

Calcineurin-Inhibitor Minimization in Liver Transplant Patients with Calcineurin-Inhibitor-Related Renal Dysfunction: A Meta-Analysis

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Abstract

Background: Introduction of calcineurin-inhibitor (CNI) has made transplantation a miracle in the past century. However, the side effects of long-term use of CNI turn out to be one of the major challenges in the current century. Among these, renal dysfunction attracts more and more attention. Herein, we undertook a meta-analysis to evaluate the efficacy and safety of calcineurin-inhibitor (CNI) minimization protocols in liver transplant recipients with CNI-related renal dysfunction.

Methods: We included randomized trials with no year and language restriction. All data were analyzed using random effect model by Review Manager 5.0. The primary endpoints were glomerular filtration rate (GFR), serum creatinine level (sCr) and creatinine clearance rate (CrCl), and the secondary endpoints were acute rejection episodes, incidence of infection and patient survival at the end of follow-up.

Results: GFR was significantly improved in CNI minimization group than in routine CNI regimen group ($Z = 5.45$, $P < 0.00001$; $I^2 = 0\%$). Likely, sCr level was significantly lower in the CNI minimization group ($Z = 2.84$, $P = 0.005$; $I^2 = 39\%$). However, CrCl was not significantly higher in the CNI minimization group ($Z = 1.59$, $P = 0.11$; $I^2 = 0\%$). Both acute rejection episodes and patient survival were comparable between two groups (rejection: $Z = 0.01$, $P = 0.99$; $I^2 = 0\%$; survival: $Z = 0.28$, $P = 0.78$; $I^2 = 0\%$, respectively). However, current CNI minimization protocols may be related to a higher incidence of infections ($Z = 3.06$, $P = 0.002$; $I^2 = 0\%$).

Conclusion: CNI minimization can preserve or even improve renal function in liver transplant patients with renal impairment, while sharing similar short term acute rejection rate and patient survival with routine CNI regimen.

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Introduction

With 1-year liver allograft survival rates now exceeding 80% [1], attention is increasingly being paid on improving long-term morbidity and mortality in liver transplant recipients. Renal dysfunction is the most concerned long-term complication post-liver transplantation, because it was estimated that 18% of recipients would develop chronic renal failure or end-stage renal disease by 5 years post-transplant and renal dysfunction would significantly increase mortality risk [2–4].

Multiple factors have been reported to be involved in chronic renal impairment in liver transplant recipients [2,4,5]. Among these, high level exposure of calcineurin-inhibitors (CNI), namely cyclosporin A (CsA) and tacrolimus (Tac), is a well recognized risk factor [2,6,7]. Importantly, although chronic CNI-induced renal insufficiency is associated with structural changes in the kidney

[8–10], an improvement in renal function can be observed in patients after CNI reduction [11–13]. Nonetheless, the initial attempts to withdraw CNI led to increased acute rejection risk [14]. To tip the balance between potent immunosuppression and less CNI exposure, several prospective, randomized, and controlled trials (RCTs) of novel CNI minimization protocols were conducted recently [15–23]. However, current knowledge about these protocols is dependent on single institution studies, which was often limited by small sample sizes and individual practice patterns.

Herein, we performed a meta-analysis of the available literature to better understand the efficacy and safety of CNI minimization protocols in liver transplant patients with CNI-related renal dysfunction. This data provide important insight capable of better informing clinical physicians regarding the treatment of CNI-related renal dysfunction.

Methods

Study design, search strategy, and study selection

Before data collection, two general protocols were designed to be compared: CNI minimization regimen and routine CNI regimen. To limit publication bias, we included published trials with no language or year restrictions. Initial searches of MEDLINE, EMBASE databases and the Cochrane Database of Systematic Reviews included terms: calcineurin-inhibitor, cyclosporin A, tacrolimus or FK506, minimization, withdrawal, reduction, elimination and liver transplantation.

To be included, trials had to be randomized, not confounded by additional therapeutic differences between the two protocols. Trials should compare renal function of liver transplant recipients receiving routine CNI regimen versus CNI minimization regimen for CNI-related renal impairment. To limit the renal function in a comparable range, we only included patients with glomerular filtration rate (GFR) under 60 ml/min, serum creatinine level (sCr) more than 1.5 mg/dl or creatinine clearance rate (CrCl) under 70 ml/min before enrollment according to the National Kidney Foundation (NKF) recommendation for chronic kidney disease (CKD) and the staging index used in clinical practice. To make sure that routine CNI regimen and CNI minimization protocols are practiced as their names, in each trial the CNI dose in the minimization group should be initially reduced by at least 25% of the dose as is used in the routine regimen group to achieve a lower target trough levels, or CNI were completely withdrawn and converted to non-CNI based protocols.

Quality assessment of trials included

A quality assessment was carried out for all the retrieved RCTs. Quality in a systematic review essentially refers to the absence of biases. To assess the methodological validity of the studies included in this review the following aspects were evaluated: allocation concealment, intention to treat analysis or not, blinding and description of handling of missing data. Articles were assessed by two reviewers (DW and YS) independently. Disagreements were resolved by consultation with a third reviewer (ZG).

Data extraction and outcome measures

For the trials included in our meta-analysis, we sought data for demography information, renal function (GFR, sCr and CrCl), acute rejection (AR), incidence of infections (including cytomegalovirus (CMV), varicella zoster virus (VZV), herpes simplex infection and nasopharyngitis, bronchitis, pneumonia, stomatitis events and urinary tract infection (UTI)), and patient survival for all patients. The primary outcomes of our meta-analysis were renal function, and the secondary outcomes were AR, incidence of various infections and patient survival. The data were extracted by two investigators (YK and YS) independently. The conduct and reporting were in accordance with the Quality of Reporting of Meta-Analyses statement.

Statistical analysis

For every outcome, we used the statistical software Review Manager 5.0 (The Cochrane Collaboration, Oxford, United Kingdom) to analyze the collected data and to compare each treatment group with the routine CNI regimen group. The primary outcomes (GFR, sCr and CrCl) and the secondary outcomes (AR, incidence of infections, patient survival) were analyzed as continuous and dichotomized variables using random effect model, and their results were reported as mean difference (95% confidence interval) and odds ratio (95% confidence

interval), respectively. The statistic strength was measured by overall effect size Z and heterogeneity index I^2 .

