Meta-Analysis of Calcineurin-Inhibitor-Sparing Regimens in Kidney Transplantation

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ABSTRACT

Calcineurin-inhibitor-sparing strategies in kidney transplantation may spare patients the adverse effects of these drugs, but the efficacy of these strategies is unknown. Here, we conduct a meta-analysis to assess outcomes associated with reducing calcineurin inhibitor exposure from the time of transplantation. We search Medline, Embase, and Cochrane Register of Controlled Trials for randomized controlled trials published between 1966 and 2010 that compared *de novo* calcineurin-inhibitor-sparing regimens to calcineurin-inhibitor-based regimens. In this analysis, we include 56 studies comprising data from 11337 renal transplant recipients. Use of the contemporary agents belatacept or tofacitinib, in combination with mycophenolate, decreased the odds of overall graft failure (OR 0.61; 95% CI 0.39–0.96; $P = 0.03$). Similarly, minimization of calcineurin inhibitors in combination with various induction and adjunctive agents reduces the odds of graft failure (OR 0.73; 95% CI 0.58–0.92; $P = 0.009$). Conversely, the use of inhibitors of mammalian target of rapamycin (mTOR), in combination with mycophenolate, increases the odds of graft failure (OR 1.43; 95% CI 1.08–1.90; $P = 0.01$). Calcineurin-inhibitor-sparing strategies are associated with less delayed graft function (OR 0.89; 95% CI 0.80–0.98; $P = 0.02$), improved graft function, and less new-onset diabetes. The more contemporary protocols did not seem to increase rates of acute rejection. In conclusion, this meta-analysis suggests that reducing exposure to calcineurin inhibitors immediately after kidney transplantation may improve clinical outcomes.

J Am Soc Nephrol 22: 2107–2118, 2011. doi: 10.1681/ASN.2010111160

Discovery of the immunosuppressive properties of the calcineurin inhibitor (CNI) ciclosporin by Borel in 1976,[1] and its introduction to the clinical arena by Calne in 1978,[2] heralded a new era in kidney transplantation. Randomized controlled studies from the early 1980s showed ciclosporin was associated with either significant reductions in absolute acute rejection rates or more "benign" presentations of rejection compared with azathioprine, the mainstay immunosuppressant hitherto.[3–5]

However, the intrinsic nephrotoxicity of ciclosporin became apparent in these early trials and is now well established, persisting despite introduction of the alternative CNI tacrolimus,[6] and so subsequent studies attempted to reduce overall CNI exposure while maintaining reduced rejection rates. Trials of the mid and late 1980s evaluated weaning CNIs months or years following transplantation.[7] However, kidney function in the early period post transplantation is a potent determinant of subsequent graft outcome,[8] and, therefore, later studies focused on reducing or completely eliminating CNIs *from the time of transplantation itself*, a strategy made possible with the development of "non-nephrotoxic" immunosuppressants.

An ever increasing array of such agents may facilitate reduced CNI exposure early post transplan-

Received November 14, 2010. Accepted June 1, 2011.

Published online ahead of print. Publication date available at www.jasn.org.

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tation. The 1990s saw the emergence of the antiproliferative agents mycophenolate mofetil and the mammalian target of rapamycin inhibitor (mTORI), sirolimus. Post 2000, the immunosuppressive armamentarium (both in standard practice and clinical trials) expanded to include the sirolimus analog, everolimus; the anti-CD52 leuco-depleting antibody, alemtuzumab; the protein kinase C inhibitor, sotrastaurin (AEB071); the lymphocyte sequestering agent, FTY 720; the janus kinase 3 inhibitor, tofacitinib (CP-690,550); the CD28 co-stimulation blocker, belatacept.

CNI exposure in current clinical practice is lower than that employed historically; however, the safety and efficacy of reducing CNI exposure from the time of transplantation has not been subjected to a full and robust data synthesis, with many protocols remaining experimental. The purpose of this systematic review and meta-analysis was, therefore, to evaluate the clinical outcomes associated with strategies designed to improve allograft function/survival by reducing, avoiding or delaying introduction of CNI.

RESULTS

The results of the literature search are illustrated in Figure 1. Fifty-six randomized clinical trials, providing data for 11,337 renal transplant recipients were identified (Table 1), with the median end-of-study time point of 12 mo. On a JADAD scoring scale for study quality 19 studies scored 1/5, 15 studies scored 2/5, and 18 studies scored 3/5 (four trials were not scored due to being in abstract format).

Total CNI avoidance,[3,5,9–38] CNI minimization,[17,39–55] and delayed introduction of CNI[49,50,55–62] were investigated in 32 ($n = 5791$), 17 (n = 4131), and 10 studies (n = 1519) respectively. Two studies[50,55] investigated CNI delay followed by minimization: to avoid "double counting" these were analyzed as "delay" studies initially, but if subgroup analyses were necessary (due to heterogeneity), then the same study was considered separately in both the "minimization" and "delay" subanalyses. One four-arm trial[17] was suitable for consideration as two separate studies (one minimization; one avoidance with mTORI/mycophenolate) without double-counting any of the participants. Study arms consisting of low intensity belatacept (as opposed to moderate intensity) and low dose tofacitinib (as opposed to high dose) were selected for evaluation against standard CNI exposure protocols, as future experience is likely to focus on these regimens.

