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REVIEW

Recurrent hepatitis C virus infection post liver transplantation: impact of choice of calcineurin inhibitor

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Conflicts of interest
C. Duvoux has received research grants from Astellas, Roche and Novartis and has also been a speaker for Astellas, Roche and Novartis. R. Firpi has received research grants from Novartis, Vertex, Pharmasset, Bayer, BMS, Gilead, GSK, HGS, and Merck, and has received honoraria for participation in advisory boards from Vertex and Genentech. G. L. Grazi has participated in advisory boards for Novartis and received travel and/or research grants from Novartis and Astellas. G. Levy has participated in advisory boards for Novartis and has been a consultant for Astellas, Roche and Abbott Labs. E. Renner has provided consultation for Astellas, Novartis, Roche, and Vertex, has been a speaker for Novartis, is a member of the Scientific Committee for the SUSTAIN trial (Novartis) and has received unrestricted research grants from Novartis and Roche. F. Villamil has participated in Novartis advisory boards for the SUSTAIN and REVERT trials, and received travel and/or research grants from Novartis, Astellas, Janssen, Roche Argentina, and Gador.

Summary

Recurrence of hepatitis C virus infection following liver transplantation (LT) for hepatitis C is universal. After LT, hepatitis C is associated with accelerated fibrosis progression and reduced graft and patient survival. Furthermore, responses to antiviral therapy in patients with recurrent hepatitis C virus post-transplant are consistently sub-optimal. Calcineurin inhibitors (CNIs) like cyclosporine A (CsA) and tacrolimus continue to dominate immunosuppressive regimens in this population; however, there is still uncertainty as to whether either offers an advantage in terms of patient outcomes. Although tacrolimus demonstrates improved efficacy in the general LT population, differences have begun to emerge between these agents regarding diabetogenic potential, antiviral activity, and fibrosis progression in patients with hepatitis C. This review critically evaluates the existing literature, providing an overview of the reported differences, concluding that despite conflicting evidence, a potential benefit of CsA in patients with hepatitis C is supported by the data and warrants further investigation. Future studies examining the role of CNIs in hepatitis C virus-positive LT recipients are required to accurately examine the effects of CNIs on outcomes such as fibrosis progression, survival, and effects on response to antiviral therapy, to provide robust information that allows clinicians to make an informed choice concerning which CNI is best for their patients.

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Introduction

Hepatitis C virus (HCV)-related liver disease is one of the leading indications for liver transplantation (LT) [1], with an increasing number of transplants being performed for HCV-related cirrhosis. The calcineurin inhibitors (CNIs) cyclosporine A (CsA) and tacrolimus remain the cornerstone of modern immunosuppressive regimens following LT. Existing evidence on the impact of these two CNIs on outcomes in HCV-positive LT patients is conflicting; some reports favor CsA [2,3], while others favor tacrolimus [4]. To date, it is unclear whether either CsA or tacrolimus offers an advantage to HCV-positive patients. This review critically examines whether the choice of CNI affects outcome following LT for HCV in terms of fibrosis progression and response to antiviral therapy, either directly or indirectly through effects on factors such as insulin resistance (IR). In addition, the article highlights existing knowledge gaps and approaches that might be taken to confirm whether the choice of CNI affects outcome of HCV post-LT.

Methodology

This manuscript is not intended to be a systematic or meta-analysis of the existing data; the PubMed database was searched using specific search terms (detailed as footnotes at the end of tables) and relevant papers were selected for inclusion, and used to identify further relevant papers.

HCV infection and LT

Up to 46% of LTs have been attributed to HCV [5,6] and this figure is likely to be even higher when hepatocellular carcinoma as an indication for LT is taken into consideration. Recurrence of HCV infection post-transplant is universal, with viral replication beginning within a few hours of transplantation [7] and histologic damage demonstrable as early as 9 days post-transplant [8]. Acute hepatitis develops between 2 and 16 weeks post-transplant [9], preceded by a sharp increase in HCV viral load. Chronic hepatitis is estimated to occur in 80–100% of patients, with from 10% to over 40% of this population progressing to cirrhosis within 5–10 years of transplant [10,11].

Liver transplantation patients with HCV recurrence have worse prognosis than their noninfected counterparts because of accelerated fibrosis progression [12,13] (Fig. 1). Furthermore, a retrospective analysis of 11 036 patient records from the United Network for Organ Sharing database demonstrated an increased risk of mortality (hazard ratio 1.23; 95% CI 1.12, 1.35) and graft failure (hazard ratio 1.30; 95% CI 1.12, 1.39) for HCV-positive compared with HCV-negative LT recipients [14] (Fig. 2). Evidence also suggests that graft and patient outcomes are inferior compared with results in the previous decade because of the increased use of older donors and change from CsA-based to tacrolimus-based immunosuppressive regimens [15]. A single-center retrospective study of 522 LT recipients demonstrated that graft and patient survival had significantly decreased in HCV-positive patients, while a significant increase was observed in HCV-negative patients during the same time frame [15]. Moreover, patients transplanted more recently have faster fibrosis progression post-transplant [12]. Some evidence suggests a correlation between HCV RNA levels at month 4 and histological recurrence [16], although 1-year protocol liver biopsies are still recommended to identify patients at risk of rapid disease progression and to allow better targeting of antiviral therapy [17,18].

