Hospital and UCL, London, UK

Summary

HCV related liver disease is the most common indication for liver transplantation. Recurrence of HCV infection is universal and has a substantial impact on patient and graft survival. Immunosuppression is a major factor responsible for the accelerated recurrence and compressed natural history of recurrent HCV infection. Accumulating experience has provided data to support certain strategies for immunosuppressive regimens.

From the available evidence, more severe recurrence results from repeated bolus corticosteroid therapy and anti-lymphocyte antibodies used to treat rejection. Low dose and slow tapering of steroids are better than high dose maintenance and/or rapid tapering. Recent meta-analyses favour steroid-free regimens but these are complicated to interpret as the absence of steroids may simply represent less immunopotency.

There is no difference in HCV recurrence between tacrolimus and cyclosporine regimens, but tacrolimus increases graft and patient survival in HCV transplanted patients. There may be a beneficial effect of maintenance azathioprine given for 6 months or longer. There is no conclusive evidence for benefit of mycophenolate and interleukin-2 receptor blockers. Few data are available for mTOR inhibitors. Better evidence is needed to establish the optimal immunosuppressive regimen for HCV recipients and more randomized trials should be performed.

© 2011 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Introduction

Hepatitis C virus (HCV) related cirrhosis is currently the most frequent indication for liver transplantation (LT). However, graft reinfection with HCV is universal in all patients who are HCV-RNA PCR positive at transplantation, and the progression of fibrosis is accelerated compared to non-transplanted patients [1], resulting in cirrhosis in about 30% of recipients by 5 years [2], with rapid decompensation and reduced survival thereafter [3,4]. Chronic HCV infection in the liver allograft looks similar to HCV in the non-transplant liver but there are significantly higher levels of viral replication [5].

A variety of pre-transplant, operative, and post-transplant factors have been associated with severity of HCV recurrence. Immunosuppression is a major factor and is still an area of uncertainty, due to the lack of good evidence for what may be better or worse [6]. Immunosuppression for HCV patients represents a fine balance between suppressing immunity and maintaining optimal host viral responses. A major problem is that a universal measure reflecting immunopotency of different immunosuppressive regimens does not exist. In addition, there are few randomized trials. Thus, evaluating the relationship between severity of HCV recurrence and immunosuppression remains a descriptive process, assessing type, dose, and duration of immunosuppressive drugs.

Moreover, other important factors such as age and quality of the donor organ need to be taken into account [7], and often these are not evaluated when assessing different immunosuppressive regimens [8]. There is little uniformity in immunosuppression regimens and wide variations in the timing and description of the evaluation of HCV recurrence: both make it difficult to draw useful conclusions for clinical practice. A recent international survey reviewed immunosuppression policy for HCV-transplanted patients in 81 centres [9]: a third had specific differences in immunosuppression between HCV *vs.* non-HCV recipients and most of the rest used variations of tacrolimus-based therapies.

Calcineurin inhibitors (CNIs)

Mechanisms of action and experimental data

The CNIs cyclosporine A (CyA) and tacrolimus (Tac) are the principal maintenance immunosuppressives. They inhibit calcineurin, a key enzyme for IL-2 production by T-cells, which recruits and activates CD4 T-cells, and via cytokine induction, affects cytotoxic CD8 cells, NK cells, and B cell activation [10]. Thus, the amount of IL-2 determines the magnitude of the immune response and rejection. HCV infection may influence CNI metabolism. A retrospective evaluation [11] of HCV and alcoholic liver disease (ALD) patients showed that, for both CNI, significantly lower doses were needed to achieve similar blood concentrations in HCV compared to ALD patients.

In vitro, CyA inhibits HCV replication by specific blockade of cyclophyllins (intracellular ligands of CyA) that bind to HCV nonstructural protein 5B, but in concentrations greater than therapeutic levels in man (1–10 μg/ml). There is controversy about effects *in vivo*. Tac does not have any anti-HCV activity [12,13].

Keywords: Immunosuppression; HCV; Liver transplant.
Received 16 September 2010; received in revised form 27 May 2011; accepted 1 June 2011

* Corresponding author. Address: The Royal Free Sheila Sherlock Liver Centre, Royal Free Hospital, Pond Street, Hampstead, NW3 2QG, London, UK. Fax: +44 207 4726226.
E-mail address: andrew.burroughs@nhs.net (A.K. Burroughs).

Clinical studies – observational

In a consecutive series of 193 HCV transplant recipients at the Royal Free Hospital [14] with 96 receiving Tac and 92 CyA, there were no differences in fibrosis progression nor survival. From the same population of HCV transplant recipients, 96 patients with at least 3 years follow-up were evaluated to assess changes in fibrosis and identify factors potentially associated with slower fibrosis progression after LT (Ishak score and Sirius red for digital image analysis of collagen, expressed as Collagen Proportionate Area – CPA) [15]: sustained virological response (SVR) ($p = 0.01$) and Tac plus AZA ($p = 0.024$) were associated with slower fibrosis progression, based on CPA.

In a retrospective study of 516 HCV patients published in abstract form, Tac improved the 5-year survival compared to CyA (75% *vs.* 67%, $p = 0.02$) despite there being neither significant difference in 1-year survival nor in fibrosis progression [16].

In a UNOS based analysis [17], HCV recipients receiving CyA at discharge from hospital had three-year patient and graft survival of 76.8% and 71.5%, respectively *vs.* 79.9% and 75% in the Tac group. CyA treated patients were at increased risk of death (HR 1.17; 95% CI 1.01–1.36), graft failure (HR 1.19; 95% CI 1.04–1.35) and biopsy-confirmed acute rejection (HR 2.03; 95% CI 1.54–2.67).

CyA may influence the efficacy of antiviral therapy in transplant recipients; CyA was a predictor of sustained virological response (SVR) in one cohort of 99 patients, but 57% also had azathioprine (AZA) which was not evaluated [18]. In 115 patients, treatment with CyA and low dose IFNa resulted in greater inhibition of HCV replication compared to either CyA or IFNa alone [19]. In a retrospective study of 446 recipients published by Selzner 2009 *et al.* [20], 39% (172 patients) received treatment; SVR was higher in patients treated with CyA (56%) than with Tac (44%) but this failed to reach statistical significance. Recently, another retrospective analysis of 464 recipients treated for HCV recurrence in 12 Italian centres evaluated the impact of CNIs on SVR [21]. End of treatment response was significantly higher in those treated with CyA (64%) than with Tac (54.5%) ($p = 0.04$), but there was no difference either in SVR or in survival. From the same database, Rendina *et al.* [22] reported an SVR rate of 35%, which significantly improved on patient survival. The rate of acute/chronic rejection in this population under interferon was around 6%.

In 16 living donor recipients [23] with Tac/corticosteroids (Cs) induction and conversion to CyA, a subsequent pre-emptive antiviral therapy was used in 11 patients: only three showed SVR (21.4%). In 120 non-transplanted patients with chronic HCV treated with IFN-a2b in combination with CyA, SVR was increased [24]. In another study, in 10 patients there was an ALT reduction but no virological response [22].

Clinical studies – randomized trials and meta-analyses (Table 1)

An early randomized controlled trial (RCT) [25] randomized 50 HCV recipients to Tac (n = 25) or CyA; no difference in HCV recurrence nor in graft and patient survival was observed. Martin *et al.* [26] randomized 79 patients to Tac (n = 38) or CyA (n = 41); despite significantly higher HCV RNA levels at 1, 6, and 12 months in CyA-treated recipients, there was no difference in histological HCV recurrence or in patient survival.

A multicentre prospective (RCT) assessed Tac/corticosteroids (Cs) *vs.* Tac/AZA/Cs [27] in a subgroup of HCV recipients: 50 dual and 35 in triple therapy, with tapering of steroids at 3 months, and then steroid withdrawal in both arms. Despite increased biopsy proven acute rejection in the dual regimen ($p = 0.008$), there was no difference in patient and graft survival.

In another RCT, a subgroup of 20 HCV patients was randomized to Tac, and 15 to Tac/Cs [28]. A steroid taper was instituted starting at a very high dose of 100 mg bd of methylprednisolone on day 1 post LT, decreasing to 20 mg/day by day 6, and weaned completely between 9 and 12 months. The incidence and severity of graft hepatitis C at 3 years was reduced in Tac alone (47%) compared with Tac + Cs (67%) (p = n.s.), but at 5 years there were no statistically significant differences in fibrosis score nor in survival rates.

The Valencia group [29] reported an RCT comparing Tac *vs.* CyA both combined with steroids in 90 HCV-positive recipients: severe or cholestatic hepatitis in CyA (12/44) and in Tac (15/46) groups was similar.