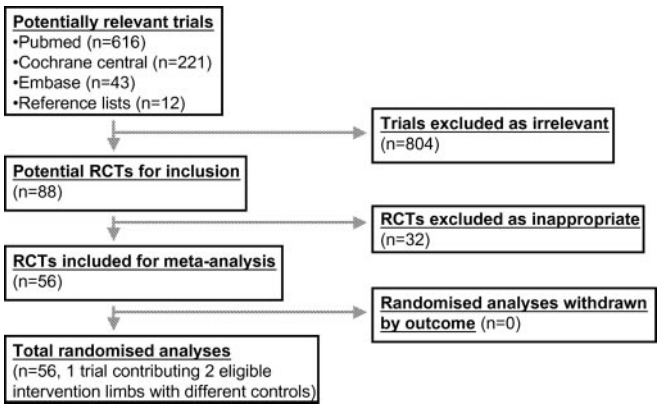

Figure 1. PRISMA flow diagram identifying *de novo* CNI sparing trials for inclusion in meta-analysis.

In the intervention arm, examples of non-CNI immunosuppressants included sirolimus or everolimus (18 studies, $n = 3155$), belatacept (three studies, $n = 950$), tofacitinib (CP-690550) (two studies, $n = 257$), FTY720 (two studies, $n = 898$), sotrastaurin (one study, $n = 142$) and alemtuzumab induction (four studies, $n = 242$). In the control arm 20 studies utilized tacrolimus as the maintenance CNI ($n = 3289$) and 35 used ciclosporin ($n = 7568$), with one study[53] incorporating both calcineurin inhibitors. The individual immunosuppressant regimens and study lengths for all of the randomized controlled trials are summarized in Table 1.

Graft Failure

In the pooled analysis, no difference was identified between standard and reduced CNI exposure regarding overall graft failure (OR 1.05 [95% CI 0.85–1.29], $P = 0.66$, $I^2 = 54\%$) or death-censored graft failure (OR 1.11 [95% CI 0.89–1.38], $P = 0.36$, $I^2 = 44\%$). However, significant interstudy heterogeneity was evident and, therefore, further subgroup analyses were conducted.

No difference in overall graft failure (OR 1.51 [95% CI 0.91–2.50], $P = 0.11$, $I^2 = 80\%$) or death-censored graft failure (OR 1.59 [95% CI 0.94–2.68], $P = 0.08$, $I^2 = 78\%$) was apparent when azathioprine or mycophenolate monotherapy was compared with CNI based regimens (11 studies, $n = 1896$). However, death-censored graft failure due to acute rejection was more common in the azathioprine or mycophenolate monotherapy arms (OR 2.79 [95% CI 1.39–5.61], $P = 0.004$, $I^2 = 65\%$).

The combination of mTORI and mycophenolate (16 studies, $n = 2688$) was associated with increased overall graft failure (OR 1.43 [95% CI 1.08–1.90], $P = 0.01$, $I^2 = 19\%$) (Figure 2) and death-censored graft failure (OR 1.59 [95% CI 1.12–2.25], $P = 0.009$, $I^2 = 5\%$) compared with CNI-based regimens. Similar results were seen when the analysis was repeated comparing mTOR/mycophenolate *versus* low-dose ciclosporin rather than low-dose tacrolimus for the Symphony study: OR 1.35 [95% CI 1.02 to 1.79], I^2 12%, $P = 0.03$ and OR 1.40 [95% CI 0.98 to 1.99], I^2 0%, $P = 0.07$ for overall graft failure and death-censored graft failure respectively. No difference between groups for death-censored graft failure secondary to acute rejection was demonstrated (OR 1.56 [95% CI 0.57–4.25], $P = 0.39$, $I^2 = 0\%$).

In contrast, the combination of mycophenolate with newer immunosuppressive agents (belatacept or tofacitinib) (five studies, $n = 1207$) was associated with reduced overall graft failure (OR 0.61 [95% CI 0.39–0.96], $P = 0.03$, $I^2 = 0\%$) (Figure 3). No difference in death-censored graft failure rates were observed (OR 0.77 [95% CI 0.46–1.31], $P = 0.34$, $I^2 =$