Results

Characteristics of included studies

We included 32 trials with 1383 patients in the current meta-analysis, including 10 RCTs [14–23] with 625 patients and 22 observational trials [12,13,24–43] with 758 patients. Figure 1 shows the flow diagram of study identification. Half of the 10 RCTs achieved CNI withdrawal or completely conversion in the end of study [14,15,19,20,22]. For those CNI was not completely withdrawn, MMF was started mostly at 500 mg twice a day [16–18,23] and in one study at 1000 mg twice a day [21] and eventually achieved 1000–2000 mg twice a day. Accordingly, CNI dose was gradually reduced by at least 25% to reach CsA trough level of 25–50 ng/mL or Tac trough level of 2–4 ng/mL [16–18,23]. Since the included 32 trials used different measures of primary outcome, we analyzed the data according to 3 different outcome measures: GFR, sCr and CrCl. Additionally, 22 observational trials compared renal function collected at baseline pre- and post-conversion. Although it was inappropriate to combine these data in a meta-analysis with that of 10 RCTs, considering these trials did follow our principle of this analysis, we included them anyway and did a separate meta-analysis for reference and supplement. Table 1 shows the basic characteristics of the included studies (the first 10 were RCTs and the remaining 22 were observational studies). In general, the CNI minimization protocols used in these 32 trials were divided into 3 categories: mycophenolate mofetil (MMF) -based, sirolimus (SRL) -based and everolimus (ERL) -based regimens. We compared them both individually and collectively with the routine CNI regimen in this meta-analysis to limit heterogeneity and gain a better understanding of the efficacy and safety profiles of different protocols.

Methodological quality

In general, the methodological quality of the included 10 RCTs was not bad. However, only 3 studies described the allocation sequences. And 3 studies used the intention-to-treat analysis to avoid detection and attrition bias. All RCTs except one were open-labeled study. Table 2 summarizes the risk evaluation of bias.

CNI minimization improves renal function

Firstly, we conducted a meta-analysis of 10 RCTs regarding GFR in patients receiving CNI minimization (including MMF and sirolimus subgroups) versus routine CNI regimen, which was shown in Figure 2. The forest plot graph of the comparison showed that in MMF subgroup, GFR of recipients was significantly higher than in routine CNI regimen group ($Z = 5.16$, $P < 0.00001$; $I^2 = 0\%$). While in sirolimus subgroup, the improvement of GFR over routine CNI regimen group was not statistically significant ($Z = 1.73$, $P = 0.08$; I^2 not applicable). In total, in the included RCTs, GFR was significantly improved in CNI minimization group as compared to routine CNI regimen group ($Z = 5.45$, $P < 0.00001$; $I^2 = 0\%$). Similarly, a meta-analysis of the included observational trials regarding GFR (shown in Figure S1) demonstrated that in MMF subgroup, GFR was significantly higher than in the routine CNI regimen group ($Z = 3.95$, $P < 0.0001$; $I^2 = 71\%$). And in sirolimus subgroup, the improvement of GFR over the routine CNI regimen group was also significant ($Z = 3.17$, $P = 0.002$; $I^2 = 90\%$). Collectively, in the included observational studies, GFR was significantly

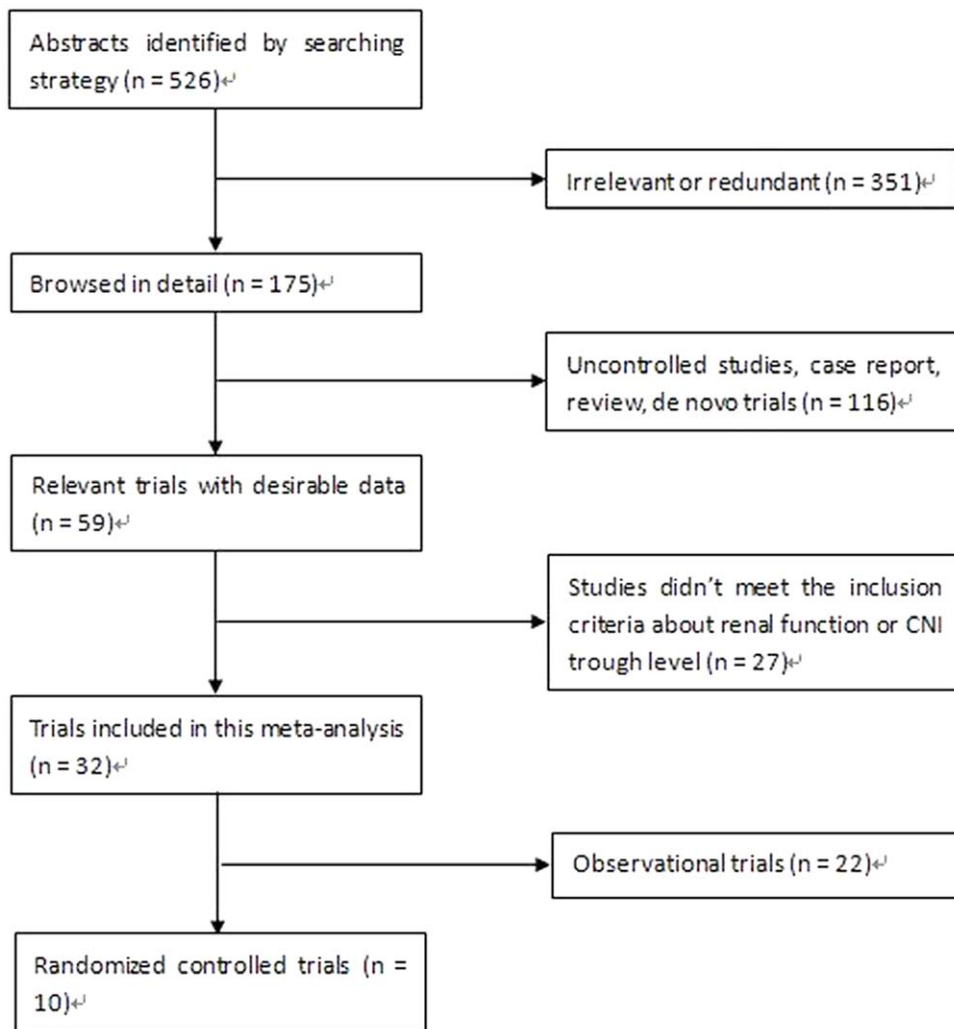


Figure 1. Flow diagram of study identification.
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improved in the CNI minimization group ($Z = 3.59$, $P = 0.0003$; $I^2 = 94\%$).

Then, we conducted a meta-analysis of sCr of patients receiving CNI minimization (including MMF and sirolimus subgroups) versus routine CNI regimen, which was shown in Figure 3. The forest plot graph of the comparison showed that in MMF subgroup, the sCr level of patients was significantly lower than in routine CNI regimen group ($Z = 4.19$, $P < 0.0001$; $I^2 = 0\%$). While in sirolimus subgroup, the decrease of sCr level was not statistically significant in comparison with routine CNI regimen group ($Z = 0.58$, $P = 0.56$; I^2 not applicable). Collectively, in the included RCTs, the sCr level was significantly decreased in CNI minimization group ($Z = 2.84$, $P = 0.005$; $I^2 = 39\%$). In addition, a meta-analysis of the included observational trials regarding sCr level (Figure S2) showed that in MMF subgroup, the sCr level was significantly lower than in routine CNI regimen group ($Z = 6.76$, $P < 0.00001$; $I^2 = 82\%$). And in sirolimus subgroup, there was a significant decrease of sCr level compared to routine CNI regimen group ($Z = 7.91$, $P < 0.00001$; $I^2 = 56\%$). And in everolimus subgroup, the sCr level was also significantly decreased ($Z = 2.68$, $P = 0.007$; I^2 not applicable). Totally, in the included observational studies, the sCr level was significantly

decreased in CNI minimization group ($Z = 6.63$, $P < 0.00001$; $I^2 = 91\%$).