More recently Manousou *et al.* [30] randomized patients to Tac monotherapy (n = 54) *vs.* triple therapy (n = 49) (Tac 0.1 mg/kg/day, low dose prednisone 20 mg/day and AZA 1 mg/kg). Steroids were tapered to zero by 3–6 months, and follow-up was a mean of 53.5 months. The predetermined end-point of stage 4 Ishak fibrosis was reached in 17 monotherapy and 10 triple therapy ($p = 0.04$), with slower fibrosis progression in triple therapy patients ($p = 0.048$). Allocated therapy and histological acute hepatitis were independently associated with stage 4 fibrosis. HVPG increased to 10 mmHg or higher, more rapidly in monotherapy *vs.* triple therapy patients ($p = 0.038$). In this study, long-term maintenance immunosuppression with AZA and shorter term prednisolone together with Tac resulted in a slower onset of histologically proven severe fibrosis and portal hypertension in comparison to Tac alone. This was independent of other known factors affecting fibrosis (e.g. donor age) and the fact that more rejection using protocol biopsies was seen in the triple therapy group [31]. There were no survival differences between the two treatment arms. This trial, for which the premise was that no steroid maintenance and less immunosuppression i.e. Tac monotherapy would result in less severe HCV recurrence, showed that AZA and low dose steroids have a beneficial modulating influence on HCV recurrence.

In 38 HCV recipients randomized to continue Tac, or switch to CyA, the latter led to a modest reduction of HCV RNA titre and appeared to enhance the response to PEG IFN/ribavirin [32]. A randomized trial [33] of 81 recurrent HCV recipients evaluated disease progression using paired liver biopsies and HVPG measurements. SVR occurred in a higher proportion of patients treated with CyA compared to Tac but the difference was not significant.

In a recent prospective study of 253 HCV recipients in Valencia, Berenguer *et al.* compared Tac *vs.* CyA-based immunosuppression in HCV recipients. There was no difference between the two CNIs with regard to the severity of recurrent HCV, the occurrence of severe cholestatic hepatitis and neither in patient nor graft survival at 1 and 7 years [34].

A meta-analysis of 16 RCT compared Tac (1899 patients) *vs.* CyA (1914 patients) in LT [35,36]. Data on HCV cohorts showed that Tac was superior to cyclosporine in improving graft (RR 0.78, 95% CI 0.68–0.89) and patient survival (RR 0.85, 95% CI 0.73–0.99) and preventing acute rejection (RR 0.82, 95% CI 0.77–0.88) and steroid resistant rejection (RR 0.57, 95% CI 0.46–0.71), but Tac increased the risk of diabetes.

Another meta-analysis [7] of five randomized studies showed no difference in patient and graft survival with respect to

Table 1. Major recent studies with calcineurin inhibitors.

Authors, year	No. of patients	Variables	Outcome	Results	*p* value
Martin *et al.*, 2004	Tac-based n = 38 CyA-based n = 41	Tac/AZA/Cs *vs.* CyA/AZA/Cs	Histological HCV recurrence Change in viral load Graft/Patient survival	• Cumulative probability of HCV recurrence in Tac 0.32 *vs.* 0.31 in CyA group • HCV RNA significantly higher in CyA *vs.* Tac group • Similar graft/patient survival	n.s. 0.032 n.s.
Gonzalez *et al.*, 2005	Dual 50 (55%) Triple 35 (40%)	Tac/Cs (D) *vs.* Tac/Cs/AZA (T)	BPAR 12 and 24 mo Patient/graft survival	BPAR: D 24 *vs.* T 7 12 mo patient and graft survival 24 mo patient and graft survival	0.008 n.s. n.s.
Margarit *et al.*, 2005	Tac 28 (HCV 20-71%) Tac/Cs 32 (HCV 15-47%)	Tac *vs.* Tac/Cs	Graft status at 3 yr Survival	• Severity graft hepatitis Tac 47% *vs.* Tac/Cs 67% • 5 year survival both 61%	n.s. n.s.
Berenguer *et al.*, 2006	90 HCV	CyA *vs.* Tac	Time to acute hepatitis Severe hepatitis Death	CyA 92 days *vs.* Tac 59 days 12 (27%) *vs.* 15 (32%) 6 (13%)-6 (13%)	0.02 n.s.
Manousou *et al.*, 2009	103 HCV	Tac 54 (MT) Tac/AZA/Cs 49 (TT)	Ishak stage 4 fibrosis HVPG ≥10 mmHg	MT 17 *vs.* TT 10 TT slower fibrosis MT 10 *vs.* TT 2	0.04 0.048 0.038
Berenguer *et al.*, 2010*	253 HCV	CyA-based 136 Tac-based 117	Severe disease Patient survival 1 yr Patient survival 7 yr SVR after antiviral therapy	CyA 27% *vs.* Tac 26% CyA 83% *vs.* Tac 78% CyA 67% *vs.* Tac 64% CyA 38% *vs.* Tac 39%	0.68 0.4 0.9
Irish *et al.*, 2010	8809 HCV patients UNOS database – Retrospective analysis	CyA *vs.* Tac (maintenance immunosuppression prior to discharge)	1 yr patient and graft survival 3 yr patient and graft survival BPAR	• CyA patient 84.6 ± 1.3% – graft 88 ± 1.2% Tac patient 86.5 ± 0.4% – graft 89.9 ± 0.3% • CyA patient 71.5 ± 1.7% – graft 76.8 ± 1.7% Tac patient 75.5 ± 0.5% – graft 79.9 ± 0.5% • CyA-treated 19.9% *vs.* Tac-treated 9%	#CyA *vs.* Tac HR for death 1.3 (95% CI: 1.07-1.58) HR for graft failure 1.26 (95% CI: 1.06-1.5) HR for BPAR 2.03 (95% CI: 1.54-2.67)

BPAR, biopsy proven acute rejection; n.s., not significant; HR, hazard ratio.
*Series of patients prospectively allocated to either Tac or CyA; "quasi-randomized" study with CyA or Tac in combination with prednisone or mycophenolate mofetil.
#Propensity score-adjusted result.

maintenance CNI. Data on histological HCV progression based on protocol biopsies was available in only 1 study. In studies favouring CyA [37–39], use of azathioprine was more frequent with CyA, than with Tac, but was not evaluated specifically.

In conclusion, CyA has anti-HCV effects *in vitro* but the clinical benefit has not been established. Tac is the preferred CNI in liver transplant recipients as it increases graft and patient survival compared to CyA, in both non-HCV and HCV cohorts. Further studies are needed on CNI and antiviral therapy combined.

Corticosteroids

Viral and molecular background

Steroids are used as parenteral boluses for acute cellular rejection, as part of induction protocols, and as maintenance immunosuppression together with other drugs. HCV viral load increases after steroid boluses, and multiple boluses are associated with worse recurrent disease. However, the effects of steroid maintenance are still controversial [40]. In an *in vitro* replicon model using Huh-7 ET cells, clinically relevant concentrations of dexamethasone and prednisolone did not enhance, but slightly reduced HCV replication [41]. *In vivo*, steroids may influence severity of HCV recurrence by modulating lymphocyte response and immune surveillance.

Steroid boluses

Steroid boluses are associated with increased viral replication: 4–10-fold *in vivo* following 3 daily i.v. injections of 1 g of methylprednisolone [42]. In 241 recipients with HCV, transplanted between 1988 and 1996 in two different centres [43], the fibrosis progression rate increased when i.v. methylprednisolone boluses were more than 3. The same group reported that recurrent cirrhosis was associated with 3 ± 2.2 boluses *vs.* 1.5 ± 2.1 in those without cirrhosis [44].

In 234 HCV transplanted patients [45], repeated treatment for rejection with Cs significantly increased the risk of accelerated

progression of graft hepatitis and graft failure, but a single pulse of methylprednisolone was not statistically associated with increased fibrosis. In another study [46], a comparison of number of boluses during 1999–2000 (mean 11) *vs.* 2001–2004 (mean 4) showed no difference between severe and mild HCV recurrence with respect to boluses, but maintenance steroids were used differently in these periods.

Maintenance steroids

Clinical studies – observational

A survey [9] of 36 centres in the US and 45 non US, documented that 6 (7.4%) used steroid-free protocols, 9 (11%) discontinued steroids within a week, 56% within 3 months and 98% within the first year. The duration of steroid therapy was significantly shorter in US, than non-US programs. (10.8 *vs.* 29.4 weeks, p <0.001).

In the comparative study [46] mentioned above, the 1999–2000 cohort, in which neither slow tapering of steroids nor dual maintenance with steroids were used, showed a significantly higher rate of severe disease (48%) compared to the 2001–2004 (29%) cohort, despite presumably the use of younger donors in the earlier transplant era. In the most recent cohort, use of triple or quadruple regimens was far less (10% *vs.* 25%; p = 0.001), with fewer boluses of methylprednisolone (4 *vs.* 11; p = 0.002), and the duration of maintenance prednisone was longer (350 days *vs.* 249 days; p <0.0001) [46]. This suggests that initial less potent immunosuppression and/or longer maintenance use of steroids is associated with less severe disease.