Table 1. Data for selected randomized controlled trials

Study (year)	Intervention arm	Control arm	CNI sparing strategy	Study length
Andres (2009)	IL2 + C+MMF + P	IL2 + lowC + MMF + P	DELAY + MINIMISATION	6 months
Andres (2009)	IL2 + T+MMF + shortP	T + MMF + P	DELAY	6 months
Asberg (2006)	IL2 + MMF + P	C + MMF + P	AVOIDANCE	12 months
Buchler (2007)	ATG + S+MMF + P	ATG + C+MMF + P	AVOIDANCE	12 months
Budde (2010)*	AEB + lowT + P	AEB + T+P	MINIMISATION	3 months
Busque (2009)	IL2 + J+MMF + P	IL2 + T+MMF + P	AVOIDANCE	12 months
Canadian Multicentre study (1983)	AZA + P	C	AVOIDANCE	1–17 months
Chan (2008)	IL2 + E+lowT + P	IL2 + E+T + P	MINIMISATION	6 months
Chan (2009) (A)	A + T	IL2 + T+MMF	MINIMISATION	12 months
Charpentier (2003)	ATG + delayT + AZA + P	T + AZA + P	DELAY	6 months
Ciancio (2005)	A + lowT + lowMMF	ATG + T+MMF + P	MINIMISATION	12 months
De Sevaux (2001)	lowC + MMF + P	C + MMF + P	MINIMISATION	6 months
Durrbach (2008)	ATG + S+MMF + P	ATG + C+MMF + P	AVOIDANCE	6 months
Durrbach (2010)	IL2 + B+MMF + P	IL2 + C+MMF + P	AVOIDANCE	12 months
Ekberg-CAESAR (2007)	IL2 + lowC + MMF + P	C + MMF + P	MINIMISATION	12 months
Ekberg-SYMPHONY (2007)**	IL2 + S+MMF + P	IL2 + lowT + MMF + P	AVOIDANCE	12 months
Ekberg-SYMPHONY (2007)**	IL2 + lowC + MMF + P	C + MMF + P	MINIMISATION	12 months
European Multicentre study (1983)	AZA + P	C	AVOIDANCE	11 months
Flechner (2002)	IL2 + S+MMF + P	IL2 + C+MMF + P	AVOIDANCE	18.1 months
Flechner "318" (2009)	IL2 + S+MMF + P	IL2 + C+MMF + P	AVOIDANCE	N/A
Flechner "ORION" (2009)	IL2 + S+MMF + P	IL2 + T+MMF + P	AVOIDANCE	N/A
Gaston (2009)	Induction + lowCNI + MMF + P	Induction + CNI + MMF + P	MINIMISATION	24 months
Gelens (2006)	IL2 + S+MMF + shortMP	T + MMF + shortMP	AVOIDANCE	9.2 months
Gheith (2007)	AZA + P	C + P	AVOIDANCE	20 yr
Glotz (2010)	ATG + S+MMF + P	(ATG)+T + MMF + P	AVOIDANCE	12 months
Grimbert (2002)***	ALG + AZA + P	ALG + delayC + AZA + P	AVOIDANCE	12 months
Groth (1999)	S + AZA + P	C + AZA + P	AVOIDANCE	12 months
Hall (1998)	AZA + P	C	AVOIDANCE	36 months
Hamdy (2005)	IL2 + S+MMF + P	IL2 + T+S + P	AVOIDANCE	24 months
Hernandez (2007)	IL2 + lowC + MMF + P	ATG + C+AZA + P	MINIMISATION	24 months
Kamar (2006)	IL2 + delayC + MPS + P	IL2 + C+MPS + P	DELAY	12 months
Kandaswamy (2005)	ATG + lowT + S+shortP	ATG + T+S + shortP	MINIMISATION	24 months
Kasiske (1997)	ATG + delayC + AZA + P	C + AZA + P	DELAY	90 days
Kreis (2000)	S + MMF + P	C + MMF + P	AVOIDANCE	12 months
Larson (2006)	ATG + S+MMF + P	ATG + T+MMF + P	AVOIDANCE	33 months
Lo (2004)	ATG + S+MMF + P	ATG + S+lowT + P	AVOIDANCE	333 days
Margreiter (2008)	A + T	T + MMF + P	DELAY	12 months
Martinez-Mier (2006)	IL2 + S+MMF + P	IL2 + C+MMF + P	AVOIDANCE	15.8 months
McMaster (1983)	AZA + P	C	AVOIDANCE	6 months
Najarian (1984)	ALG + AZA + P	C + P	AVOIDANCE	24 months
Nashan (2004)	IL2 + lowC + E+P	IL2 + C+E + P	MINIMISATION	36 months
Noel (2009)	ATG + T+MMF + P	IL2 + T+MMF + P	DELAY	12 months
Novick (1986)	ALG + AZA + P	C + P	AVOIDANCE	12 months
Ponticelli (1988)	AZA + P	C	AVOIDANCE	36 months
Rosenthal (1983)	AZA + P	C	AVOIDANCE	24 months
Ruggenenti (2007)	A + S+MMF	A + C+MMF	AVOIDANCE	12 months
Russ (2003)	lowT + S+P	T + S+P	MINIMISATION	6 months
Salvadori (2006)	highFTY720 + lowC	lowFTY720 + C	MINIMISATION	12 months
Salvadori (2009)	stanE + lowC	highE + vlowC	MINIMISATION	36 months