Finally, we conducted a meta-analysis of CrCl of patients receiving CNI minimization (including MMF, sirolimus and everolimus subgroups) versus routine CNI regimen, which was shown in Figure 4. The forest plot graph of the comparison showed that in MMF subgroup, the improvement of CrCl over routine CNI regimen group was not statistically significant ($Z = 1.23$, $P = 0.22$; $I^2 = 0\%$). And in sirolimus subgroup, once again, we could not document a significant improvement of CrCl ($Z = 1.73$, $P = 0.08$; $I^2 = 0\%$), neither did we in everolimus subgroup ($Z = 0.61$, $P = 0.54$; I^2 not applicable). In total, in the included RCTs, CrCl was not significantly improved in CNI minimization group over routine CNI regimen group ($Z = 1.59$, $P = 0.11$; $I^2 = 0\%$). In contrast, a meta-analysis of the included observational trials regarding CrCl (Figure S3) showed that in MMF subgroup, CrCl was significantly higher than in routine CNI regimen group ($Z = 3.69$, $P = 0.0002$; $I^2 = 80\%$). While in everolimus subgroup, improvement of CrCl over routine CNI regimen group was not significant ($Z = 1.88$, $P = 0.06$; I^2 not applicable). Totally, in the included observational studies, CrCl was significantly improved in CNI minimization group ($Z = 4.02$, $P < 0.0001$; $I^2 = 75\%$).

Table 1. Basic characteristics of the studies included in this meta-analysis.

Author	Year	Country	Number of Patient	Gender (Male/Female)	Drug Used (CNI minimization/Routine CNI regimen)	Initial CNI trough levels (ng/mL)	Duration of Follow-up
H.J.Schlitt et al ¹⁴	2001	Germany	28	19/9	MMF/CNI+AZA+steroids	CsA 100–120/Tac 8–10	6 m
P.De Simone et al ¹⁵	2009	Italy	145	85/60	Everolimus+CNI ↓ +steroids/CNI*	CsA 105.5/Tac 5.65	12 m
V.R.Cicinnati et al ¹⁶	2007	Germany	75	51/24	CNI ↓ +MMF+steroids/CNI*	CsA 110.6/Tac 6.59	12 m
S.Beckebaum et al ¹⁷	2004	Germany	32	22/10	CNI ↓ +MMF/CNI*	CsA 116/Tac 5.6	12 m
G.P.Pageaux et al ¹⁸	2006	France	56	45/11	CNI ↓ +MMF/CNI*	CsA 162/Tac 4.4	12 m
U.Eisenberger et al ¹⁹	2009	Switzerland	16	12/4	Sirolimus*/CNI*	CsA 160/Tac 10/8	12 m
C.C.Rogers et al ²⁰	2009	U.S.A.	82	46/36	Sirolimus*/CNI*	CsA 100–150/Tac 6–8	12 m
M.Biselli et al ²¹	2009	Italy	60	50/10	CNI ↓ +MMF/CNI*	CsA 120/Tac 7	12 m
S.Shenoy et al ²²	2007	U.S.A.	40	29/11	Sirolimus*/CNI*	CsA 150/Tac 6.3	12 m
S.Beckbaum et al ²³	2009	Germany	90	63/27	CNI ↓ +MMF/CNI*	N/A	12 m
A.Kornberg et al ¹²	2005	Germany	43	29/14	CNI ↓ +MMF/CNI*	CsA 143.6/Tac 9.9	6 m
M.Cantarovich et al ¹³	2003	Canada	19	N/A	CsA ↓ +MMF/CsA*	CsA 132	12 m
L.B.Pulido et al ²⁴	2008	Spain	31	N/A	MMF*/CNI*	CsA 65.63/Tac 3.72	12 m
D.Reich et al ²⁵	2005	U.S.A.	15	10/5	MMF+steroids/CNI*	CsA 188.8/Tac 10/8	13 m
F.Di Benedetto et al ²⁶	2009	Italy	31	N/A	Sirolimus+steroids/CNI+steroids	N/A	36 m
C.Ponton et al ²⁷	2010	Spain	88	74/14	CNI ↓ +MMF/CNI*	N/A	6 m
C.Creput et al ²⁸	2007	France	49	37/12	CNI ↓ +MMF/CNI+AZA+steroids	CsA 100–250/Tac 5–10	36 m
R.O.Koch et al ²⁹	2004	Austria	32	22/10	CNI ↓ +MMF+steroids/CNI+AZA+steroids	CsA 32/Tac 2.7	6 m
M.L.Raimondo et al ³⁰	2003	U.K.	16	10/6	MMF*/CNI+AZA+steroids	N/A	12 m
J.M.M.Planas et al ³¹	2004	Spain	50	32/18	MMF/CNI*	CsA 93/Tac 6.5	18 m
U.Tannuri et al ³²	2007	Brazil	11	5/6	CNI ↓ +MMF/CNI+ steroids	Tac 6–8	24 m
R.Pfzmann et al ³³	2002	Germany	47	N/A	CNI ↓ +MMF/CNI+steroids	CsA 100–180/Tac 5–10	6 m
K.D.Fairbanks et al ³⁴	2003	U.S.A.	21	10/11	Sirolimus*/CNI*	N/A	16 m
E.Q.Sanchez et al ³⁵	2005	U.S.A.	35	N/A	Sirolimus+CNI ↓ +MMF/CNI+MMF+steroids	N/A	24 m
Y.J.Yang et al ³⁶	2008	China	16	16/0	Sirolimus*/CNI*	N/A	6 m
G.Orlando et al ³⁷	2007	Italy	42	34/8	MMF/CNI*	CsA 129/Tac 2.3	12 m
P.De Simone et al ³⁸	2009	Italy	70	51/19	Everolimus*/CNI*	CsA 100–150/Tac 3–8	12 m
S.Dharancy et al ³⁹	2009	France	52	43/9	MMF*/CNI+AZA+steroids	CsA 150–250/Tac 6–12	12 m
I.Morard et al ⁴⁰	2007	Switzerland	9	N/A	Sirolimus*/CNI*	CsA 96/Tac 7	23 m
M.Vivarelli et al ⁴¹	2010	Italy	28	N/A	Sirolimus+steroids/CNI*	N/A	12 m
J.Castroagudin et al ⁴²	2011	Spain	30	26/4	Everolimus*/CNI*	N/A	12 m
A.H.Cotterell et al ⁴³	2002	U.S.A.	8	5/3	Sirolimus+CNI ↓ /CNI*	N/A	12 m

*Regimen based on one drug with concomitant drug(s) that was either prednisone, or AZA, or MMF. Abbreviations: CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; AZA, azathioprine; N/A, not available.