Many risk factors for accelerated HCV recurrence and/or its severity have been reported with varying degrees of validation. These factors include donor age [15,17,19,20], recipient age [20], early symptomatic HCV recurrence [21], cytomegalovirus infection [20], diabetes mellitus (DM) [20], therapy for acute rejection [12,20], potent T-cell-depleting therapies such as OKT3 or alemtuzumab [22], and tacrolimus-based regimens [19]. Evidence also suggests a potential role for corticosteroid use (in terms of use versus complete avoidance and rapid versus slow tapering), viral load and genotype, and human leucocyte antigen mismatch, although further data are required [20].

Currently, the recommended antiviral therapy for HCV-positive LT recipients consists of pegylated interferon (IFN) alfa and ribavirin once patients develop stage 1 or 2 fibrosis [11,23,24]. While achievement of sustained virologic response (SVR) has been shown to improve outcomes in HCV-positive LT recipients [25,26], antiviral therapy can be poorly tolerated and SVR is consistently lower in this population (~30%) compared with nontransplant

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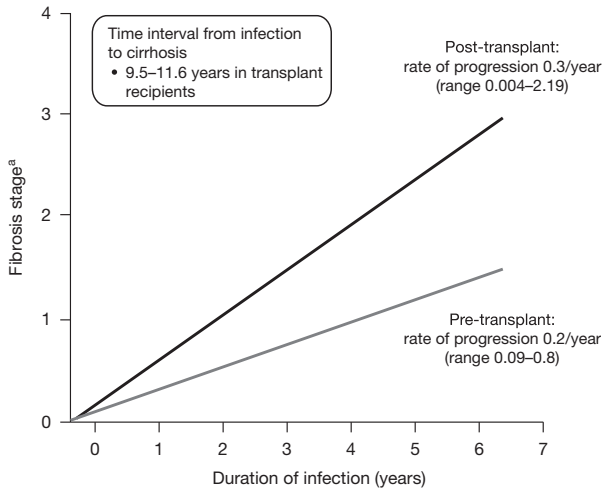
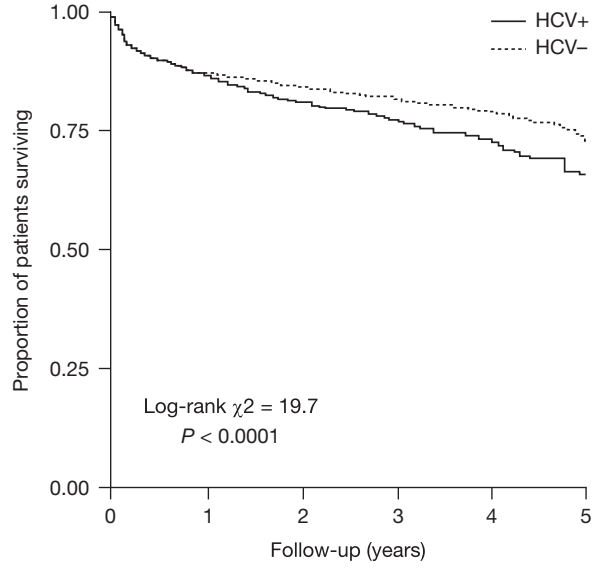


[a]Fibrosis staging: 0, none; 1, periportal fibrous expansion; 2, porto-portal septa; 3, bridging fibrosis; 4, cirrhosis

Figure 1 Fibrosis progression in HCV-positive patients [12]. HCV, hepatitis C virus. Reprinted with permission from the European Association for the Study of the Liver © 2000.



No. at risk						
HCV+	4439	3035	1951	1134	519	93
HCV–	6597	4784	3343	2117	1003	220

Figure 2 HCV-positive and -negative survival rates [14]. HCV, hepatitis C virus. Reprinted with permission from the American Gastroenterological Association © 2002.

patients [27,28]. Recently approved antiviral therapies such as boceprevir [29] and telaprevir [30,31] have improved SVR in the nontransplant population (over 60% in combination with pegylated IFN alfa–ribavirin). Evidence on the efficacy and safety of these agents in the HCV-positive LT population is currently limited [32], along with any potential interactions with CNIs. Therefore, the impact of these new compounds on HCV infection in LT recipients warrants further specific study.

On the other hand, despite a lack of consensus regarding the optimal immunosuppressive regimen for HCV-positive LT recipients, emerging data suggest that the choice of CNI may have an impact on the rates and severity of recurrence of HCV and response to antiviral therapy and therefore could be a consideration in the management of hepatitis C recurrence post-transplant.

Efficacy of CNIs on prevention of rejection, graft loss, and patient survival in LT recipients

Across all LT indications, evidence from several trials and meta-analyses suggest that tacrolimus offers an advantage over CsA in terms of efficacy [33–37]. These original studies primarily compared tacrolimus with the original galenic formulation of CsA (Sandimmune) or used C_0 monitoring; subsequently, a multicenter, randomized, controlled study of 495 *de novo* LT recipients (LIS2T) demonstrated equivalent incidence of biopsy-proven acute rejection at 3 months and equivalent patient and graft survival at 6 and 12 months with tacrolimus and CsA microemulsion with C_2 monitoring [2,3].