Table 1. Continued

Study (year)	Intervention arm	Control arm	CNI sparing strategy	Study length
Schaefer (2006)	ATG + S+MMF + P	ATG + T+MMF + P	AVOIDANCE	12 months
Tedesco-Silva (2006)	highFTY720 + lowC	lowFTY720 + C	MINIMISATION	12 months
Thomas (2007)	ATG + T+MMF + P	A + T	DELAY	377 days
Vathsala (2005)	A + lowC	C + AZA + P	DELAY + MINIMISATION	6 months
Vincenti (2005)	IL2 + B+MMF + P	IL2 + C+MMF + P	AVOIDANCE	12 months
Vincenti (2010)	IL2 + B+MMF + P	IL2 + C+MMF + P	AVOIDANCE	12 months
Vincenti (2010) (A)	IL2 + J+MMF/MPS + P	IL2 + C+MMF/MPS + P	AVOIDANCE	6 months
Wienand (1993)	ALG + delayC + shortAZA + P	ALG + C+shortAZA + P	DELAY	12 months

*3-month data taken prior to CNI withdrawal protocol.
**Different intervention limbs of the same study (SYMPHONY) (Reference 17).
***12-yr study (survival data taken at 1-yr).
T, Tacrolimus; C, Ciclosporin; CNI, Calcineurin inhibitor; S, Sirolimus; E, Everolimus; B, Belatacept; J, JAK 3 inhibitor (tofacitinib, CP-690550); FTY720, Experimental name; ATG, Anti-thymocyte globulin; ALG, Anti-lymphocyte globulin; IL2, Interleukin 2 receptor antagonist; A, Alemtuzumab; MMF, Mycophenolate mofetil; MPS, Mycophenolate sodium; AZA, Azathioprine; MP, Methylprednisolone; P, Prednisolone; (A), Abstract at conference.

0%). No difference in death-censored graft failure secondary to acute rejection between these protocols and CNI containing protocols was evident (OR 0.68 [95% CI 0.31–1.48], $P = 0.33$, $I^2 = 0\%$).

CNI minimization, compared with standard exposure CNI, (17 studies, $n = 4131$) was associated with reduced overall graft failure (OR 0.73 [95% CI 0.58–0.92], $P = 0.009$, $I^2 = 0\%$) (Figure 4) and death-censored graft failure (OR 0.73 [95% CI 0.55–0.97], $P = 0.03$, $I^2 = 0\%$). No difference in graft failure secondary to rejection was seen (OR 0.67 [95% CI 0.34–1.31], $P = 0.24$, $I^2 = 0\%$).

No effect of delayed CNI introduction (10 studies, $n = 1519$) on overall graft failure (OR 1.04 [95% CI 0.75–1.44], $P = 0.81$, $I^2 = 28\%$) or death-censored graft failure (OR 1.01 [95% CI 0.70–1.44], $P = 0.97$, $I^2 = 4\%$) was demonstrated. No difference in graft failure secondary to rejection (OR 1.03 [95% CI 0.41–2.56], $P = 0.95$, $I^2 = 0\%$) was seen in these studies.

Patient Survival

There was no effect of reduced CNI exposure on mortality in the pooled analysis (OR 0.92 [95% CI 0.76–1.11], $P = 0.39$, $I^2 = 0\%$) with no evidence of heterogeneity.

Delayed Graft Function

Reduced CNI exposure from the time of transplantation was associated with reduced DGF rates in the 45 studies ($n = 9456$) with available data (OR 0.89 [95% CI 0.80–0.98]; $P = 0.02$, $I^2 = 23\%$) (Figure 5).

Graft Function

Reduced CNI exposure was associated with improved graft function compared with standard CNI exposure (WMD + 5.31 ml/min [95% CI 2.82–7.81 ml/min], $P < 0.001$) in the pooled analysis (Figure 6). However, significant interstudy heterogeneity was observed ($I^2 = 67\%$) and further subanalyses were conducted:

No difference in graft function between regimens based on azathioprine or mycophenolate monotherapy *versus* CNI-based regimens was seen (WMD + 7.51 ml/min [95% CI −5.15–20.17 ml/min], $P = 0.24$, $I^2 = 62\%$). Conversely, the