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CNI minimization does not compromise short term acute rejection and patient survival, but increases infection rates

To evaluate the safety of CNI minimization protocols, we then compared acute rejection episodes, incidence of infections and patient survival between CNI minimization and routine CNI regimen group.

For the meta-analysis of CNI minimization versus routine CNI regimen in acute rejection, there was no significant difference between two groups in all subgroups and total analysis (MMF subgroup: $Z = 0.14$, $P = 0.89$; $I^2 = 28\%$; sirolimus subgroup: $Z = 0.28$, $P = 0.78$; $I^2 = 21\%$; everolimus subgroup: $Z = 0.01$, $P = 0.99$; I^2 not applicable; and total: $Z = 0.01$, $P = 0.99$; $I^2 = 0\%$) (shown in Figure 5).

We also conducted a meta-analysis of the incidence of various infections between two groups (shown in Figure 6). The difference of incidence of infections between MMF/everolimus subgroup and routine CNI regimen group was comparable ($Z = 1.96$, $P = 0.05$; $I^2 = 0\%$; $Z = 1.36$, $P = 0.18$; I^2 not applicable). While in

sirolimus subgroup, the infection incidence was significantly higher than in routine CNI regimen group ($Z = 2.02$, $P = 0.04$; $I^2 = 0\%$). In total, the infection incidence was significantly higher in CNI minimization group ($Z = 3.06$, $P = 0.002$; $I^2 = 0\%$).

For the meta-analysis of patient survival at the end of the follow-up, there was no significant difference between CNI minimization and routine CNI regimen group in all subgroups and in total (MMF subgroup: $Z = 0.42$, $P = 0.67$; $I^2 = 0\%$; sirolimus subgroup: $Z = 0.59$, $P = 0.56$; I^2 not applicable; everolimus subgroup: $Z = 0.47$, $P = 0.64$; I^2 not applicable; and total: $Z = 0.28$, $P = 0.78$; $I^2 = 0\%$) (shown in Figure 7).

Discussion

CNI provide potent immunosuppression for solid organ transplant patients, however, simultaneously exhibit nephrotoxicity

Table 2. Risk of bias in the randomized controlled trials.

Authors	Allocation Sequence Described	Intention To Treat Analysis	Blinding			Handling Of Missing Data
			Patient	Personnel	Assessor	
Schlitt et al (2001) ¹⁴	No	Yes	Yes	No	No	Unclear
Simone et al (2009) ¹⁵	Yes	Yes	No	No	No	Unclear
Cicinnati et al (2007) ¹⁶	No	Yes	No	No	No	Unclear
Beckebaum et al (2004) ¹⁷	No	No	No	No	No	Unclear
Pageaux et al (2006) ¹⁸	No	No	No	No	No	Unclear
Eisenberger et al (2009) ¹⁹	Yes	No	No	No	No	Unclear
Rogers et al (2009) ²⁰	No	No	No	No	No	Unclear
Biselli et al (2009) ²¹	No	No	No	No	No	Unclear
Shenoy et al (2007) ²²	Yes	No	No	No	No	Last Value Forward
Beckebaum et al (2009) ²³	No	No	No	No	No	Unclear

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as a major side effect. CNI cause both acute (functional) nephrotoxicity and chronic (structural) nephrotoxicity. Whereas acute nephrotoxicity is reversible by withdrawal of the CNI, chronic nephrotoxicity due to CNIs is thought to be irreversible and even progressive [44]. Withdrawal of CNI during early stages of renal dysfunction results in improvement of renal function when pathologic changes are still reversible [11–13]. The principle of CNI minimization protocols is to reduce CNI exposure, by converting CNI to non-nephrotoxic immunosuppressive drugs with or without low dose CNI. MMF, and mammalian target of rapamycin (mTOR) inhibitors (mTORis), namely SRL and ERL, are the commonest options in CNI minimization protocols for their non-nephrotoxicity and potent immunosuppression effects [14–23].

In the current meta-analysis (32 controlled studies included with a total of 1383 patients), both GFR and sCr comparison presented

a significant improvement of renal function in CNI minimization group in both RCTs and observational studies analysis. Although there was significant improvement of CrCl in CNI minimization over routine CNI regimen in observational studies, we could not document such improvement in RCTs analysis. According to the National Kidney Foundation recommendation, GFR is the best estimate of kidney function and is used in the NKF staging of CKD, thus it is rational to conclude that CNI minimization is capable to restore renal function of liver transplant patients with CNI-related renal impairment.

To explore whether CNI reduction is safe in liver transplant recipients, we performed a meta-analysis of acute rejection episodes, infection rates and patient survival. There was no significant difference in acute rejection episodes between CNI minimization and routine CNI regimen, suggesting the immuno-