Recently, several reports, including a meta-analysis, have reported equivalent patient and graft survival with CsA and tacrolimus in the HCV-positive patient population [38–40]. Also, a recent large database analysis has suggested an increased risk of death/graft loss with CsA compared with tacrolimus [4]. However, it should be noted that these studies did not stipulate the use of CsA microemulsion with C_2 monitoring. Interestingly, in the LIS2T study, use of CsA microemulsion with C_2 monitoring was associated with a lower incidence of death/graft loss at 6 and 12 months compared with patients who received tacrolimus in patients transplanted for HCV [2,3]. The reason for the difference in survival rates is not clear: most of the deaths and graft losses were not obviously associated with HCV recurrence. In the light of evidence from the LIS2T trial, these conflicting data highlight the current controversy surrounding the potential benefits of one CNI over another, and the need for evidence comparing the longer term outcomes of C_2-monitored CsA-based versus tacrolimus-based regimens following LT for HCV.

Other important differences between CsA and tacrolimus on other endpoints in HCV patients, such as effect of antiviral therapy, fibrosis progression, and development of IR/DM could contribute to different outcomes following LT, and will be discussed in detail in the remainder of this article.

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Effect of CNIs on viral replication

Preclinical data

An *in vitro* study demonstrated that CsA, but not tacrolimus, inhibited HCV replication in cultured hepatocytes (Fig. 3a) [41]. A subsequent similar study confirmed these results, demonstrating CsA-dependent viral suppression at clinically achievable drug concentrations [42]. Furthermore, several groups have shown that CsA has an additive inhibitory effect on HCV clearance when combined with IFN compared with tacrolimus [41,43,44].

Both CsA and tacrolimus bind immunophilins: while CsA binds cyclophilin, tacrolimus binds FK-binding protein [45]. It has been hypothesized that CsA may suppress HCV replication by preventing cyclophilin B nonstructural protein 5B binding (Fig. 3b) [46], although recent studies have also demonstrated a potential role for cyclophilin A and nonstructural protein 5A binding [47]. Based on these findings, nonimmunosuppressive derivatives of CsA and other cyclophilin inhibitors, such as alisporivir (Debio-025), are currently being investigated as potential treatment for HCV [48]. Indeed, alisporivir in combination with pegylated IFN alfa/ribavirin is associated with SVR of $\leq$69% in chronic hepatitis C genotype 1 treatment-naive patients [49].

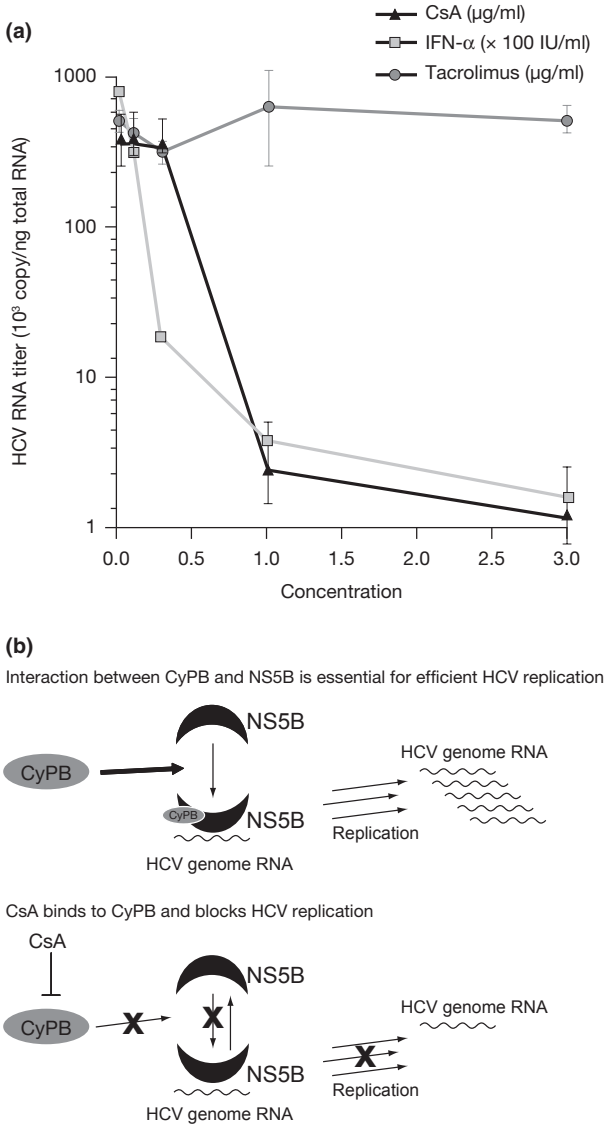

Figure 3 (a) CsA and IFN alpha suppress hepatitis C viral replication, while tacrolimus does not [41]. CsA, cyclosporine A; IFN, interferon; HCV, hepatitis C virus; RNA, ribonucleic acid. Reprinted with permission from WILEY © 2003 by the American Association for the Study of Liver Diseases. (b) The interaction of CsA, cyclophilins, and HCV machinery [46]. CyPB, cyclophilin B; NS5B, nonstructural protein 5B binding; HCV, hepatitis C virus; RNA, ribonucleic acid; CsA, cyclosporine A. Reprinted with permission from John Wiley & Sons © 2007.