These data concur with our data [14], which documented that short term maintenance steroids (up to 6 months) was associated with less fibrosis progression (O R 0.4, 95% CI 0.23–0.83). Another study evaluated 39 patients: rapid tapering of steroids (group A), was compared to slow tapering and withdrawal, 25 months after LT (group B) [47]. At 12 months after LT, advanced fibrosis was greater in group A compared to group B (42.1% *vs.* 7.6%). Moreover, one and two-year advanced fibrosis-free survivals were 65.2% *vs.* 93.7% (p = 0.03) and 60.8% *vs.* 93.7% (p = 0.02) in group A compared to group B, respectively (Table 2).

In 80 patients, at both 6 and 12 months after transplantation [48], higher median daily prednisone doses resulted in less moderate/severe recurrent HCV compared to lower doses: at 12 months 35.7% *vs.* 66% (p = 0.02).

Clinical studies – randomized trials and meta-analyses

In our recent randomised trial of 103 LT patients, [30], despite more histologically proven and treated rejection episodes (identified by protocol biopsies) [31], treatment with Tac and maintenance steroids and long term AZA resulted in slower onset of histologically proven severe fibrosis and portal hypertension, without statistical differences in the rates of renal dysfunction, retransplantation and death. The fibrosis progression rate in this group is the lowest reported to date (Ishak stage 4 at 3 years 23%). This suggests the "beneficial" effect of maintenance steroids is more important than the detrimental effect of bolus steroids.

Another RCT compared steroid maintenance (Cya–AZA–basiliximab–steroids) (n = 74) *vs.* steroid-free (Cya, AZA, basiliximab) (n = 66) regimens in HCV liver transplanted patients [49]. At 12 months, there was no significant difference in histological recurrence (41% steroid *vs.* 37.5% in non-steroid, p = 0.35). The steroid-free regimen was associated with lower treatment failure rate (death, graft loss, withdrawal for adverse effects).

Steroid avoidance

Clinical studies – observational

Among 28 HCV patients after living donor transplantation, 18 received CNI, MMF, and anti-CD25 antibody, and 10 received steroid based immunosuppression (CNI and steroids ± MMF ± antiCD25 antibody) [50]. The steroid-free group had less CMV infection (p = 0.049), and less HCV recurrence at 1 year, 18% *vs.* 46% (p = 0.009).

Clinical studies – randomized trials and meta-analyses

Steroid avoidance was evaluated in a multicenter RCT [51] with 312 patients: (1) Tac and steroids; (2) Tac, steroids, and MMF; and (3) daclizumab induction, Tac, and MMF. Patient and graft survival did not differ significantly between treatment arms. Freedom from HCV recurrence at one year was 62%, 60%, and 67% in the three groups respectively; freedom from rejection was significantly higher in the corticosteroid-free immunosuppression group. Long-term follow-up is not yet available.

Llado *et al.* randomized 198 LT patients to receive basiliximab and CyA either with prednisone 0.5 mg/kg/day, tapering to 0.15 mg/kg/day at day 90, or without prednisone [52]. Amongst these, there were 89 HCV-infected patients, with a cumulative percentage of protocol biopsies with grade 3 or 4 fibrosis (Scheuer classification) at 6 months, 1 year, and 2 years of 0%, 8%, and 22% in the non-Cs group, compared to 8%, 19%, and 31% in the Cs group, respectively (p = n.s.) (Table 3). The authors concluded that immunosuppression without steroids in HCV patients was safe, and improved histological short term evolution of HCV recurrence with a reduction in bacterial infections and metabolic complications.

Kato *et al.* randomized 70 HCV patients to receive Tac and daclizumab *vs.* Tac and steroids during 1999–2001, and Tac with MMF and daclizumab *vs.* Tac with MMF and steroids during 2002–2005 [53]. No significant differences were found in mean fibrosis stage, between the various treatment groups, either averaging across the two time periods or during these periods themselves. Patients on steroid-free regimens had significantly less diabetes mellitus and wound infections.

In a recent RCT in 110 patients, early Cs-free immunosuppression was the goal [54]. All patients received Tac/Cs and at 2 weeks post-transplant were randomized to placebo (14 HCV) or steroids (16 HCV). Recurrent cirrhosis was not influenced by continuous steroid therapy but was more frequent in those receiving steroid boluses.

A meta-analysis [55] evaluated 30 publications, comprising 19 RCT comparing steroid-free with steroid-based immunosuppression, including both HCV and non-HCV recipients. There were no differences in death, graft loss and infection rates. HCV recurrence was lower with steroid avoidance, and although no individual trial reached statistical significance, meta-analysis showed an advantage of approximately 10% (RR 0.90; 95% CI: 0.82–0.99, p = 0.03). However, there was considerable clinical heterogeneity, and it was difficult to assess the global immunopotency of each regimen. No evaluation of concomitant immunosuppression was made. Data on fibrosis progression and on steroid dose and withdrawal were not reported.

A more recent meta-analysis [56] evaluated steroid withdrawal in LT for any indication in 2590 patients from 21 RCT. There were no differences between Cs-free and Cs-based protocols in nearly all analyzed outcomes. In 14 studies (1418 patients) evaluating an HCV transplanted population, there was

Table 2. Maintenance corticosteroids for HCV-positive recipients: the case for.

Author, year	Immunosuppression	No. patients (total)	Steroids	Interpretation (fibrosis, survival)
Brillanti *et al.*, 2002	CyA/Tac, Pred, AZA CyA/Tac, Pred	80 n = 44	n = 36 <7.5 mg at 6 mo Slow tapering to 24 mo or indefinitely	- Slow tapering maintenance steroids beneficial - Prednisolone at 6 mo associated with disease-free graft survival
Samonakis *et al.*, 2005	CyA or Tac-based combination 47 monotherapy	193	Median maintenance steroids 4 mo	Short term steroids, less fibrosis progression
Berenguer *et al.*, 2006	CyA or Tac-based combination	HG, 52 RC, 90	Pred >6 mo: 65% HG, 81% RC Pred >1 yr: 21% HG, 46% RC	Severe disease less in RC due to avoid rapid tapering and potent induction immunosuppression
Vivarelli *et al.*, 2007	Tac-based	23 16	**Group A**: rapid tapering/ withdrawal 3 mo **Group B:** slow tapering/ withdrawal 25 mo	Slow tapering of steroids associated with reduced progression of recurrent HCV
Kato *et al.*, 2007	Tac+Dacl *vs.* Tac+Cs Tac+Dacl+MMF *vs.* Tac+Cs+MMF	31 39	Complete taper off within 3 mo in the control groups	- No difference in fibrosis scores - Acute rejection episode the 1st year correlated with higher fibrosis - No difference in graft survival
Manousou *et al.*, 2009	Tac *vs.* Tac/AZA/Pred	54 49	Pred tapered/stopped between 3-6 mo	Short term steroids associated with slower onset of histologically proven severe fibrosis and portal hypertension

Tac, tacrolimus; AZA, azathioprine; Pred, prednisolone; CyA, cyclosporin A; Dacl, daclizumab; MMF, mycophenolate mofetil; HG, historical group; RC, recent cohort.

an advantage of steroid-free protocols with regards to HCV recurrence (RR 1.15), acute graft hepatitis (OR 3.15), and treatment failure (death, graft loss, withdrawal) (OR 1.87). Cumulative meta-analysis showed a relatively consistent reduction in the HCV recurrence rate between 2005 and 2008. However, dose and rapidity of steroid withdrawal were not evaluated and again results are difficult to interpret due to heterogeneity in the studies (Table 3).

In conclusion, randomized trials in which the only variable is maintenance steroids are needed. The "protective role" of slow steroid withdrawal shown in several studies also requires further investigation, as steroids may reduce the immune mediated damage of infected liver cells. Although HCV viraemia levels normally increase during steroid treatment, a rapid decrease in steroid immunosuppression could expose HCV-infected cells to a partially restored immune system and attack. This could determine a worse graft evolution in patients undergoing the faster CS tapering protocols [46] [14].

Azathioprine (AZA) and mycophenolate mofetil (MMF)

Mechanisms of action and experimental data

AZA and MMF are antimetabolites. AZA is a prodrug of 6-mercaptopurine, which by inhibiting inosine monophosphate dehydrogenase (IMPDH) reduces purine synthesis, affecting DNA, RNA, other nucleotides, and proteins [57]. T and B lymphocytes are dependent on *de novo* synthesis of purines for their proliferation.

Mycophenolic acid (MPA)-the active metabolite of MMF, is a selective non-competitive inhibitor of IMPDH. AZA is more myelotoxic and hepatotoxic than MMF, but MMF causes diarrhea in 30% of patients, and tissue invasive CMV infection, especially at 3 g/day. These side effects are reduced with 2 g/day. Both drugs have *in vitro* antiviral activity against HCV. In one *in vivo* crossover study, MMF as a substitute for AZA increased viral load but there was no ALT change [58]. Whether either drug has an effect on the severity of HCV recurrence is still debated.