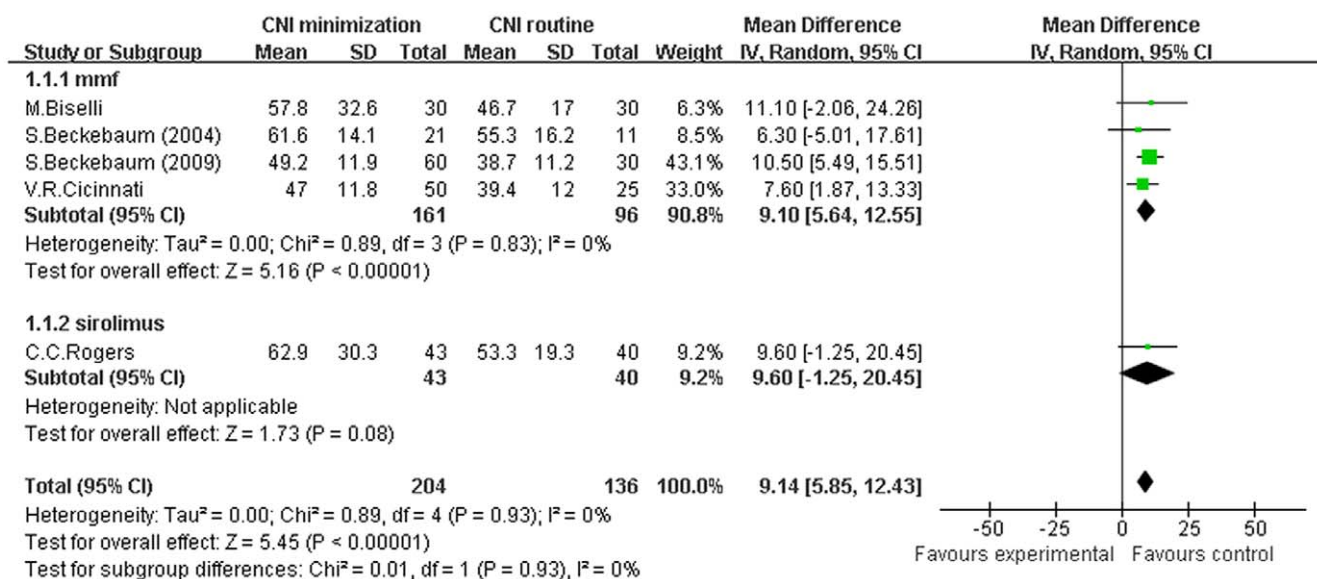


Figure 2. Meta-analysis of CNI minimization versus routine CNI regimen in GFR (RCTs). In MMF subgroup, the GFR of recipients was significantly higher than in routine CNI regimen group. In sirolimus subgroup, the improvement of GFR over routine CNI regimen group was not statistically significant. In the total 10 RCTs, the GFR was significantly improved. Z = total effect size, I² = heterogeneity index. Columns in green represent the mean difference of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI.

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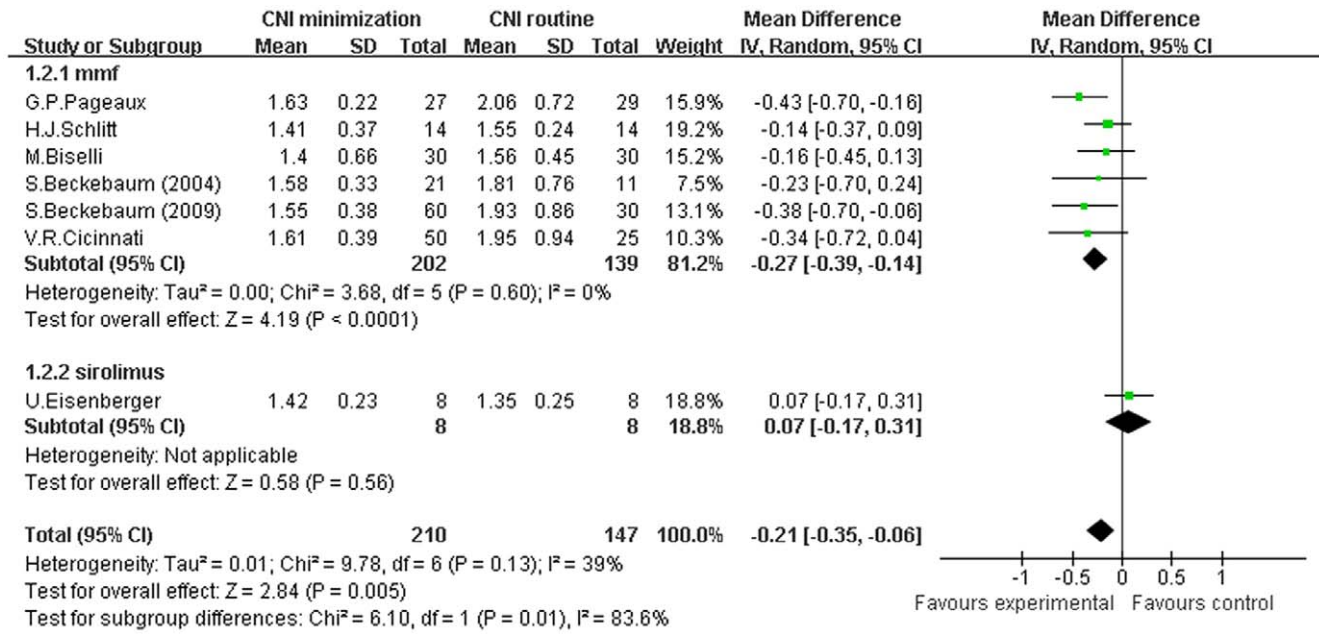


Figure 3. Meta-analysis of CNI minimization versus routine CNI regimen in sCr (RCTs). In MMF subgroup, the sCr level was significantly lower than in routine CNI regimen group. In sirolimus subgroup, the decrease of sCr level was not statistically significant. In the total 10 RCTs, the sCr level was significantly decreased in CNI minimization group. Z=total effect size, I²=heterogeneity index. Columns in green represent the mean difference of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. doi:10.1371/journal.pone.0024387.g003