Clinical data

Although the current *in vitro* data are promising, the evidence for translation into clinical benefit is somewhat lacking [50], and there has been limited study of the impact of CNIs on HCV viral load post-transplant. Martin and colleagues observed that HCV RNA levels increased by 1 log at 6 months with CsA compared with tacrolimus following LT for HCV with equivalent histologically diagnosed HCV recurrence and survival rates [51]. This could be explained in the clinical setting by the immunosuppressive effect of CsA overwhelming the *in vitro* antiviral properties. This is one of the few studies to prospectively assess HCV replication in patients receiving tacrolimus or CsA, but unfortunately gives no indication of the HCV genotypes examined, how many patients received high doses of steroids for treatment of rejection and how HCV RNA was measured. Despite these data, evidence suggests that CsA may provide a benefit versus tacrolimus in terms of time to recurrence (Table 1). The LIS2T study demonstrated a significantly longer time to recurrence (as confirmed by biopsy) with CsA (100 ± 50 days) versus tacrolimus (70 ± 40 days; $P < 0.05$) (Table 1) [3]. This finding was also observed in a randomized controlled study comparing the impact of CNIs at year 1 fibrosis progression [52] and is in agreement with a subsequent retrospective study of 396 patients undergoing transplant for HCV-induced liver disease, which reported significantly higher incidence of histological HCV recurrence-free survival in patients treated with CsA versus tacrolimus [53]. Since earlier HCV recurrence has been associated with accelerated fibrosis progression, this finding indirectly supports the concept that CsA may provide benefits with regard to fibrosis progression in the long term.

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Table 1. Reported time to recurrence in trials comparing cyclosporine A and tacrolimus.

Study	*n*	Time to recurrence		*P* value	Confirmation of HCV recurrence
		CsA	Tacrolimus		
LIS2T [3] (prospective)	495	100 ± 50 days (*n* = 250)	Tacrolimus: 70 ± 40 days (*n* = 245)	<0.05	Biopsy
Berenguer *et al.* 2006 [52] (prospective)	90	92 days (range 39–343) (*n* = 46)	59 days (range 35–185) (*n* = 44)	0.02	Annual biopsy or when clinically indicated
Kim *et al.* 2012 [53] (retrospective)	396	55.4%, 18.6% and 16.7% recurrence-free survival at 1, 3, and 5 years, respectively	30.8%, 10.3% and 8.1% recurrence-free survival at 1, 3, and 5 years, respectively	<0.001 for all time points	Histologic fibrosis score
Testa *et al.* 2000 [121] (retrospective)	300	35.2% within 1 year post-transplant	61.5% within 1 year post-transplant	0.017*	Histologic findings (biopsy) associated with positive hepatitis C serologic test results and/or viremia in the absence of endotheliitis
Foxton *et al.* 2006 [19] (retrospective)	163	HR for prediction of time to bridging fibrosis/cirrhosis, tacrolimus versus CsA: 2.113; 95% CI 1.156, 3.863	0.015 (univariate Cox proportional hazards model)		
Sheiner *et al.* 1995 [122] (retrospective)	96	186 ± 25 days (*n* = 72)	68 ± 14 days (*n* = 9)	0.05 versus CsA*	Post-transplant biopsy only in response to changes from patients' baseline LFTs†
Ben-Ari *et al.* 2003 [123]	45	17.0 ± 15.5 months (*n* = 15)	10.5 ± 10.1 months (*n* = 19)	NS	Positive HCV antibody detection, PCR, elevated serum ALT and histological evidence

ALT, alanine transferase; CsA, cyclosporine A; HCV, hepatitis C virus; HR, hazard ratio; CI, confidence interval; LFT, liver function test; NS, not significant; PCR, polymerase chain reaction.

*No difference in incidence of recurrence.

†Clinical recurrence of hepatitis C defined as elevated LFTs and a positive liver biopsy specimen.

PubMed search terms used: ('HCV' or 'hepatitis C virus' or 'hepatitis C') and 'fibrosis' and ('recurrence' or 'recurrent') and ('CsA' or 'cyclosporine' or 'cyclosporin') and ('tacrolimus' or 'tac' or 'FK506'): 34 abstracts returned; papers selected dependent on relevance and used to identify other relevant papers.

Sustained virologic response represents 'virologic cure' [23] and has been linked to several favorable post-transplant outcomes, including fibrosis stabilization/improvement [54], lower fibrosis stage [25], and increased survival after LT [25,26]. However, post-transplant SVR is highly variable, and can be low, ranging from 23% to 50% (average of 36.5%) [25,55,56], which is substantially lower than in nontransplant patients [23]. As such, improving SVR rates represents an important goal in HCV-positive patients following liver transplant.

Some reports suggest that, in line with the *in vitro* antiviral data suggesting a synergy between CsA and IFN [41,43,44], a higher SVR can be achieved in patients receiving IFN and CsA. For instance, in a prospective study in 120 HCV-positive nontransplant patients SVR was significantly higher with CsA and antiviral therapy compared with antiviral therapy alone (55.2% versus 31.8%; $P = 0.01$) and comparable with that seen with IFN plus ribavirin at the time [57]. While these data did not change the clinical management of HCV-infected patients in the nontransplant setting (as treating nontransplant patients with an immunosuppressant represents a controversial therapeutic approach), they did provide evidence of the potential benefits of CsA in combination with antiviral therapy for HCV, consistent