Clinical studies – azathioprine

Ten studies have data on AZA and severity of HCV recurrence [57] [59]: in six it was decreased, in four there was similar severity and no study was associated with increased severity (Table 4). This effect was statistically significant in a cohort of 66 patients [60]: less recurrence (p <0.005), less progression (p = 0.014). In another study of 92 patients [61], AZA was associated with less cirrhosis, death or retransplantation (RR 0.37 [95% CI 0.14–0.92], p = 0.033). A short (<6 months) duration of maintenance with AZA was a risk factor for severe recurrent disease [8]. Our data showed that long term AZA was associated with a lower risk of allograft failure and mortality [14].

Moreover, in our RCT [30], long term maintenance immunosuppression with AZA (and short term low dose prednisone) with Tac resulted in slower progression to severe fibrosis and portal hypertension in comparison to Tac monotherapy. Another multicentre trial had 65 HCV transplant recipients randomized to Tac/steroids *vs.* Tac/steroids/AZA [27]. AZA was associated with less acute rejection but no differences in graft and patient survival.

In another randomized study [62], a direct comparison between MMF and AZA in 54 HCV-positive transplant recipients was not evaluated, but there were no differences in patient and graft survival at 1 year.

Clinical studies – MMF

Seventeen studies have data on MMF and severity of HCV recurrence: only two found decreased severity, (in one there was no multivariate analysis), nine studies documented similar severity, and six had increased severity [57].

A prospective study [63] of 21 patients receiving quadruple induction CyA-based immunosuppression augmented by MMF

(n = 12) or by AZA (n = 9) showed that MMF tended to delay recurrent hepatitis C and to limit initial graft dysfunction, but there was more severe progression of graft fibrosis with MMF than with AZA.

In a randomized study [64] of four MMF dose regimens for 8 weeks in 30 patients, no subject became HCV-RNA negative nor had 1 log decrease. In a retrospective matched study of 80 patients with histologically proven HCV recurrence, 40 patients treated with MMF and CNI tapered for 24 months, and 40 non-MMF patients [65], the MMF group had reduced fibrosis progression, graft inflammation and ALT levels.

A retrospective evaluation [66] of 3463 HCV transplanted patients assessed long term outcomes of MMF based on discharge medication data. Those with MMF, Tac, and steroids compared to other Tac regimens without MMF had significantly increased four-year survival (79.5% *vs.* 73.8%; $p = 0.002$) and graft survival (74.9% *vs.* 69.5%; $p = 0.024$) and less acute rejection (27.3% *vs.* 32.1%; $p = 0.047$). However, MMF did not show benefit in terms of histologically HCV recurrence.

The published data [57] of RCTs and cohort studies showed that in 2 RCTs comparing MMF to AZA for acute rejection, only one had less treated rejection with MMF (38.5% *vs.* 47.7%; $p = 0.025$), with no difference in patient and graft survival. No RCTs have compared MMF and AZA in patients with CNI-related chronic renal dysfunction. Among two studies evaluating MMF substituting AZA, one was stopped due to severe rejection. Recurrent HCV was less severe in 5/9 studies with AZA compared with only 2/17 using MMF, six of which documented worse recurrence.

There was no data on HCV recurrence in another large study based on discharge medications in which patients on MMF at discharge had less progressive renal dysfunction [67]. A retrospective case-control study [68] in 30 patients evaluated the effect of MMF monotherapy on recurrent HCV. Fifteen patients were switched from CNIs to MMF monotherapy, due to renal dysfunction and metabolic side-effects and were evaluated after 48 weeks; they were matched with 15 patients on CNIs. Patients on MMF had no worsening in fibrosis *vs.* an increase in fibrosis in the CNIs group ($p < 0.0002$).

In conclusion, there is still controversy regarding the best anti-proliferative agent for HCV recipients. Evidence from well designed randomized studies is still limited as regards outcomes. Observational studies suggest that maintenance AZA is associated with less fibrosis progression compared to MMF.

mTOR inhibitors: sirolimus and everolimus

Experimental data and mechanism of action

These inhibit the mammalian target of rapamycin (mTOR) blocking interleukin-2 and interleukin-15 induction of proliferation of T and B cells. Cell growth and angiogenesis are linked with mTOR activity; mTOR inhibition decreases HCC growth. Sirolimus also reduces TGFβ and procollagen, important factors in the development of liver fibrosis. Recent data in animal models suggest that sirolimus and everolimus are associated with significantly less fibrosis progression and portal hypertension than treatment with CNIs [69].

Clinical studies

In a non randomized study of 67 HCV transplant patients, 39 received a regimen including sirolimus, and 28 received CNIs [70]: a significant decrease in HCV RNA levels and better survival was documented in the sirolimus group. However, doses of drugs were not specified and protocol biopsies were not performed. In 35 transplant recipients with HCV and HCC, who had developed renal dysfunction, sirolimus was used: there was less fibrosis and less likelihood to develop advanced disease and to require antiviral therapy [71]. However, data on other immunosuppressives was not reported.

There are very few HCV specific data on everolimus [72] [73]. Only well designed RCT will confirm if mTOR inhibitors are useful in HCV transplant recipients. The bone marrow suppression may make it more difficult if antiviral therapy is needed. If the SILVER study demonstrates benefit in terms of HCC recurrence [74] [75], then mTOR inhibitors may become standard therapy for HCV recipients with HCC.

Anti-lymphocyte preparations

OKT3, ATG, antiCD25

Anti-lymphocyte preparations are used to treat steroid resistant acute rejection in some induction regimens [76].

Clinical studies – observational

OKT3 has been associated with fibrosing cholestatic hepatitis accompanied by very high serum viral loads [8,77–78]. In a group of 31 HCV transplanted patients, RATG was used initially (n = 16); thereafter basiliximab or daclizumab (n = 15) [79]. Steroids and Tac were maintenance therapy. Patient and graft survivals at 6 months were excellent and similar in both groups. HCV recurrence was higher with IL-2 receptor inhibitors (80%) compared to RATG (56%; p = n.s.). In 7 HCV transplanted patients evaluating ATG for treating acute rejection [80], all patients developed severe recurrence with significant rises in HCV RNA levels, and one died.

Alemtuzumab use in 38 HCV transplant recipients [81] resulted in patient and graft survival at one year of 71% and 70%, *vs.* 65% and 54%, respectively, with conventional treatment, the latter being quite low. Only 6% pretreated with alemtuzumab developed rejection during the first four months compared to 30% in the conventionally treated group. HCV replication was worse with alemtuzumab, but no data for histological recurrence were provided.

Anti-CD25 use was evaluated retrospectively [82] in 152 HCV recipients. Together with preemptive antiviral therapy, there was improved short term mortality of HCV recipients, who were transplanted with aged organs but no histological data was given.

Clinical studies – randomized trials

Thymoglobulin induction (n = 22) plus Tac was compared to Tac plus steroids (n = 30) without induction [83]. Patient survival, rejection rates and HCV recurrence did not differ, but in the thymoglobulin group mean time to histological recurrence was shorter, despite lower baseline HCV RNA loads.

Table 3. Maintenance corticosteroids for HCV-positive recipients: the case against.

Author, year	Immunosuppression	No. patients (total)	Steroids	Interpretation (fibrosis, survival)
Filiponi *et al.*, 2004	CyA/AZA/Pred/ Basiliximab CyA/AZA/Placebo/ Basiliximab	74 66	Tapered to 5 mg (3 mo)	Similar patient and graft survival at 1 year follow-up Steroid-free group: lower rate of treatment failure
Klintmalm *et al.*, 2007	Tac/Pred Tac/Pred/MMF Tac/MMF/Daclizumab	80 79 153	Tapered to 5 mg (3 mo)	Steroid-free regimen safe and effective at 1 year follow-up – Recurrence not influenced by immunosuppression
Llado *et al.*, 2008	CyA/Basiliximab/Pred CyA/Basiliximab	46 43	Withdrawn at 3 mo	Steroid-free regimen safe and effective at 2 year follow-up – ↓ Infectious and metabolic complications – lower severe fibrosis (n.s.)
Marubashi *et al.*, 2009	Tac-CyA/MMF/ anti-CD25/Cs Tac-CyA/MMF/ anti-CD25	10 18	Tapered off at 3 mo	Living Donor Liver Transplants – HCV recurrence less frequent in steroid free ($p = 0.009$)
Segev *et al.*, 2008	Cs-based *vs.* Cs-free immunosuppression	Meta analysis 30 publications – 19 RCT		Not exclusively liver transplanted – Heterogeneous trials – HCV recurrence lower with Cs avoidance
Sgourakis *et al.*, 2009	Cs-based *vs.* Cs-free immunosuppression	Meta analysis 21 RCT		**Not exclusively HCV transplants**: Cs-free protocols advantage on metabolic profile, CMV, rejection **HCV population**: Cs-free protocols benefit on HCV recurrence, acute graft hepatitis and treatment failure

Tac, tacrolimus; Cya, cyclosporine A; AZA, azathioprine; Pred, prednisolone; MMF, mycophenolate mofetil; Cs, corticosteroids; RCT, randomized controlled trials; n.s., not significant.