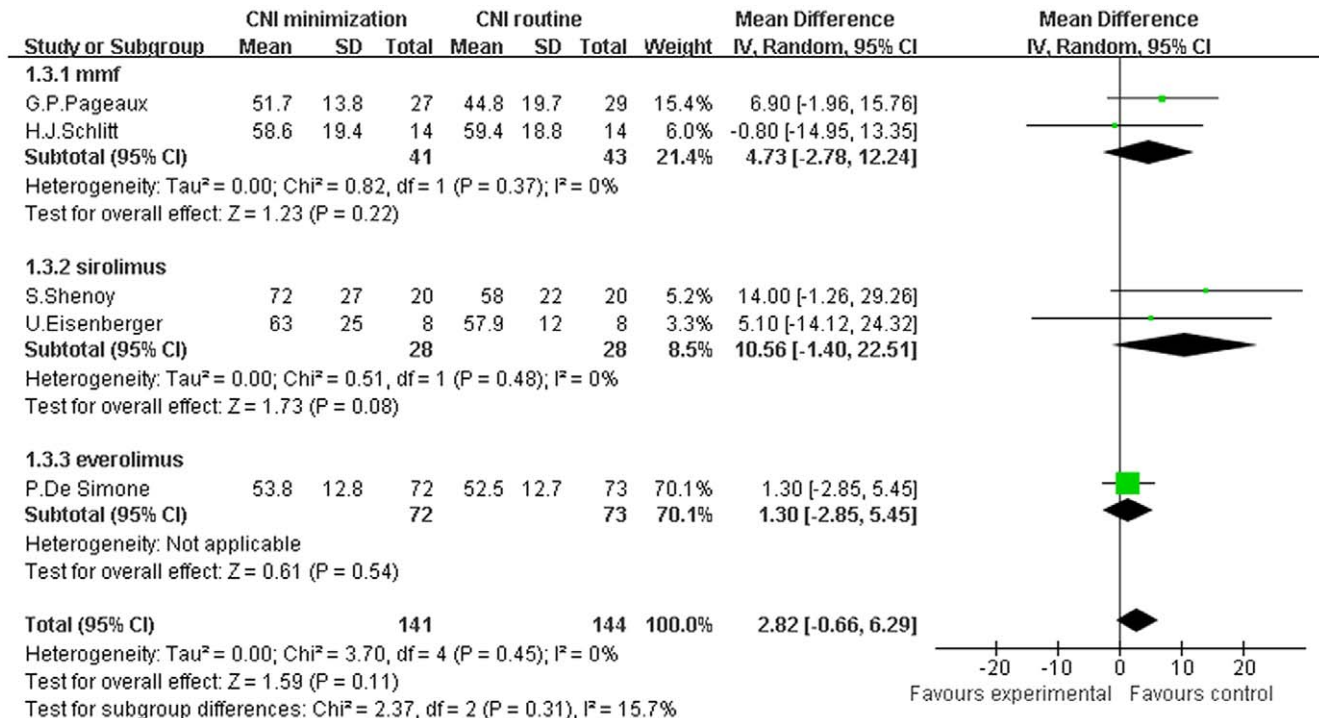


Figure 4. Meta-analysis of CNI minimization versus routine CNI regimen in CrCl (RCTs). In MMF, sirolimus and everolimus subgroup, the improvement of CrCl over routine CNI regimen group was not statistically significant, as well as in the total 10 RCTs. Z=total effect size, I²=heterogeneity index. Columns in green represent the mean difference of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. doi:10.1371/journal.pone.0024387.g004

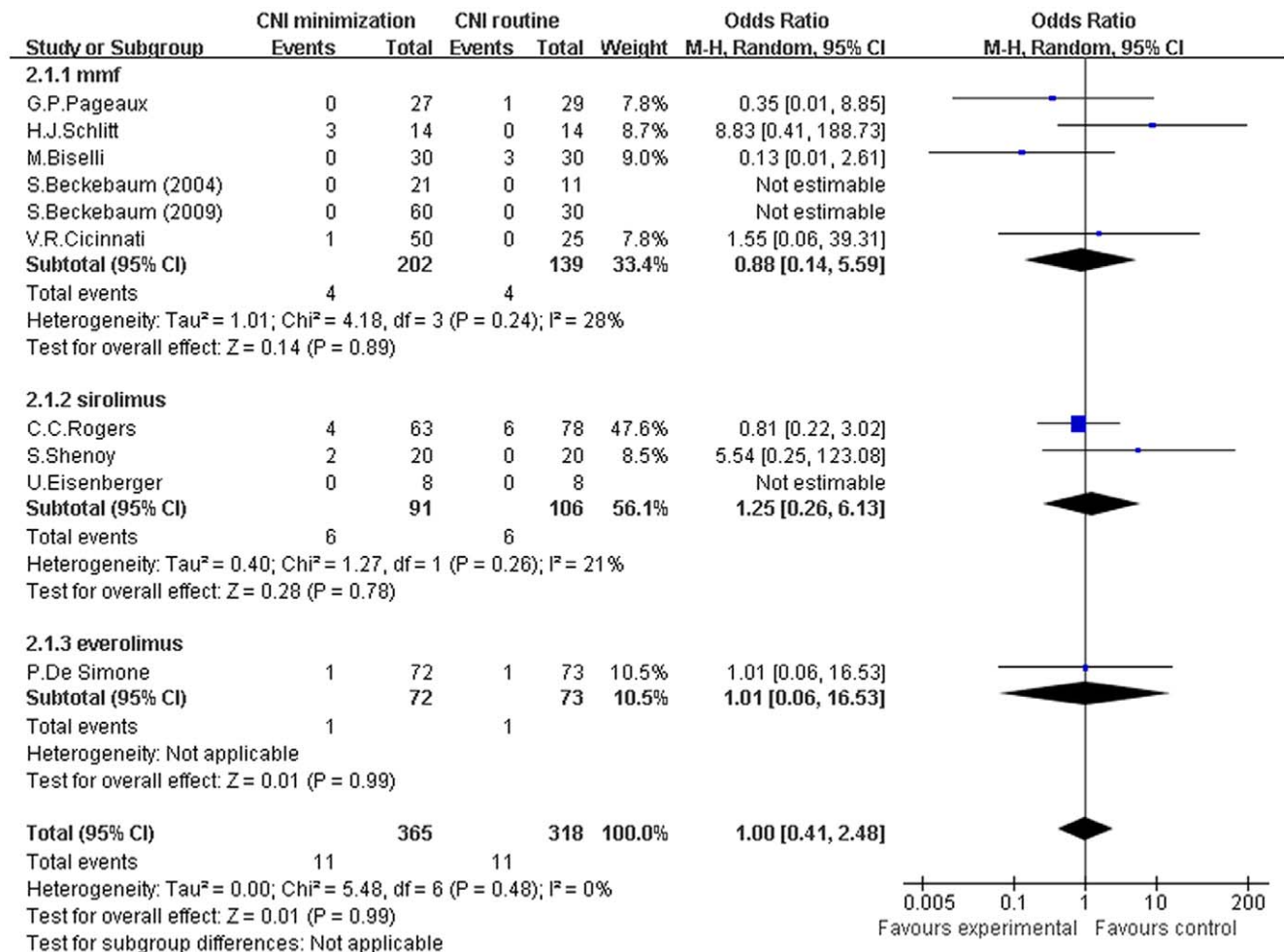


Figure 5. Meta-analysis of CNI minimization versus routine CNI regimen in acute rejection (RCTs). There was no significant difference between CNI minimization and routine CNI regimen group in all subgroup and total analysis. Z = total effect size, I² = heterogeneity index. Columns in blue represent the odds ratio of each study and column size represents the study weight in the analysis. Lines represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. doi:10.1371/journal.pone.0024387.g005

suppression effect was not compromised in patients taking on CNI minimization. However, the incidence of infections is higher in CNI minimization group than in routine CNI group, although most studies did not provide the exact incidence of specific infections, suggesting increased immune load by CNI minimization protocols when introducing MMF or mTORis. On the other hand, there was no significant difference in patient survival between two groups, which can be interpreted in three ways. Firstly, the follow-up durations of these studies are not long enough to show any change of the survival rate. Secondly, the improvement in renal function is not sufficient enough to alter patient survival [45]. Finally, the renal benefit may be counteracted by the increased infection risks. Therefore, whether the improved renal function can be translated into a better survival and whether long term use of CNI minimization protocols would lead to a higher acute rejection or infection rate still need further study.

Notably, the CNI minimization protocols in the included studies are heterogenous. Since the additional drugs and different combinations can alter the outcomes, we divided them into 3 subgroups, namely MMF-based, SRL-based and ERL-based subgroups. In both meta-analysis of RCTs and observational trials, the MMF-based regimen presents an obvious benefit on

renal function protection. It has been reported that replacement of CNI by MMF in liver transplant patients with renal dysfunction can also improve other CNI associated side-effects, such as hypertension and hyperuricemia [46]. Concern about this CNI minimization protocol is from the fact that there are conflicting results regarding the risk of allograft rejection with withdrawal of CNI and subsequent MMF monotherapy [14,29,47–50]. However, in the current meta-analysis, MMF-based CNI minimization protocol is not associated with higher rejection and infection rates. One of the major reasons explaining the discrepancy of rejection rate between the previous reports and current analysis is that most recent protocols are combination of MMF and low-dose CNI but not MMF monotherapy. Collectively, we recommend that MMF can serve as a good option to reduce CNI exposure in liver transplant recipients with renal dysfunction, without increasing rejection and infection rates.