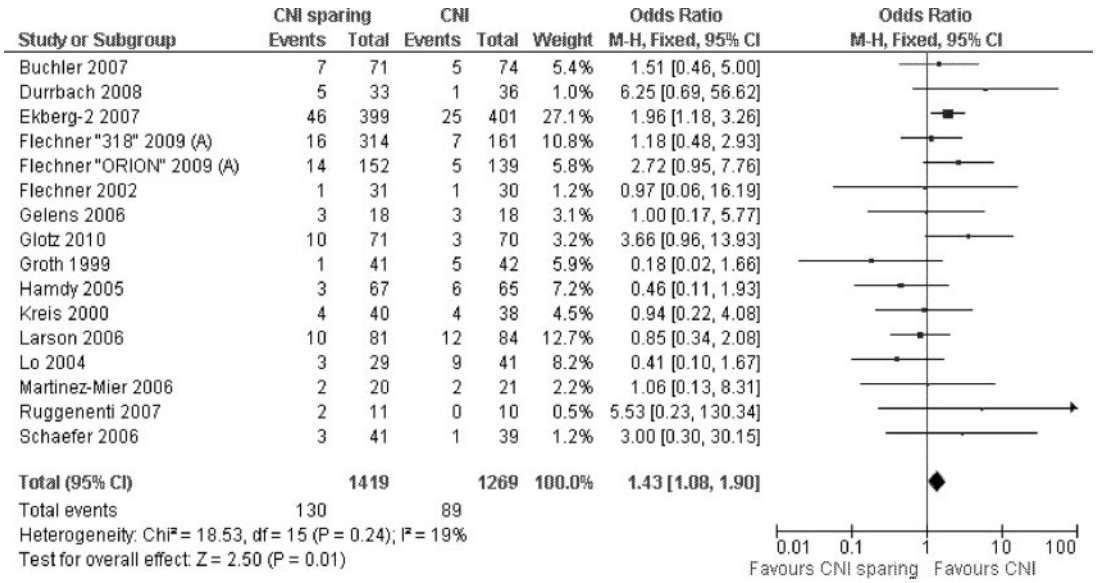

Figure 2. Forest plot of overall graft survival with CNI avoidance strategies using mTORI/mycophenolate combination.

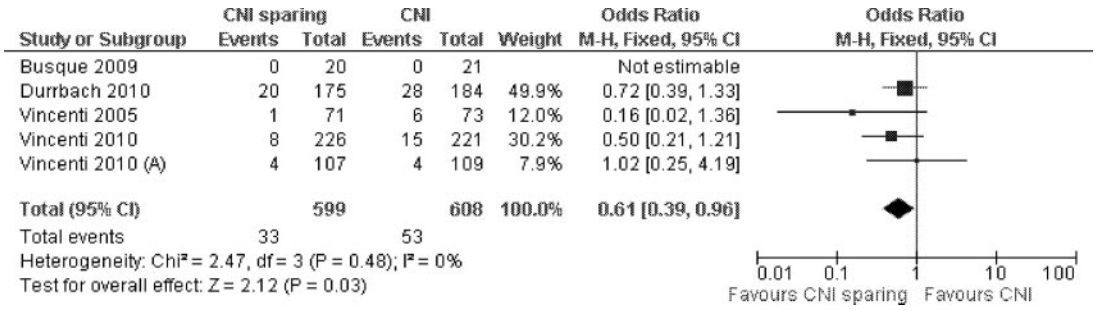

Figure 3. Forest plot of overall graft survival with CNI avoidance strategies using new agents (belatacept or tofacitinib).

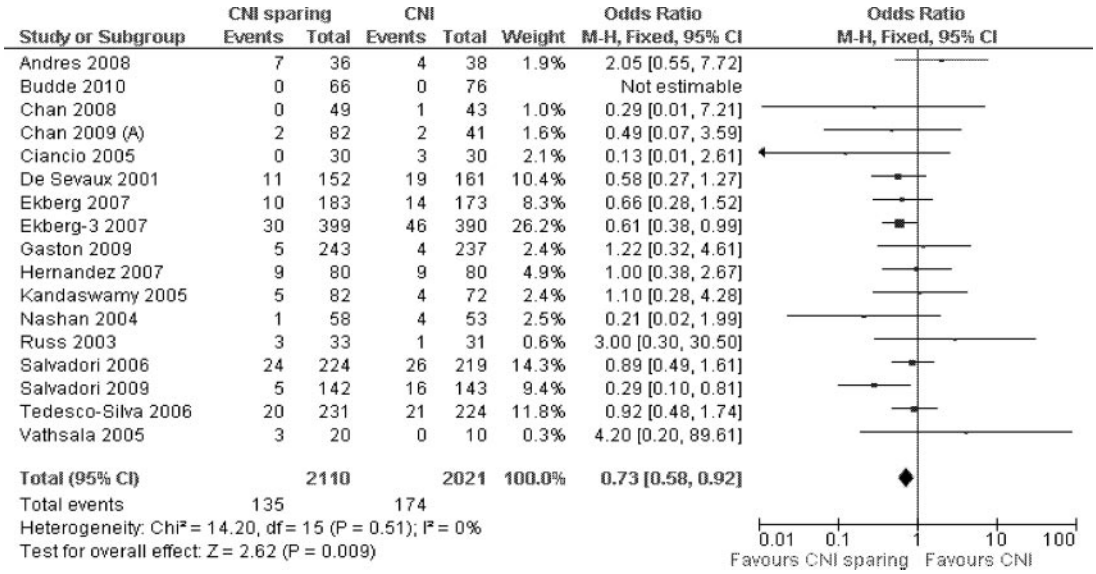

Figure 4. Forest plot of overall graft survival with CNI minimization strategies.

combination of mTORI and mycophenolate was associated with improved graft function (WMD + 6.58 ml/min [95% CI −0.08–13.23 ml/min], $P = 0.05$, $I^2 = 83\%$), as was the combination of mycophenolate with either belatacept or tofacitinib (WMD + 8.54 ml/min [95% CI 3.64–13.43 ml/min], $P < 0.001$, $I^2 = 50\%$). Although residual heterogeneity was evident in all these subanalyses, this reflected varying degrees of renal function improvement in the reduced CNI exposure groups, with only three studies in the overall cohort demonstrating a point estimate failing to demonstrate superior function with these protocols.