Interleukin-2 receptor blockers: basiliximab and daclizumab

Anti-IL-2 receptor antibodies are specific to the α–chain of the IL-2 receptor and result in fewer side effects in comparison to ATG. They are effective at preventing acute rejection in the early post-transplant period.

Clinical studies – observational

A multicentre, open label exploratory 6-month study had 70 HCV(−) and 31 HCV(+) patients [84]. Two 20 mg doses of basiliximab were given on day 0 and day 4, together with CyA, AZA, and Cs. Rejection was more frequent in HCV-positive [29%] than HCV-negative patients [20%], ($p = 0.44$). More patients in the HCV group required addition of Tac therapy compared to the HCV-negative group, although there were no differences in grade of rejection. The recurrence rate of HCV hepatitis was 48% at 6 months, with no graft loss up to 12 months from HCV recurrence.

A retrospective comparison of basiliximab induction [85] (study group 46 patients, HCV n = 10) compared to historical controls (46 patients, HCV n = 13) who received Tac-based immunosuppression reported less histological HCV recurrence in the basiliximab group (24% *vs.* 71%), but no protocol biopsies were performed.

Daclizumab was given to 21 HCV+ and 20 HCV−, at risk for neurological or renal complications from CNI [86], together with MMF and steroids, followed by Tac and steroid taper. Patients with HCV given daclizumab had an earlier onset of hepatitis, jaundice, with greater histological activity and more rapid progression and higher viral loads: 45% developed advanced disease within 1 year.

Induction therapy was evaluated in the UNOS data base [76]. HCV+ without induction (n = 17,362), HCV+ with induction (n = 3479), HCV− without induction (n = 20,417) and HCV− with induction (n = 4357). In a multivariate analysis, induction in both HCV-positive and negative groups was associated with improved patient (HR 0.91, 95% CI 0.83–0.99) and graft survival (HR 0.88, 95% CI 0.81–0.95). However, the benefit of induction was most pronounced in those with renal insufficiency or with organ perfusion support at transplant, and the differences were quite small in other patient groups (Table 5).

Clinical studies – randomized trials

A randomized double blind placebo controlled trial reported data in a subgroup of 133 recipients with HCV (64 basiliximab, 69 placebo, both groups receiving CyA and Cs) [87]. Clinically suspected acute rejection episodes were confirmed by liver biopsy. Analysis of the combined end point of biopsy-confirmed rejection episodes, HCV recurrence, death or graft loss showed significant differences in favour of basiliximab for the HCV cohort, at both 6 and 12 months ($p = 0.009$ and $p = 0.035$, respectively). However, the reduced HCV recurrence rate was only 3.3% less with basiliximab.

In another RCT [49], 140 HCV+ recipients were randomized to basiliximab + steroids or basiliximab + placebo, both groups receiving CyA and AZA. Histological recurrence rates of hepatitis C were similar (41.2% basiliximab and 37.5% non-basiliximab groups, $p = 0.354$) but the steroid-free regimen was associated with significantly lower rate of treatment failure (death, graft loss, withdrawal for adverse events).

A large multicentre RCT [51] randomized 312 HCV recipients in three arms Tac + Cs (n = 80), Tac + Cs + MMF (n = 79) or daclizumab induction, Tac, MMF (n = 153). At 1 year, patient and graft survival was not different. Freedom from recurrent HCV was 61.8 ± 6.2%, 60.1 ± 6.1%, and 67.0 ± 4.3% in arms 1, 2, and 3, respectively (p = n.s.).

Another RCT [53] randomized HCV transplanted patients to a steroid-free maintenance regimen using daclizumab induction, or

Table 4. Studies with azathioprine in respect with HCV recurrence.

Author, year	No. of patients (total/HCV)	Outcome	Variables	Results	*p* value
Hunt *et al.*, 2001	65/65	HCV recurrence – Progression of HCV recurrence	Use *vs.* nonuse of AZA	6/17 *vs.* 37/48 1/6 *vs.* 18/37	<0.005 0.014
Berenguer *et al.*, 2002	522/283	Cirrhosis (fibrosis stage 4)	Induction without AZA	Associated in univariate analysis	-
Berenguer *et al.*, 2003	554/554	Severe HCV recurrence (fibrosis stage ¾ within first two years)	AZA <12 mo	OR 3.24 [95% CI: 1.51-6.96]	0.003
Samonakis *et al.*, 2005	193/193	Overall survival 3 mo survival Severe fibrosis	AZA at 3 mo No maintenance AZA No maintenance AZA	OR 0.3 [95% CI: 0.18-0.64] OR 0.3 [95% CI: 0.16-0.64] -	- - 0.029
Garcia Gonzalez *et al.*, 2005	177/85	Acute rejection Patients and graft survival	Use *vs.* non-use of AZA	Lower acute rejection Survival: no difference	0.008 n.s.
Eid *et al.*, 2007	92/92	Cirrhosis (fibrosis stage 4)	Use of AZA Tac+Cs *vs.* Tac/AZA/Cs	OR 0.37 [95% CI: 0.14-0.92]	0.033
Manousou *et al.*, 2009	103/103	Stage 4 fibrosis Portal hypertension	Use of AZA Tac *vs.* Tac/AZA/Cs	Slower onset of severe fibrosis and portal hypertension	-

AZA, azathioprine; OR, odds ratio; Tac, tacrolimus; Cs, corticosteroids; n.s., not significant.

steroid maintenance without induction. Daclizumab had no impact on hepatic fibrosis progression. Occurrence of acute rejection was strongly associated with increased hepatic fibrosis at 1 year.

In conclusion, OKT3, ATG, and alemtuzumab for preventing or treating rejection are associated with severe HCV recurrence. Data for IL-2 receptor antagonists are contradictory, most studies showing no harm, but some showing worse recurrence.

Weaning off immunosuppression and tolerance

Long-term immunosuppression leads to nephrotoxicity, metabolic disorders, opportunistic infections, and neoplasms. Some liver transplant recipients can be weaned off all immunosuppressive drugs [88] as they have tolerance to the graft [89] as the liver seems to have an "immunological privilege" compared to other transplanted solid organs.

Patients transplanted for HCV cirrhosis represent an ideal group for weaning strategies. In 34 patients with recurrent HCV disease [90], complete and permanent immunosuppression withdrawal was achieved in 24%, but 35% rejected during tapering, and another 41% developed rejection within eight months. Weaned patients showed stabilization or improvement of histological fibrosis, lower necroinflammation, and improved liver function, after a mean follow-up of 45 months. Low blood CyA trough levels during the first post-transplant week and initial steroid-free immunosuppression were independent predictors of sustained weaning, suggesting this subgroup had less propensity for rejection.

During follow-up [91] over 6.5 years, seven of the eight originally tolerant patients, remain alive and in good condition, while 1 died of severe HCV recurrence at 10 years after transplantation and 6 years after stopping immunosuppression. Four of the 26 patients in whom weaning failed died of HCV recurrence, lung carcinoma, and acute myocardial infarction, after a mean follow-up of 115 months. The 10-year survival from liver transplantation was comparable (89% *vs.* 87.5%) to non-weaned patients, but there was no difference in HCV recurrence histologically.

Unfortunately, induction of tolerance is not a clinical reality, but in the future, molecular signatures (such as peripheral blood mononuclear cells' gene expression profiles) might identify patients in whom immunosuppression can be stopped and this will be particularly important in HCV recipients [89] .

Key Points

- Corticosteroid pulses and antilymphocyte therapies given for acute cellular rejection are associated with more severe HCV recurrence; a single course of MP (up to 3 boluses) for rejection does not appear deleterious
- Tacrolimus and cyclosporine regimens are similar in terms of HCV recurrence, but tacrolimus based regimens have better graft and patient survival
- Induction therapy may not have adverse effects on HCV recurrent disease but further evidence is needed to confirm this
- Observational studies show benefit of low dose and slow tapering of steroids and long term maintenance azathioprine
- MMF is beneficial as a CNI sparing agent in recipients with nephrotoxicity, whereas its effect on severity of HCV recurrence may be harmful compared to azathioprine containing regimens, but this requires further evidence
- Rapid changes in immunosuppression or alteration in strength of immunosuppression are likely to be deleterious for recurrence but the evidence for this is scarce
- The development of tolerance and weaning off immunosuppression is an appealing prospect for HCV recipients, but as yet is not applicable for clinical practice

Table 5. Induction with anti-lymphocyte preparations.