In addition, mTORis are potent anti-proliferative agents that have clear therapeutic potential in liver transplantation [51–55]. However, only 4 SRL-based RCTs were included in this meta-analysis, and no significant improvement of renal function was documented, neither did the ERL-based RCTs. But in the meta-analysis of observational trials, SRL-based regimen yielded a

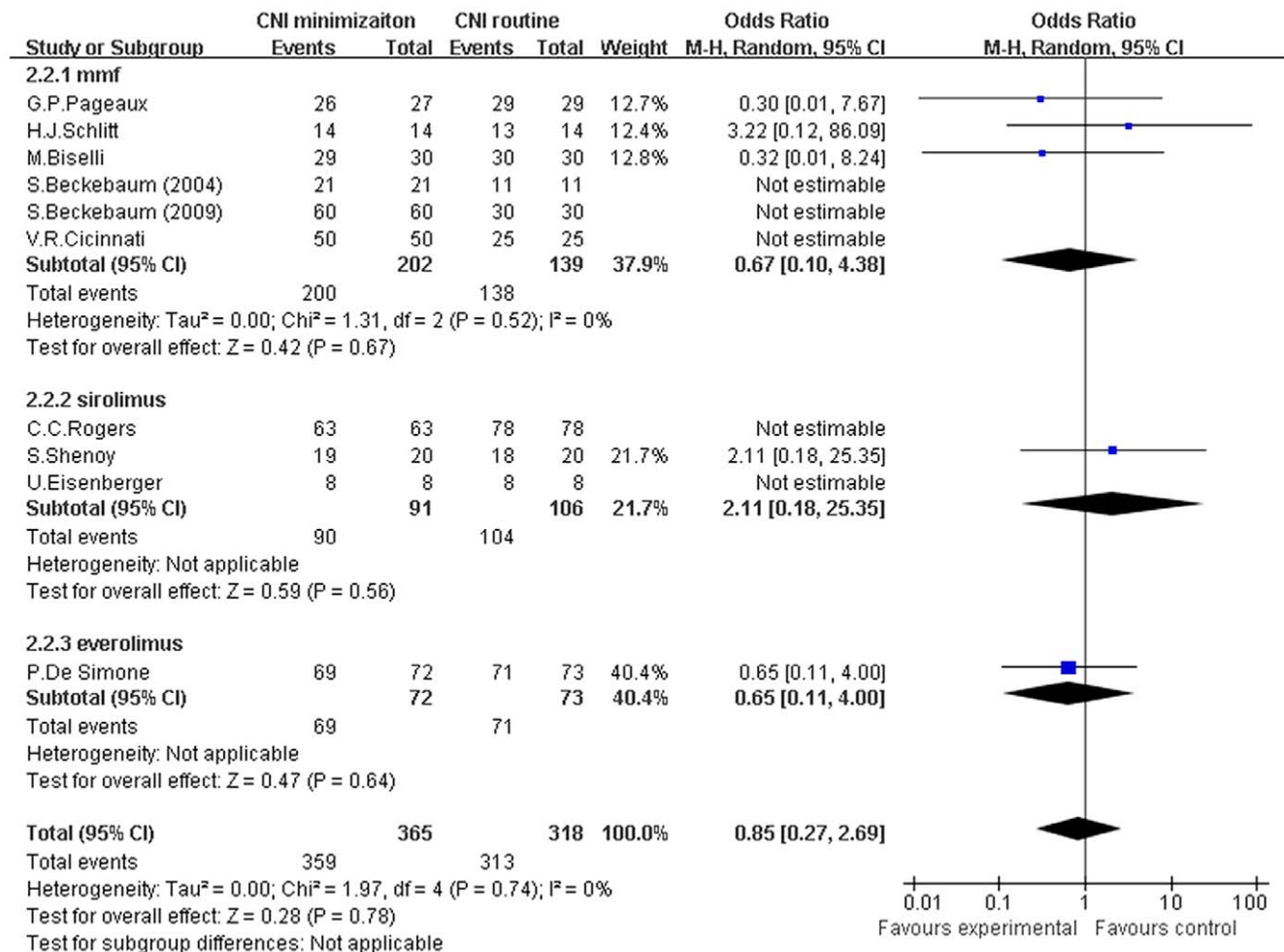


Figure 6. Meta-analysis of CNI minimization versus routine CNI regimen in infection incidence (RCTs). In MMF and everolimus subgroup, the difference of infection incidence was not statistically significant. In sirolimus subgroup, the infection incidence was significantly higher than in routine CNI regimen group. In total, the infection incidence was significantly higher in CNI minimization group. Z=total effect size, I²=heterogeneity index. Columns in blue represent the odds ratio of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. doi:10.1371/journal.pone.0024387.g006

significant improvement of renal function as shown in GFR and sCr, in spite of relatively high heterogeneities. Both SRL-based and ERL-based regimens are equally potent and safe as routine CNI regimen in immunosuppression according to our meta-analysis. Moreover, in non-renal dysfunction population, SRL, either used in combination with prednisone alone or MMF-prednisone in CNI-withdrawal protocols, resulted in improved renal function and acceptable acute rejection rate, although with increased rates of thrombocytopenia, digestive hemorrhage, pleural effusion and other adverse events [56]. In terms of ERL, Simone et al recently reported that ERL, in combination with low-dose CNI, was associated with low acute rejection rate and particularly good renal function [15]. However, in another study, the use of combination CsA and mTORis led to potential long-term CNI nephrotoxicity [57]. Since the number of SRL-based and ERL-based RCTs included in this meta-analysis is small, more high-quality RCTs based on SRL and ERL should be conducted to draw a clear conclusion on whether mTORis-based CNI minimization protocols are effective and safe in patients with impaired renal function. However, according to the results from the meta-analysis of observational trials and considering their well

known anti-tumor effects [58–60], mTORis may be a good alternative for MMF to reduce or replace CNI in liver transplant recipients with a pre-transplant diagnosis of hepatocellular carcinoma (HCC) and post-transplant renal dysfunction. However, clinicians should pay attention to the increased risks of infections when SRL is used.