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with further investigations in post-transplant populations [25,58,59]. A literature search identified 14 studies that compared the impact of CsA versus tacrolimus on SVR following LT (Table 2), of which eight were retrospective. Eleven of these studies reported numerically higher SVR with CsA compared with tacrolimus [25,43,54,58,60–66], with statistical significance achieved in four [25,43,58,60]. Tacrolimus was associated with numerically higher, but not statistically significant SVR in two studies [55,67] and one study showed equivalent results for CsA and tacrolimus [38]. A recent meta-analysis of the effectiveness of antiviral treatment in patients receiving CsA versus those receiving tacrolimus concluded that CsA has a small but significant advantage over tacrolimus in terms of SVR, especially in patients with genotype 1 and 1/4 (risk ratio 1.64; $P = 0.007$; Fig. 4) [25,26,43,54,55,58–60,64,67–75]. These results remain conflicting, as most data are from studies that are retrospective and insufficiently powered, meaning a higher level of evidence is still required for confirmation. These data highlight the difficulty in demonstrating differences in SVR, and the importance of well-designed, appropriately powered trials. Although data are limited, pilot studies have suggested that switching patients who do not respond to antiviral therapy for HCV recurrence from tacrolimus to CsA may improve virologic response; in a study of 21 patients who failed to respond to antiviral therapy when receiving tacrolimus, eight were switched to CsA, leading to SVR in five (63%) [76].

Overall, the current available data clearly indicate a need for further randomized trials prospectively investigating the effects of CsA versus tacrolimus on SVR to provide robust evidence on any potentially differential effects. Such trials should be designed with the recently reported role of interleukin (IL) 28b polymorphisms in predicting response to antiviral therapy in mind [77].

Table 2. Reported SVR in trials comparing cyclosporine A and tacrolimus.

	SVR rate,%		
Study (*n*)	CsA	Tacrolimus	*P* value
ReViS-TC Study Group 2011 [58]* (410)	48	37	0.037
Berenguer *et al.* 2010 [38]* (253)	38	39	NS
Firpi *et al.* 2006 [43]* (115)	46	27	0.03
Selzner *et al.* 2009 [25]* (172)	56	44	0.05
Cescon *et al.* 2009 [60]* (99)	43	14	0.001
Rayhill *et al.* 2006 [61]* (97)	50	22	0.16
Berenguer *et al.* 2006 [62]* (67)	39	28	NS
Firpi *et al.* 2010 [63] (38)	39	35	NS
Lodato *et al.* 2008 [64]† (53)	35	14	NS
Hanouneh *et al.* 2008 [65]* (53)	44	30	NS
Oton *et al.* 2006 [67]* (52)	28	56	0.053‡; 0.12§
Carrión *et al.* 2007 [54] (51)	45	28	NS
Dumortier *et al.* 2004 [66] (20)	67	53	NS
Fernández *et al.* 2006 [55] (47)	17	26	NS

CsA, cyclosporine A; NS, not significant; SVR, sustained virologic response.
*Retrospective study.
†Genotype 1 only.
‡Univariate analysis.
§Multivariate analysis.
PubMed search terms used: ('sustained virological response' or SVR) and (CsA or cyclosporine or cyclosporin) and (tacrolimus or tac or FK506): 10 results returned. Papers selected dependent on relevance and used to identify other relevant papers and additional studies were added from Author's knowledge.

Effect of CNIs on fibrogenesis

There are data to suggest that a profibrotic milieu exists in HCV-positive transplant recipients. For example, levels of the cytokines IL-2 and IL-4 have been implicated in progressive liver damage in immunocompetent patients with chronic HCV infection [78] and increased levels of IL-4 have been demonstrated in patients with severe recurrent HCV post-LT [79]. Although CNIs markedly reduce IL-2 production, there is less effect on IL-4 [80]. As IL-4 is known to increase collagen production *in vitro* [81], this may explain the rapid development of fibrosis in HCV patients post-LT.

Preclinical data

Preliminary *in vitro* data suggest that CsA may have antifibrotic activity. It has been shown that CsA – but not tacrolimus – inhibits both collagen synthesis and smooth muscle alfa-actin expression in rat liver cells at clinically relevant concentrations [82,83]. In addition, one study has demonstrated CsA inhibition of the profibrotic effects of IL-4 and transforming growth factor beta on human intrahepatic fibroblasts [84]. A differential effect of the two CNIs on the effects of profibrotic cytokines may explain the differences in rates of fibrosis progression in patients treated with CsA or tacrolimus and requires further investigation.

Fas-mediated apoptosis has been demonstrated to play a role in liver fibrosis [85] and HCV-related hepatocellular damage [86,87]. Given the integral role apoptosis and the Fas system play in the pathology of HCV infection, agents that inhibit apoptosis may be of benefit in transplant patients who have recurrent disease. CsA has demonstrated anti-apoptotic activity *in vitro* and *in vivo* [88,89], and reports have suggested that CsA may protect against Fas-mediated apoptosis *in vivo* [90]. While initial *in vitro* evidence suggested tacrolimus did not have an anti-apoptotic