Author, year	HCV patients	Outcome	Variables	Results	*p* value
Nelson *et al.,* 2001	21 HCV+ 20 HCV-	HCV recurrence – Histologically progressive disease	Daclizumab, MMF, Cs >Tac/Cs taper	Worse fibrosis with daclizumab – No difference in mortality	<0.05 n.s.
Neuhaus *et al.,* 2002	133 HCV+ 64 basiliximab/ 69 placebo	Rejection episodes, graft loss or death, HCV recurrence	Basiliximab or placebo + CyA/Cs	Analysis favors basiliximab [all end points]	0.02
Calmus *et al.,* 2002	70 HCV- 31 HCV+	Rejection episodes, Patient and graft survival at 12 mo	Basiliximab, CyA, AZA, Cs	Rejection more frequent in HCV+ 48% recurrence at 6 mo	0.44
Marcos *et al.,* 2004	38 HCV out of 76 adult recipients	Rejection – Patient and graft survival	anti CD25 (alemtuzumab) + standard immunosuppression	Worse for HCV, increased replication, no data on histological recurrence	
Filipponi *et al.,* 2004	140 HCV	Histological recurrence, treatment failure, acute rejection	Basiliximab+Cs or Basiliximab+Placebo+ CyA/AZA	Treatment failure higher in Bas/ Cs group – Rejection less in Bas/ Cs group Recurrence n.s. differences	0.03 0.04 0.354
Kamar *et al.,* 2005	31 patients	Rejection – Patient and graft survival at 6 mo	RATG (n = 16) Basiliximab/Daclizumab (n = 15) – Cs/Tac	Similar results in both groups	n.s.
Llado *et al.,* 2006	89 HCV/198 patients	Rejection – HCV recurrence	Basiliximab+CyA With Cs/without Cs	Rejection rate similar HCV recurrence same Fibrosis, Viremia	0.67 1 n.s.
Klintmalm *et al.,* 2007	312 HCV patients	Rejection – HCV recurrence – Patient and graft survival	Tac+Cs *vs.* Tac+Cs+MMF *vs*. Daclizumab+Tac+MMF	Excellent patient/graft survival – Acute rejection and donor age risk factors for HCV recurrence	n.s. 0.001
Kato *et al.,* 2007	Period 1: 31 Period 2: 39	Mean fibrosis stage at 1 year protocol biopsy – Acute rejection – Steroid side effects	Tac+Daclizumab *vs.* Tac+Cs – Tac+MMF+Daclizumab *vs.*Tac+MMF+Cs	No benefit in reducing mean fibrosis at 12 mo Cs group more wound infection and more diabetes	n.s. 0.01 0.003
Urbani *et al.,* 2008	302 HCV patients	Mortality, rejection	CyA/AZA/Cs *vs.* CyA/Cs *vs.* CyA*/Alemtuzumab	Single drug immunosuppressive regimen associated with survival	0.05
Moonka *et al.,* 2010	17,362 induction- 3479 induction+	Patient and graft survival	HCV *vs.* non HCV, Induction ±	Improved Patient [HR 0.91] and Graft survival [HR 0.88]	0.024 <0.008

*With extracorporeal photopheresis.
Tac, tacrolimus; Cya, cyclosporine A; MMF, mycophenolate mofetil; Cs, corticosteroids; Bas, basiliximab.

Conclusions

There are still more questions than answers regarding immunosuppression for HCV recipients, despite the advent of new drugs and a plethora of studies. However, unfortunately most studies are small and solely observational. Moreover, the few meta-analyses of RCT have significant clinical heterogeneity.

Appropriate RCT are still needed, and should evaluate protocol biopsies at determined intervals, the number of histologically proven rejection episodes, the interaction of immunosuppressive agents with HCV replication, donor age, quality, and all factors should be evaluated in relation to defined clinical outcomes, e.g. severity of recurrent disease, graft, and patient survival. Currently, the selection of the appropriate immunosuppression for the HCV recipient demands a critical approach in examining and understanding the available literature.

From our review it is clear that the ideal immunosuppression is not currently known, but less potent maintenance immunosuppression and avoiding repeated cellular rejection and its treatment result in slower progression of fibrosis due to recurrent HCV. Tac is the CNI of first choice and low dose steroids with prolonged tapering may have a modulating effect on liver injury. Long term azathioprine may have advantages over mycophenolate. There is insufficient data to recommend mTOR inhibitors at present.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding of conflict of interest with respect to this manuscript.

References

[1] Terrault NA, Shiffman ML, Lok AS, Saab S, Tong L, Brown Jr RS, et al. Outcomes in hepatitis C virus-infected recipients of living donor vs. deceased donor liver transplantation. Liver Transpl 2007;13:122–129.
[2] Watt K, Veldt B, Charlton M. A practical guide to the management of HCV infection following liver transplantation. Am J Transplant. 2009;9:1707–1713.
[3] Berenguer M, Prieto M, Rayon JM, Mora J, Pastor M, Ortiz V, et al. Natural history of clinically compensated hepatitis C virus-related graft cirrhosis after liver transplantation. Hepatology 2000;32:852–58.
[4] Kalambokis G, Manousou P, Samonakis D, Grillo F, Dhillon AP, Patch D, et al. Clinical outcome of HCV-related graft cirrhosis and prognostic value of hepatic venous pressure gradient. Transpl Int 2009;22:172–181.