Undoubtedly, there are some limitations in the current meta-analysis as others. Firstly, we included studies using different regimens without comparing between themselves, it make us difficult to figure out which combination is the best one although the current data show that the MMF-based CNI minimization protocol received the greatest supports. Secondly, most of the studies we included didn't undertake follow-ups longer than 12 months, giving us insufficient data on how CNI minimization would affect long-term graft or patient survival. Finally, as shown in Table 2, the risk of bias of the included randomized trials was relatively high, since no study was double blind designed and only 3 of 10 studies conducted intention-to-treat analysis, which may attenuate the power of the current study.

In conclusion, this meta-analysis included all current relevant studies from various countries covering different populations. It

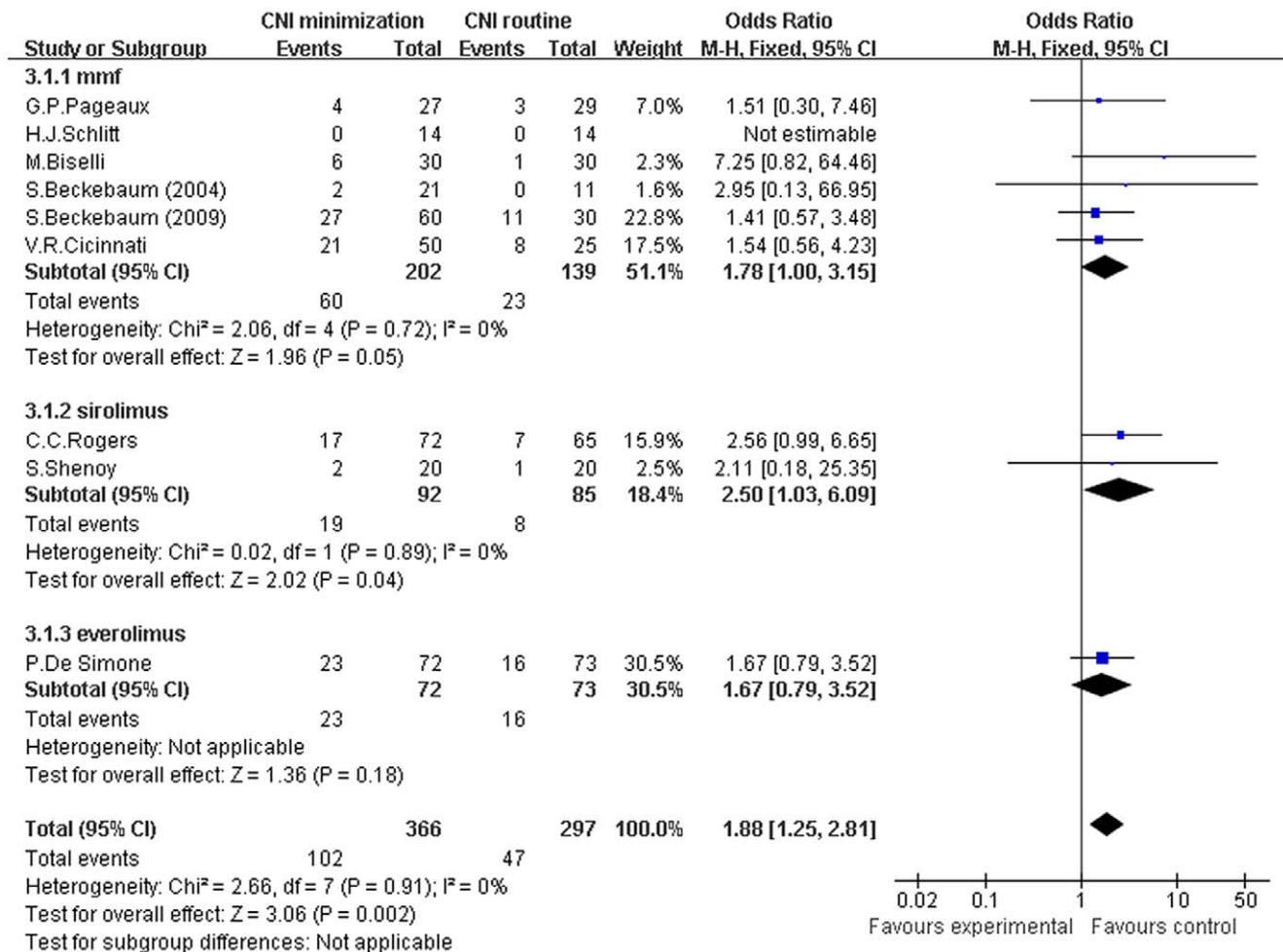


Figure 7. Meta-analysis of CNI minimization versus routine CNI regimen in patient survival (RCTs). There was no significant difference between CNI minimization and routine CNI regimen group in all subgroup and total analysis. Z = total effect size, I² = heterogeneity index. Columns in blue represent the odds ratio of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. doi:10.1371/journal.pone.0024387.g007

can make up to the shortage of small sample size and limited population of individual studies, providing stronger evidence on the clinical application of CNI minimization protocols. It is convincing that CNI minimization can improve renal function in liver transplant patients with CNI-related renal impairment, while has an equal or similar effect on acute rejection and patient survival as routine CNI regimen. However, it should be cautious to use SRL-based minimization regimens in patients with high risks of infections. Studies in the future should try to figure out whether this improved renal function can prolong long-term patient or graft survival, and which minimization protocol is the standard one in various combinations.

Supporting Information

Figure S1 Meta-analysis of CNI minimization versus routine CNI regimen in GFR (observational trials). In MMF and sirolimus subgroups, GFR was significantly higher than in the routine CNI regimen group, so was in the total analysis. Z = total effect size, I² = heterogeneity index. Columns in green represent the mean difference of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the

overall effect size and diamond width represents the overall 95% CI. (TIF)

Figure S2 Meta-analysis of CNI minimization versus routine CNI regimen in sCr (observational trials). In MMF, sirolimus and everolimus subgroups, the sCr was significantly decreased in CNI minimization group, so was in the total analysis. Z = total effect size, I² = heterogeneity index. Columns in green represent the mean difference of each study and column size represents the study weight in the analysis. Lanes represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. (TIF)

Figure S3 Meta-analysis of CNI minimization versus routine CNI regimen in CrCl (observational trials). In MMF subgroup, CrCl was significantly higher in the CNI minimization group than in the routine CNI regimen group. In everolimus subgroup, improvement of CrCl over routine CNI regimen group was not statistically significant. In the total analysis, the CrCl was significantly improved in CNI minimization group. Z = total effect size, I² = heterogeneity index. Columns in green

represent the mean difference of each study and column size represents the study weight in the analysis. Lines represent the 95% CI of each study. Diamonds in black represent the overall effect size and diamond width represents the overall 95% CI. (TIF)

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Author Contributions

Conceived and designed the experiments: YK DW YS ZG XH. Performed the experiments: YK DW YS. Analyzed the data: YK DW YS WL XL ZG XH. Contributed reagents/materials/analysis tools: YK DW YS. Wrote the paper: YK DW YS ZG XH.

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