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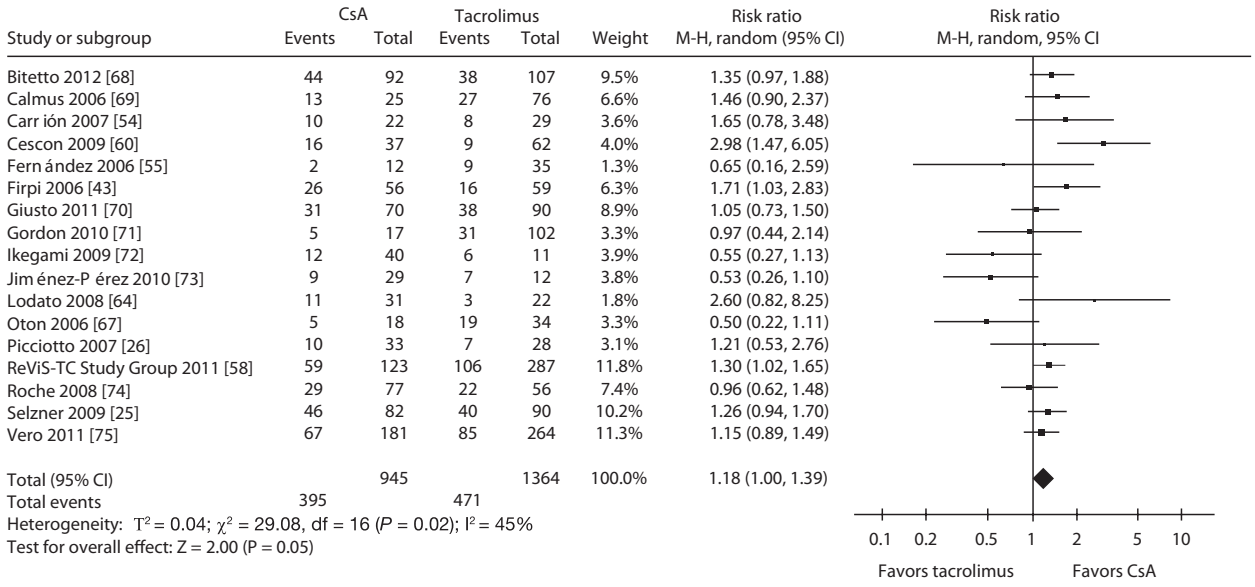

Figure 4 Meta-analysis of 17 observational studies of IFN-based combination therapy for recurrent hepatitis C (all genotypes) [59] post-transplant comparing the proportion of patients with SVR on CsA and tacrolimus. CI, confidence interval; CsA, cyclosporine A; IFN, interferon; M-H, Mantel-Haenszel; SVR, sustained virologic response. Reprinted with permission from John Wiley & Sons © 2012.

effect [89], more recent data suggest it may be anti-apoptotic [91], indicating the need for further study to investigate whether the potential anti-apoptotic effects of CsA represent a viable hypothesis for the differences in fibrosis outcomes in patients receiving CsA or tacrolimus.

Clinical data

In the recent prospective analysis of 253 HCV-positive LT recipients [38], no difference between CsA and tacrolimus was observed in terms of fibrosis progression at 1 year. However, this timeframe is rather short and other studies have suggested that CsA may provide benefits in terms of HCV recurrence and associated liver damage compared with tacrolimus. On the other hand, several reports suggest that CsA may have a potential advantage over tacrolimus (Table 3). A retrospective study identified tacrolimus as a risk factor for time to bridging fibrosis/cirrhosis and also suggested improved graft outcomes for LT recipients with HCV recurrence on CsA versus tacrolimus-based immunosuppression [19]. Some data suggest that the mid- to long-term risk of fibrosis progression may be greater with tacrolimus (Table 3): one prospective study of 96 patients transplanted for HCV-related cirrhosis identified tacrolimus use at 1 year post-transplant as an independent risk factor for accelerated fibrosis progression [92] in line with the study by Foxton *et al.* [19]. An observational follow-up of 95 patients from the open-label LIS2T study reported a statistically lower incidence of histologically proven HCV-related hepatitis [93], while retrospective studies have demonstrated a higher incidence of graft survival without fibrosing cholestatic hepatitis [61] and reduced incidence of moderate to severe fibrosis [94] with CsA versus tacrolimus. Of 15 studies that identified and addressed the issue of the impact of CNI on fibrosis progression following hepatitis C recurrence [15,19,38,52,53,53,61,92,93,95–101] (Table 3), eight did not show any difference between CsA and tacrolimus [38,52,93,95–98,101]. Two of these studies had a 1-year follow-up [38,52]; a time period which may be insufficient for the demonstration of any clinically relevant impact. No study favored tacrolimus and seven suggested a beneficial impact of CsA [15,19,53,61,92,99,100], which was significant in five cases.

At present, the few data available on the impact of CsA and tacrolimus on cholestatic fibrosis suggest no difference between the two CNIs [38,40,52]. However, a recent small retrospective study (n = 37; 5 cases of fibrosing cholestatic hepatitis) reported improved outcomes with a combination of early antiviral treatment, close monitoring of biopsies/viral load, and conversion from tacrolimus to CsA [102].

Collectively, these data give an indication that the use of CsA may potentially lower the incidence and severity of fibrosis post-transplant. Again, these findings deserve confirmation through well-designed prospective clinical trials with a substantial follow-up period (at least 3 years, ideally with protocol biopsies or at least adjustment on duration of follow-up), to provide a high level of evidence. If these data are confirmed, there may be an advantage in the use of CsA, particularly in nonresponders to antiviral therapy, as a potential means of slowing fibrosis progression.

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Table 3. Existing evidence of CNIs as a risk factor for fibrosis (studies with a histological evaluation at ≥ 1 year).