[5] McCaughan GW, Shackel NA, Bertolino P, Bowen DG. Molecular and cellular aspects of hepatitis C virus reinfection after liver transplantation: how the early phase impacts on outcomes. Transplantation 2009;87:1105–1111.
[6] Roche B, Samuel D. Risk factors for hepatitis C recurrence after liver transplantation. J Viral Hepat 2007;14:89–96.
[7] Berenguer M, Royuela A, Zamora J. Immunosuppression with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. Liver Transpl 2007;13:21–29.
[8] Berenguer M, Crippin J, Gish R, Bass N, Bostrom A, Netto G, et al. A model to predict severe HCV-related disease following liver transplantation. Hepatology 2003;38:34-41.
[9] Gedaly R, Clifford TM, McHugh PP, Jeon H, Johnston TD, Ranjan D. Prevalent immunosuppressive strategies in liver transplantation for hepatitis C: results of a multi-center international survey. Transpl. Int. 2008;21:867–872.
[10] Perry I, Neuberger J. Immunosuppression: towards a logical approach in liver transplantation. Clin Exp Immunol 2005;139:2–10.
[11] Oo YH, Dudley T, Nightingale P, Haydon G, Mutimer D. Tacrolimus and cyclosporin doses and blood levels in hepatitis C and alcoholic liver disease patients after liver transplantation. Liver Transpl 2008;14:81–87.
[12] Nakagawa M, Sakamoto N, Tanabe Y, Koyama T, Itsui Y, Takeda Y, et al. Suppression of hepatitis C virus replication by cyclosporin a is mediated by blockade of cyclophilins. Gastroenterology 2005;129:1031–1041.
[13] Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. Hepatology 2003;38:1282–88.
[14] Samonakis DN, Triantos CK, Thalheimer U, Quaglia A, Leandro G, Teixeira R, et al. Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. Liver Transpl 2005;11:386–395.
[15] Manousou P, Dhillon A, Cholongitas E, Samonakis D, Isgro G, Calvaruso V, et al. J Hepatol 2011;54:S227.
[16] O'Leary JG KG, Neri M, Jennings LW, McKenna GJ, Davis GL, Trotter JF. Tacrolimus improves survival in hepatits C patients after liver transplantation Hepatology 2010;52:1065 (834 A).
[17] Irish W, Arcona S, Bowers D, Trotter JF. Cyclosporine versus Tacrolimus Treated Liver Transplant Recipients with Chronic Hepatitis C: outcomes Analysis of the UNOS/OPTN Database. Am J Transpl 2011;11:1676–1685.
[18] Cescon M, Grazi GL, Cucchetti A, Vetrone G, Ravaioli M, Ercolani G, et al. Predictors of sustained virological response after antiviral treatment for hepatitis C recurrence following liver transplantation. Liver Transpl 2009;15:782–789.
[19] Firpi RJ, Zhu H, Morelli G, Abdelmalek MF, Soldevila-Pico C, Machicao VI, et al. Cyclosporine suppresses hepatitis C virus in vitro and increases the chance of a sustained virological response after liver transplantation. Liver Transpl 2006;12:51–57.
[20] Selzner N, Renner EL, Selzner M, Adeyi O, Kashfi A, Therapondos G, et al. Antiviral treatment of recurrent hepatitis C after liver transplantation: predictors of response and long-term outcome. Transplantation 2009;88:1214–1221.
[21] Vero VSM, Pasulo L, Ponziani FR, Vigano R, Marino M, Donato MF, et al. J Hepatol 2011;54:S235.
[22] Rendina MCN, Fagiuoli S, Ponziani F, Vigano R, Iemmolo RM, Donato MF, et al. Acute and chronic rejection during interferon therapy in HCV recurrent transplant patients: results from AISF-RECOLT-C group. J Hepatol 2011;54:S230.
[23] Eguchi S, Takatsuki M, Soyama A, Hidaka M, Tokai H, Hamasaki K, et al. Intentional conversion from tacrolimus to cyclosporine for HCV-positive patients on preemptive interferon therapy after living donor liver transplantation. Ann Transpl 2007;12:11–15.
[24] Inoue K, Sekiyama K, Yamada M, Watanabe T, Yasuda H, Yoshiba M. Combined interferon alpha2b and cyclosporin A in the treatment of chronic hepatitis C: controlled trial. J Gastroenterol 2003;38:567–572.
[25] Zervos XA, Weppler D, Fragulidis GP, Torres MB, Nery JR, Khan MF, et al. Comparison of tacrolimus with microemulsion cyclosporine as primary immunosuppression in hepatitis C patients after liver transplantation. Transplantation 1998;65:1044–1046.
[26] Martin P, Busuttil RW, Goldstein RM, Crippin JS, Klintmalm GB, Fitzsimmons WE, et al. Impact of tacrolimus versus cyclosporine in hepatitis C virus-infected liver transplant recipients on recurrent hepatitis: a prospective, randomized trial. Liver Transpl 2004;10:1258–1262.
[27] Gonzalez MG, Madrazo CP, Rodriguez AB, Gutierrez MG, Herrero JI, Pallardo JM, et al. An open, randomized, multicenter clinical trial of oral tacrolimus in liver allograft transplantation: a comparison of dual vs. triple drug therapy. Liver Transpl 2005;11:515–524.
[28] Margarit C, Bilbao I, Castells L, Lopez I, Pou L, Allende E, et al. A prospective randomized trial comparing tacrolimus and steroids with tacrolimus monotherapy in liver transplantation: the impact on recurrence of hepatitis C. Transpl Int 2005;18:1336–1345.
[29] Berenguer M, Aguilera V, Prieto M, San Juan F, Rayon JM, Benlloch S, et al. Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected liver transplant recipients. Liver Transpl 2006;12:762–767.
[30] Manousou P, Samonakis D, Cholongitas E, Patch D, O'Beirne J, Dhillon AP, et al. Outcome of recurrent hepatitis C virus after liver transplantation in a randomized trial of tacrolimus monotherapy versus triple therapy. Liver Transpl 2009;15:1783–1791.
[31] Samonakis DN, Mela M, Quaglia A, Triantos CK, Thalheimer U, Leandro G, et al. Rejection rates in a randomised trial of tacrolimus monotherapy versus triple therapy in liver transplant recipients with hepatitis C virus cirrhosis. Transpl Infect Dis 2006;8:3–12.
[32] Firpi RJ, Soldevila-Pico C, Morelli GG, Cabrera R, Levy C, Clark VC, et al. The use of cyclosporine for recurrent hepatitis C after liver transplant: a randomized pilot study. Dig Dis Sci 2010;55:196–203.
[33] Carrion JA, Navasa M, Garcia-Retortillo M, Garcia-Pagan JC, Crespo G, Bruguera M, et al. Efficacy of antiviral therapy on hepatitis C recurrence after liver transplantation: a randomized controlled study. Gastroenterology 2007;132:1746–1756.
[34] Berenguer M, Aguilera V, San Juan F, Benlloch S, Rubin A, Lopez-Andujar R, et al. Effect of calcineurin inhibitors in the outcome of liver transplantation in hepatitis C virus-positive recipients. Transplantation 2010;90:1204–1209.
[35] McAlister VC, Haddad E, Renouf E, Malthaner RA, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. Am J Transplant 2006;6:1578–1585.
[36] Haddad EM, McAlister VC, Renouf E, Malthaner R, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus for liver transplanted patients. Cochrane Database Syst Rev 2006:CD005161.
[37] Berenguer M, Prieto M, San Juan F, Rayon JM, Martinez F, Carrasco D, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. Hepatology 2002;36: 202–10.
[38] Levy G, Grazi GL, Sanjuan F, Wu Y, Muhlbacher F, Samuel D, et al. 12-month follow-up analysis of a multicenter, randomized, prospective trial in de novo liver transplant recipients (LIS2T) comparing cyclosporine microemulsion (C2 monitoring) and tacrolimus. Liver Transpl 2006;12:1464–1472.
[39] Villamil F, Levy G, Grazi GL, Mies S, Samuel D, Sanjuan F, et al. Long-term outcomes in liver transplant patients with hepatic C infection receiving tacrolimus or cyclosporine. Transplant Proc 2006;38:2964–2967.
[40] Neumann UP, Berg T, Bahra M, Seehofer D, Langrehr JM, Neuhaus R, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. J Hepatol 2004;41:830–836.
[41] Henry SD, Metselaar HJ, Van Dijck J, Tilanus HW, Van Der Laan LJ. Impact of steroids on hepatitis C virus replication in vivo and in vitro. Ann NY Acad Sci 2007;1110:439–447.
[42] Gane EJ, Portmann BC, Naoumov NV, Smith HM, Underhill JA, Donaldson PT, et al. Long-term outcome of hepatitis C infection after liver transplantation. N Engl J Med 1996;334:815–820.
[43] Berenguer M, Ferrell L, Watson J, Prieto M, Kim M, Rayon M, et al. HCV-related fibrosis progression following liver transplantation: increase in recent years. J Hepatol 2000;32:673–684.
[44] Prieto M, Berenguer M, Rayon JM, Cordoba J, Arguello L, Carrasco D, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation: relationship with rejection episodes. Hepatology 1999;29:250–56.
[45] Neumann UP, Berg T, Bahra M, Puhl G, Guckelberger O, Langrehr JM, et al. Long-term outcome of liver transplants for chronic hepatitis C: a 10-year follow-up. Transplantation 2004;77:226–231.
[46] Berenguer M, Aguilera V, Prieto M, San Juan F, Rayon JM, Benlloch S, et al. Significant improvement in the outcome of HCV-infected transplant recipients by avoiding rapid steroid tapering and potent induction immunosuppression. J Hepatol 2006;44:717–722.
[47] Vivarelli M, Burra P, La Barba G, Canova D, Senzolo M, Cucchetti A, et al. Influence of steroids on HCV recurrence after liver transplantation: a prospective study. J Hepatol 2007;47:793–798.
[48] Brillanti S, Vivarelli M, De Ruvo N, Aden AA, Camaggi V, D'Errico A, et al. Slowly tapering off steroids protects the graft against hepatitis C recurrence after liver transplantation. Liver Transpl 2002;8:884–888.
[49] Filipponi F, Callea F, Salizzoni M, Grazi GL, Fassati LR, Rossi M, et al. Double-blind comparison of hepatitis C histological recurrence Rate in HCV+ Liver transplant recipients given basiliximab + steroids or basiliximab + placebo, in addition to cyclosporine and AZAthioprine. Transplantation 2004; 78:1488–1495.