CNI minimization and CNI delay strategies were also associated with improved graft function (WMD + 3.44 ml/min [95% CI 1.21–5.68 ml/min], $P = 0.003$, $I^2 = 0\%$ and WMD + 2.83 ml/min [95% CI 0.09–5.76 ml/min]; $P = 0.05$ $I^2 = 0\%$ respectively), with no evidence of interstudy heterogeneity.

Acute Rejection

Comparing CNI sparing to CNI-based regimens, increased acute rejection rates were seen across 53 studies ($n = 10712$) with available data (OR 1.24 [95% CI 1.01–1.53], $P = 0.04$, $I^2 = 70\%$) and further subanalyses were performed in light of the observed heterogeneity.

Azathioprine or mycophenolate monotherapy was associated with increased acute rejection rates compared with CNI-based regimens (OR 2.34 [95% CI 1.40–3.91], $P = 0.001$, $I^2 = 78\%$). No difference in rejection rates was demonstrated for the other strategies to reduce or avoid CNI exposure: OR 1.46 [95% CI 0.86–2.46], $P = 0.16$, $I^2 = 62\%$ for the combination of mTORI and mycophenolate; OR 1.04 [95% CI 0.50–2.16], $P = 0.91$, $I^2 = 70\%$ for the combination of mycophenolate and either belatacept or tofacitinib; OR 0.99 [95% CI 0.76–1.28], $P = 0.91$, $I^2 = 49\%$ for CNI minimization; OR 1.00 [95% CI 0.67–1.50], $P = 1.00$, $I^2 = 56\%$ for CNI delay.

Influence of Antibody Induction

To investigate the influence of antibody induction as a potential confounder in these analyses, a meta-regression analysis was performed to assess whether the observed effects were influenced by the use of 'differential' induction therapy, *i.e.* the use of induction in the CNI sparing arm but not in the standard arm. This analysis was most pertinent for the CNI minimization trials as the majority of studies using differential induction were limited to this subgroup. Studies using differential induction ($n = 6$) were compared with the remaining studies that either used induction in both arms ($n = 6$) or induction in neither arm ($n = 5$). No

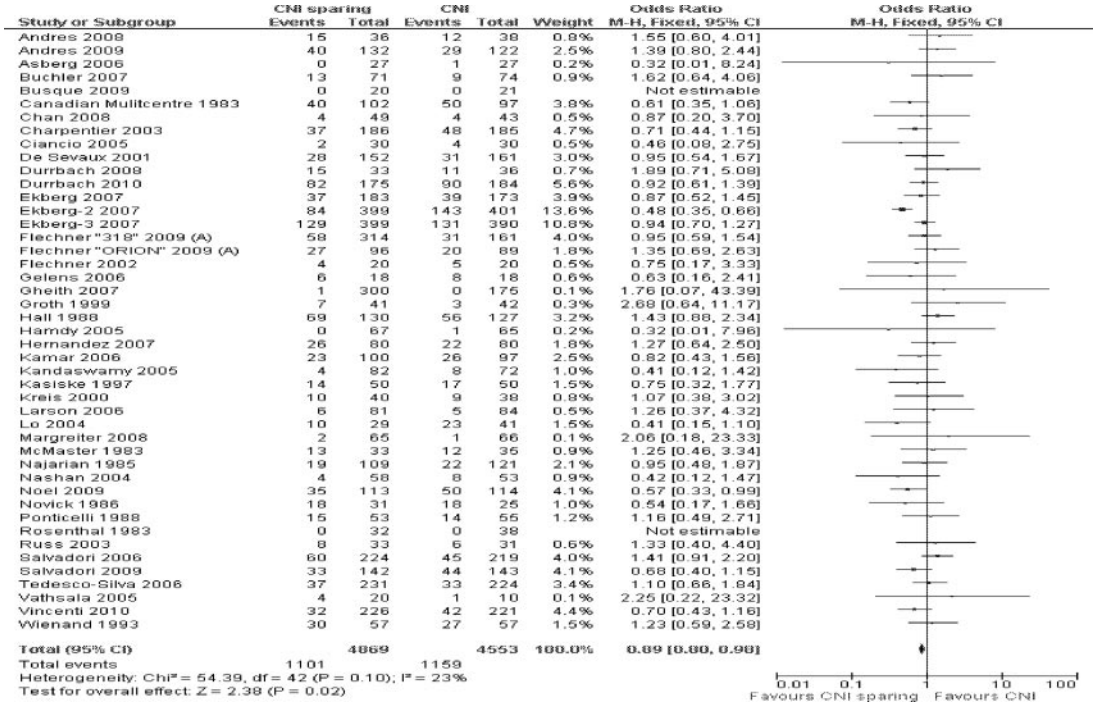

Figure 5. Figure plot showing episodes of delayed graft function comparing all CNI sparing studies with CNI-based regimens.