Study	Study type	*n*	Evidence for CNI as a risk factor for fibrosis	Follow-up period	Protocol biopsy?	*P* value	CNI identified as risk factor?
Berenguer *et al.* 2010 [38]	Prospective	253	No difference in incidence of advanced fibrosis with CsA (30%) or tacrolimus (24.5%)	1 year	Yes	NS	Neither
Cisneros *et al.* 2007 [95]	Prospective	97	No difference in rate of fibrosis progression with CsA versus tacrolimus (0.7 $\pm$ 0.2 versus 0.6 $\pm$ 0.1 Metavir units/year, respectively)	50 $\pm$ 6 months (range 12–151)	Yes	NS	Neither
Duvoux *et al.* 2002 [92]	Prospective	96	Use of tacrolimus at 1 year post-transplant identified as independent risk factor for accelerated fibrosis progression: exponential co-efficient 5.8; 95% CI 1.9, 17.8	>5 years	No	0.001 (multivariate analysis)	Tacrolimus
Berenguer *et al.* 2006 [52]	Prospective	90	No difference in incidence of severe fibrosis with CsA (65%) or tacrolimus (62%)	1 year	Yes	NS	Neither
LIS2T [93]	Observational follow-up	95	Increased incidence of histologic evidence of HCV-related hepatitis with tacrolimus (100%) versus CsA (87%) Numerically higher 3-year actuarial risk of fibrosis stage 3 or 4 with tacrolimus (80%) versus CsA (46%)	34 $\pm$ 0.9 months (CsA) and 37 $\pm$ 0.7 months (tacrolimus) (mean)	No	0.02 Not stated	Neither; trend toward tacrolimus
O'Leary *et al.* 2011 [96]	Retrospective	516	Fibrosis progression similar with CsA versus tacrolimus; tacrolimus not identified as a risk factor for advanced fibrosis	5 years	Yes	NS in both instances	Neither
Kim *et al.* 2012 [53]	Retrospective	396	Histological HCV recurrence-free survival was higher with CsA versus tacrolimus at 1 (55.4 versus 30.8%), 3 (18.6 versus 10.3%) and 5 years (16.7 versus 8.1%) Tacrolimus was identified as a risk factor for HCV recurrence in LT patients; relative hazard 1.635 (95% CI 1.240, 2.157)	5 years	Yes	<0.001 0.0005	Tacrolimus
Berenguer *et al.* 2002 [15]	Retrospective	283	Tacrolimus identified as a risk factor associated with cirrhosis	3 years (range 0–10 years)	Yes	0.009	Tacrolimus
Foxton *et al.* 2006 [19]	Retrospective	163	Tacrolimus HR for prediction of progression to bridging fibrosis/cirrhosis, compared with CsA: 2.017; 95% CI 1.096, 3.713	Median 49.4 months (range 20.6–79.5)	Yes	0.024 (multivariate Cox proportional hazards model)	Tacrolimus
Bahr *et al.* 2005 [97]	Retrospective	130	Fibrosis progression similar with CsA versus tacrolimus	Mean 5.5 years	Not stated	Not stated	Neither
Rayhill *et al.* 2006 [61]	Retrospective	97	Statistically higher incidence of graft survival without fibrosing cholestatic hepatitis with CsA versus tacrolimus	5.6 years (CsA) and 3.5 years (tacrolimus)	Yes	0.01	Tacrolimus

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Table 3. continued

Study	Study type	*n*	Evidence for CNI as a risk factor for fibrosis	Follow-up period	Protocol biopsy?	*P* value	CNI identified as risk factor?
			Trend toward higher fibrosis-free survival with CsA versus tacrolimus			0.1	
Johnson *et al.* 1996 [100]	Retrospective	74	Incidence of post-transplant cirrhosis higher with tacrolimus (31.8%) versus CsA (8.9%)	22 months (average)	No	<0.05	Tacrolimus
Oton *et al.* 2006 [101]	Retrospective	66	Although univariate analysis suggested a higher level of fibrosis with CsA versus tacrolimus ($P = 0.19$), no significant difference between fibrosis in CsA versus tacrolimus was determined in a multivariate analysis	95.3 months (CsA) and 41.1 months (tacrolimus)	Yes	0.24	Neither
Hunt *et al.* 2001 [98]	Retrospective	65	No difference in fibrosis progression demonstrated between CsA (12/43 patients) and tacrolimus (7/22 patients)	7.3–8.4 years (average)	No	0.80	Neither
van der Laan *et al.* 2010 [99]	Retrospective	60	Significantly lower Ishak fibrosis score with CsA (mean 1.7 ± 0.4) versus tacrolimus (3.1 ± 0.4)	23.6 months (CsA) and 22.3 months (tacrolimus)	No	0.023	Tacrolimus
			Incidence of moderate to severe fibrosis (Ishak score ≥ 4) higher with tacrolimus (41%) versus CsA (7%)			0.028	
			Mean time to moderate fibrosis (Ishak score ≥ 3) was 38.3 ± 15.1 months with CsA and 23.5 ± 12.6 months with tacrolimus			NS	

CsA, cyclosporine A; NS, not significant; CI, confidence interval; HCV, hepatitis C virus; HR, hazard ratio; LT, liver transplantation; NS, not significant. PubMed search terms used: ('HCV' or 'hepatitis C virus' or 'hepatitis C') and 'fibrosis' and ('CsA' or 'cyclosporine' or 'cyclosporin') and ('tacrolimus' or 'tac' or 'FK506'): 39 abstracts returned. Papers selected dependent on relevance and used to identify other relevant papers and additional studies were added from Author's knowledge; only studies with a histological examination at ≥ 1 year were considered for inclusion.