[50] Marubashi S, Dono K, Nagano H, Kim C, Asaoka T, Hama N, et al. Steroid-free living donor liver transplantation in adults: impact on hepatitis C recurrence. Clin Transpl 2009;23:904–913.
[51] Klintmalm GB, Washburn WK, Rudich SM, Heffron TG, Teperman LW, Fasola C, et al. Corticosteroid-free immunosuppression with daclizumab in HCV(+) liver transplant recipients: 1-year interim results of the HCV-3 study. Liver Transpl 2007;13:1521–1531.
[52] Llado L, Fabregat J, Castellote J, Ramos E, Xiol X, Torras J, et al. Impact of immunosuppression without steroids on rejection and hepatitis C virus evolution after liver transplantation: results of a prospective randomized study. Liver Transpl 2008;14:1752–1760.
[53] Kato T, Gaynor JJ, Yoshida H, Montalvano M, Takahashi H, Pyrsopoulos N, et al. Randomized trial of steroid-free induction versus corticosteroid maintenance among orthotopic liver transplant recipients with hepatitis C virus: impact on hepatic fibrosis progression at one year. Transplantation 2007;84:829–835.
[54] Weiler N, Thrun I, Hoppe-Lotichius M, Zimmermann T, Kraemer I, Otto G. Early steroid-free immunosuppression with FK506 after liver transplantation: long-term results of a prospectively randomized double-blinded trial. Transplantation 2010;90:1562–1566.
[55] Segev DL, Sozio SM, Shin EJ, NAZArian SM, Nathan H, Thuluvath PJ, et al. Steroid avoidance in liver transplantation: meta-analysis and meta-regression of randomized trials. Liver Transpl 2008;14:512–525.
[56] Sgourakis G, Radtke A, Fouzas I, Mylona S, Goumas K, Gockel I, et al. Corticosteroid-free immunosuppression in liver transplantation: a meta-analysis and meta-regression of outcomes. Transpl Int 2009;22: 892–905.
[57] Germani G, Pleguezuelo M, Villamil F, Vaghjiani S, Tsochatzis E, Andreana L, et al. AZAthioprine in liver transplantation: a reevaluation of its use and a comparison with mycophenolate mofetil. Am J Transplant 2009;9:1725–1731.
[58] Zekry A, Gleeson M, Guney S, McCaughan GW. A prospective cross-over study comparing the effect of mycophenolate versus AZAthioprine on allograft function and viral load in liver transplant recipients with recurrent chronic HCV infection. Liver Transpl 2004;10:52–57.
[59] Adreana LIG, Germani G, Pleguezuelo M, Burroughs AK. Immunosuppression in Liver Transplant Recipients with HCV-related Cirrhosis. Org Tiss Cells 2009;12:9–23.
[60] Hunt J, Gordon FD, Lewis WD, Pomfret E, Pomposelli JJ, Jenkins RL, et al. Histological recurrence and progression of hepatitis C after orthotopic liver transplantation: influence of immunosuppressive regimens. Liver Transpl 2001;7:1056–1063.
[61] Eid AJ, Brown RA, Paya CV, Razonable RR. Association between toll-like receptor polymorphisms and the outcome of liver transplantation for chronic hepatitis C virus. Transplantation 2007;84:511–516.
[62] Wiesner R, Rabkin J, Klintmalm G, McDiarmid S, Langnas A, Punch J, et al. A randomized double-blind comparative study of mycophenolate mofetil and AZAthioprine in combination with cyclosporine and corticosteroids in primary liver transplant recipients. Liver Transpl 2001;7:442–450.
[63] Kornberg A, Kupper B, Tannapfel A, Hommann M, Scheele J. Impact of mycophenolate mofetil versus AZAthioprine on early recurrence of hepatitis C after liver transplantation. Int Immunopharmacol 2005;5:107–115.
[64] Firpi RJ, Nelson DR, Davis GL. Lack of antiviral effect of a short course of mycophenolate mofetil in patients with chronic hepatitis C virus infection. Liver Transpl 2003;9:57–61.
[65] Bahra M, Neumann UI, Jacob D, Puhl G, Klupp J, Langrehr JM, et al. MMF and calcineurin taper in recurrent hepatitis C after liver transplantation: impact on histological course. Am J Transplant 2005;5:406–411.
[66] Wiesner RH, Shorr JS, Steffen BJ, Chu AH, Gordon RD, Lake JR. Mycophenolate mofetil combination therapy improves long-term outcomes after liver transplantation in patients with and without hepatitis C. Liver Transpl 2005;11:750–759.
[67] Lake J, Patel D, David K, Richwine J, Morris J. The association between MMF and risk of progressive renal dysfunction and death in adult liver transplant recipients with HCV. Clin Transpl 2009;23:108–115.
[68] Manzia TM, Angelico R, Toti L, Bellini MI, Sforza D, Palmieri G, et al. Long-term, maintenance MMF monotherapy improves the fibrosis progression in liver transplant recipients with recurrent hepatitis C. Transpl Int 2011;24:461–468.
[69] Patsenker E, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, et al. Potent antifibrotic activity of mTOR inhibitors Sirolimus and Everolimus but not of Cyclosporine A and Tacrolimus in experimental liver fibrosis. J Hepatol 2011;55:388–398.
[70] Wagner D, Kniepeiss D, Schaffellner S, Jakoby E, Mueller H, Fahrleitner-Pammer A, et al. Sirolimus has a potential to influent viral recurrence in HCV-positive liver transplant candidates. Int Immunopharmacol 2010;10:990–993.
[71] Nandy NRC, Nasser I, Challies TL, Karp S, Johnson S, Hanto DW, et al. Impact of Sirolimus based imuunosuppression on fibrosis progression in HCV liver transplant recipients. Hepatology 2010;55:151 (395 A).
[72] De Simone P, Carrai P, Precisi A, Petruccelli S, Baldoni L, Balzano E, et al. Conversion to everolimus monotherapy in maintenance liver transplantation: feasibility, safety, and impact on renal function. Transpl Int 2009;22:279–286.
[73] De Simone P, Metselaar HJ, Fischer L, Dumortier J, Boudjema K, Hardwigsen J, et al. Conversion from a calcineurin inhibitor to everolimus therapy in maintenance liver transplant recipients: a prospective, randomized, multicenter trial. Liver Transpl 2009;15:1262–1269.
[74] Schnitzbauer AA, Zuelke C, Graeb C, Rochon J, Bilbao I, Burra P, et al. BMC Cancer 2010;10:190.
[75] Toso C, Merani S, Bigam DL, Shapiro AM, Kneteman NM. Sirolimus-based immunosuppression is associated with increased survival after liver transplantation for hepatocellular carcinoma. Hepatology 2010;51:1237–43.
[76] Moonka DK, Kim D, Kapke A, Brown KA, Yoshida A. The influence of induction therapy on graft and patient survival in patients with and without hepatitis C after liver transplantation. Am J Transplant 2010;10:590–601.
[77] Papatheodoridis GV, Davies S, Dhillon AP, Teixeira R, Goulis J, Davidson B, et al. The role of different immunosuppression in the long-term histological outcome of HCV reinfection after liver transplantation for HCV cirrhosis. Transplantation 2001;72:412–418.
[78] McCaughan GW, Zekry A. Mechanisms of HCV reinfection and allograft damage after liver transplantation. J Hepatol 2004;40:368–374.
[79] Kamar N, Borde JS, Sandres-Saune K, Suc B, Barange K, Cointault O, et al. Induction therapy with either anti-CD25 monoclonal antibodies or rabbit antithymocyte globulins in liver transplantation for hepatitis C. Clin Transpl 2005;19:83–89.
[80] Schmitt TM, Phillips M, Sawyer RG, Northup P, Hagspiel KD, Pruett TL, et al. Anti-thymocyte globulin for the treatment of acute cellular rejection following liver transplantation. Dig Dis Sci 2010;55:3224–3234.
[81] Marcos A, Eghtesad B, Fung JJ, Fontes P, Patel K, Devera M, et al. Use of alemtuzumab and tacrolimus monotherapy for cadaveric liver transplantation: with particular reference to hepatitis C virus. Transplantation 2004;78:966–971.
[82] Urbani L, Mazzoni A, Colombatto P, Bindi L, Biancofiore G, Tascini C, et al. A novel immunosuppressive strategy combined with preemptive antiviral therapy improves the eighteen-month mortality in HCV recipients transplanted with aged livers. Transplantation 2008;86:1666–1671.
[83] De Ruvo N, Cucchetti A, Lauro A, Masetti M, Cautero N, Di Benedetto F, et al. Preliminary results of a "prope" tolerogenic regimen with thymoglobulin pretreatment and hepatitis C virus recurrence in liver transplantation. Transplantation 2005;80:8–12.
[84] Calmus Y, Scheele JR, Gonzalez-Pinto I, Jaurrieta EJ, Klar E, Pageaux GP, et al. Immunoprophylaxis with basiliximab, a chimeric anti-interleukin-2 receptor monoclonal antibody, in combination with AZAthioprine-containing triple therapy in liver transplant recipients. Liver Transpl 2002;8:123–131.
[85] Ramirez CB, Doria C, di Francesco F, Iaria M, Kang Y, Marino IR. Basiliximab induction in adult liver transplant recipients with 93% rejection-free patient and graft survival at 24 months. Transplant Proc 2006;38:3633–3635.
[86] Nelson DR, Soldevila-Pico C, Reed A, Abdelmalek MF, Hemming AW, Van der Werf WJ, et al. Anti-interleukin-2 receptor therapy in combination with mycophenolate mofetil is associated with more severe hepatitis C recurrence after liver transplantation. Liver Transpl 2001;7:1064–1070.
[87] Neuhaus P, Clavien PA, Kittur D, Salizzoni M, Rimola A, Abeywickrama K, et al. Improved treatment response with basiliximab immunoprophylaxis after liver transplantation: results from a double-blind randomized placebo-controlled trial. Liver Transpl 2002;8:132–142.
[88] Geissler EK, Schlitt HJ. Immunosuppression for liver transplantation. Gut 2009;58:452–463.
[89] Sanchez-Fueyo A, Strom TB. Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. Gastroenterology 2011;140:51–64.
[90] Tisone G, Orlando G, Cardillo A, Palmieri G, Manzia TM, Baiocchi L, et al. Complete weaning off immunosuppression in HCV liver transplant recipients is feasible and favourably impacts on the progression of disease recurrence. J Hepatol 2006;44:702–709.
[91] Orlando G, Manzia T, Baiocchi L, Sanchez-Fueyo A, Angelico M, Tisone G. The Tor Vergata weaning off immunosuppression protocol in stable HCV liver transplant patients: the updated follow-up at 78 months. Transpl Immunol 2008;20:43–47.