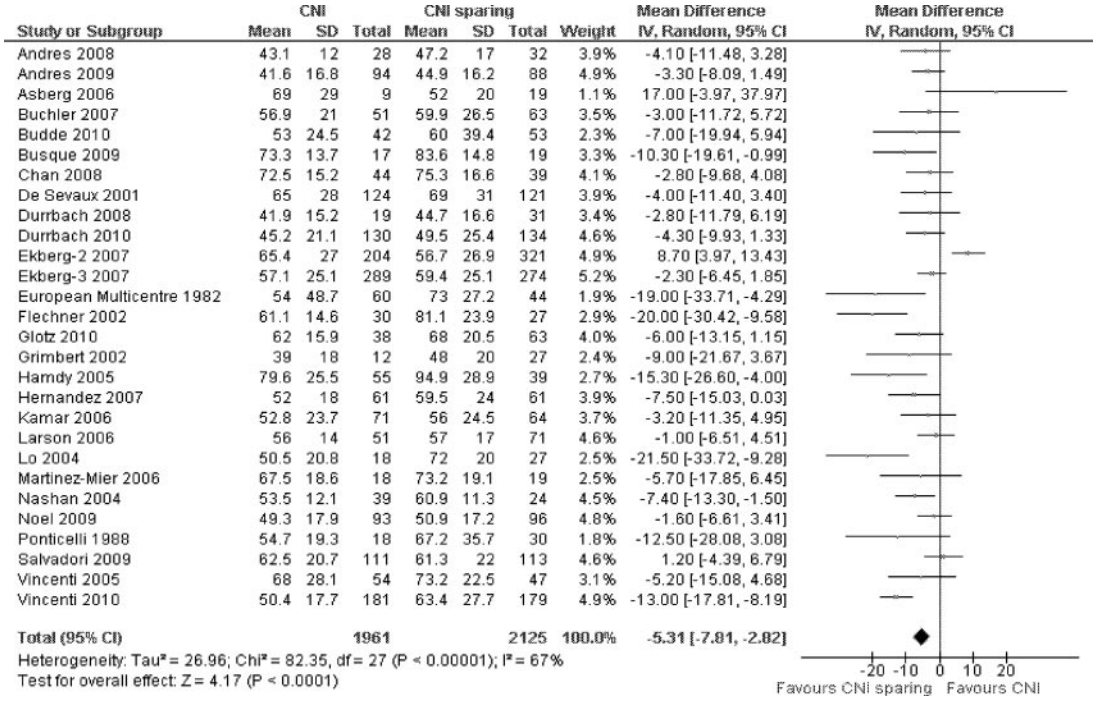

Figure 6. Figure plot showing graft function for all CNI sparing *versus* CNI-based studies.

evidence was found to suggest an effect of differential induction on graft failure or acute rejection (Table 2).

Other Outcomes

CNI sparing protocols were not associated with different rates of NODAT compared with CNI-based regimens (OR 0.88 [95% CI 0.74–1.04], $P = 0.12$, $I^2 = 1\%$) when the 38 studies ($n = 7305$) reporting this outcome were analyzed. However, the eight studies ($n = 2943$) that specifically utilized current diagnostic guidelines for NODAT[63] demonstrated reduced rates of NODAT with reduced exposure CNI (OR 0.74 [95% CI 0.55–0.99], $P = 0.04$, $I^2 = 7\%$) (Figure 7).

Table 2. Meta-regression assessing influence of differential antibody induction on outcomes in CNI minimization sub-group

Outcome	Odds Ratio (Ratio [95% CI])	*P* Value
Overall graft failure	0.82 [0.46, 1.43]	0.45
Death-censored graft failure	0.90 [0.47, 1.76]	0.75
Acute rejection	0.83 [0.36, 1.87]	0.63

No difference was observed in incidence of infections (polyoma, CMV or total infections) when comparing CNI sparing to standard CNI arms: OR 0.65 [95% CI 0.36–1.17], $P = 0.15$, $I^2 = 0\%$ for polyoma virus; OR 0.99 [95% CI 0.74–1.31], $P = 0.94$, $I^2 = 64\%$ for CMV; OR 1.02 [95% CI 0.92–1.12], $P = 0.72$, $I^2 = 22\%$ for total infections as reported in eight, 38, and 41 studies respectively.