Effect of CNI on IR and DM

The course of HCV disease is adversely affected by IR and the presence of DM, with HCV infection known to be associated with a high risk of IR and DM even in nontransplanted patients [103]. In an observational study, the incidence of new-onset DM was significantly higher in HCV-positive LT patients (47.1%) compared with noninfected patients (18.9%; $P = 0.008$) [104].

A number of analyses have shown that IR, metabolic syndrome, and pre- and post-transplant DM are independent risk factors associated with severity and progression of fibrosis in HCV following LT [19,105,106]. In one study, both pre- and post-transplant DM were associated with progression to severe fibrosis ($P = 0.039$; hazard ratio 2.68 and $P = 0.004$; hazard ratio 3.28, respectively) [19]. Furthermore, IR and DM have been reported to impact negatively on SVR in both nontransplant and transplant patients [107–109].

Cyclosporine A has repeatedly been reported to be less diabetogenic following solid organ transplant compared with tacrolimus [2,3,104]. In the observational study mentioned earlier, incidence of new-onset DM was significantly higher in patients receiving tacrolimus compared with those receiving CsA ($P = 0.0014$; Fig. 5) [104]. In addition, data from small studies also suggest that conversion from tacrolimus to CsA has the potential to reduce the prevalence and severity of post-transplant DM [110,111], with similar evidence emerging in the HCV-positive LT population [112]. Taken together, these data provide an interesting hypothesis that CsA may have the potential to reduce the risk of post-transplant DM in HCV-positive LT recipients.

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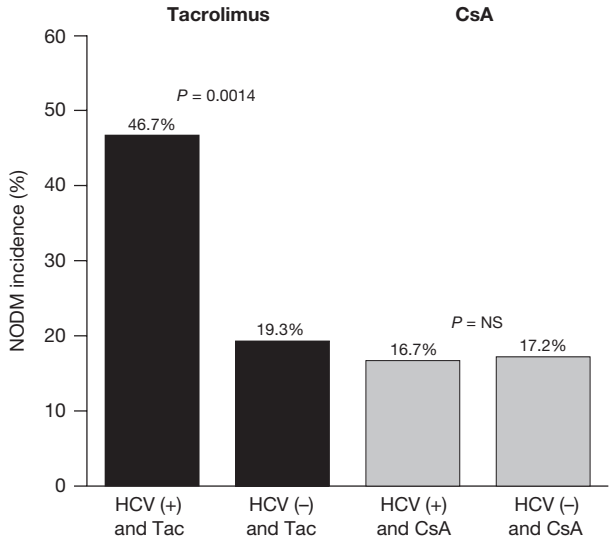

Figure 5 Incidence of NODM according to HCV infection status in patients receiving CsA or tacrolimus. CsA, cyclosporine A; HCV, hepatitis C virus; NODM, new-onset diabetes mellitus; NS, not significant; Tac, tacrolimus. Reprinted with permission from WILEY © 2007 AASLD.

This may, in turn, decrease the severity of HCV recurrence and improve the response to antiviral therapy, although further studies would be required to confirm this effect.

Effect of CNIs on the immune response and clearance of HCV

Both the innate and adaptive arms of the immune response are required for effective HCV clearance. Early in viral infection, natural killer cells and macrophages control viral replication and spread, while dendritic cells induce adaptive, antiviral $CD4^+$ and $CD8^+$ T-cell responses [113]. As strong innate and adaptive responses have been demonstrated to correlate with milder graft injury in the post-transplant period [114,115], the requirement for immunosuppression will contribute to both the recurrence of HCV and accelerated rates of progression following transplantation.

The HCV core protein is known to suppress T-cell replication. A study has reported that this HCV core protein effect is augmented by CsA [116]. If confirmed, this could provide a potential mechanism by which the use of CNIs post-transplant contributes to enhanced viral replication and increased disease recurrence. $CD4^+CD25^+FoxP3^+$ T-regulatory cells (Tregs), implicated in induction of peripheral tolerance, have been demonstrated to play a role in viral persistence by suppressing HCV-specific T-cell responses [117]. Recent *in vitro* studies have reported that CsA significantly inhibited T-regulatory function [118], while tacrolimus does not inhibit the development of Treg induction by antithymocyte globulin [119]. If confirmed, this difference in impact of the two CNIs on Treg activity might partially explain the enhanced effect of CsA on antiviral therapy by inhibiting a potent suppressive antiviral pathway; these interesting findings require further investigation.

Conclusions

There is strong evidence that *in vitro* CsA has antiviral activity and inhibits fibrogenesis and hepatocyte apoptosis. Clinically, evidence from small prospective studies and retrospective analyses suggests potential benefits of CsA compared with tacrolimus in HCV-positive LT recipients in terms of antiviral activity, time to recurrence of disease, and incidence and severity of recurrent disease. However, there are still many studies demonstrating no difference in these outcomes with the use of either CNI in HCV post-transplant. One reason for this disparity may be that in many negative studies monitoring of CsA was by measurement of C_0 (trough levels), which is known to be inferior to measurement of C_2, especially with the use of CsA microemulsion [2,3,120]. In addition, longer follow-up periods are required to truly examine any potential differences between CsA and tacrolimus in outcomes such as fibrosis progression.

The disparity seen in the literature and the quality of the existing data emphasize the need for further large randomized, controlled longer term trials in HCV-positive transplant recipients to specifically and accurately examine the effects of CNIs on outcomes such as fibrosis progression and survival, as well as efficacy of IFN-based antiviral therapy, to provide robust information that allows clinicians to make an informed choice concerning which CNI is best suited to their patients.

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