Study Withdrawals

An increase in treatment discontinuations with CNI sparing protocols was observed (OR 1.33 [95% CI 1.06–1.66], $P = 0.01$, $I^2 = 79\%$). Heterogeneity prompted further analysis and the combination of mTORI and mycophenolate was demonstrated to have significantly more treatment withdrawals compared with CNI-based regimens (OR 2.07 [95% CI 1.20–3.59], $P = 0.009$, $I^2 = 81\%$). No difference in withdrawal rate between study arms was seen for the other subgroup analyses.

DISCUSSION

This meta-analysis, examining 11,337 patients from 56 randomized controlled trials, provides insights into the risks and benefits of reducing CNI exposure immediately following kidney transplantation. This study importantly demonstrates that this strategy can be safe and efficacious in the short-to-medium period post kidney transplantation. The strength of this study is assessment of "hard" end points in renal transplantation (graft loss and mortality) which individual studies have hitherto been underpowered to address.

Although (by an iterative process) CNI exposure in current clinical practice is now lower than that employed historically, controlled clinical trials of reduced CNI exposure have not been subjected to systematic review and meta-analysis. This analysis demonstrates that all investigated protocols (avoidance, minimization and delayed introduction of CNI) are effective in improving renal function without evidence for increased rejection. However, for other "hard" endpoints, important differences between protocols emerged. Of particular interest, the newer agents belatacept or tofacitinib (as yet unapproved for clinical use) in combination with mycophenolate result in improved overall graft survival. Longer follow-up is required to confirm the durability of these beneficial effects and further trials are needed in other populations, particularly those at higher immunological risk and/or those on tacrolimus-based protocols as ciclosporin was the comparator in these particular studies. Future studies will require comparison with CNI "minimization" protocols, as this meta-analysis demonstrates that such minimization protocols also result in improved overall (and death censored) graft survival, thereby lending evidence-based support for the recent vogue to consider minimization protocols the standard of care for the majority of kidney transplant recipients.

More concerning was the increased overall and death-censored graft failure rates associated with the use of mTORIs and mycophenolate in combination, despite improved graft function *in those surviving with functioning grafts*. No increase in acute rejection rate or graft loss to acute rejection was evident, suggesting that merely increasing exposure to the constituent immunosuppressants in these protocols may not necessarily improve outcomes. In addition, these protocols were associated with high withdrawal rates and within-study crossover, potentially limiting any renoprotective effect of these agents. Thus, the benefit of improved renal function is offset by increased graft loss and questions the suitability of this combination immediately following transplantation. A previous meta-analysis of mTOR inhibitor use in renal transplant recipients also demonstrated no difference in acute

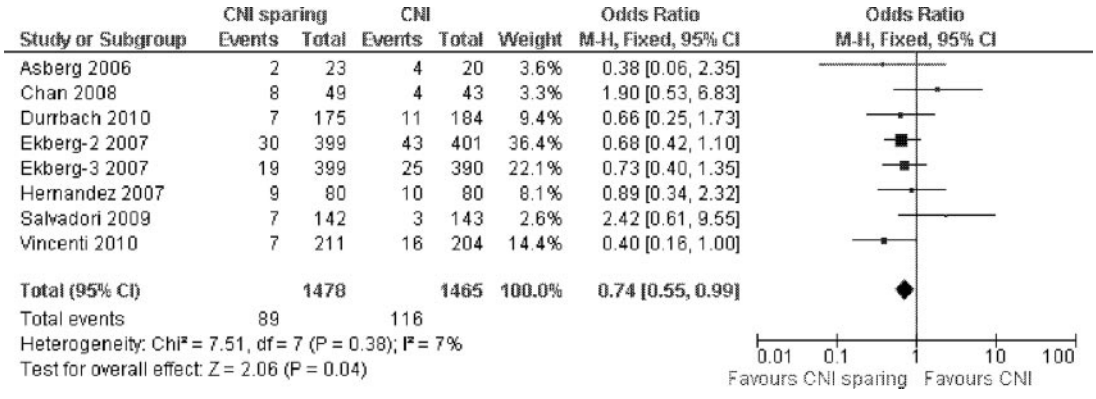

Figure 7. Forest plot of episodes of new onset diabetes after transplantation between CNI sparing and CNI-based arms (diagnosis by guidelines).

rejection and superior graft function when mTORs are used as CNI replacement.[64] However, in contrast to our results, that analysis did not demonstrate any difference in graft loss. The explanation for this incongruity is likely to be a difference in study numbers: while the previous analysis combined eight trials ($n = 750$), our empirical data comprised 16 studies ($n = 2688$) and is likely to be better powered to analyze such "hard" endpoints.

Other benefits of reduced CNI exposure were seen, including a reduction in delayed graft function, supporting the rationale for delayed introduction of CNI post transplantation. Interestingly, reduced CNI exposure was also associated with reduction in new onset diabetes, itself associated with impaired long-term patient and graft survival,[65] confirming the significant and important diabetogenic potential of CNIs. Previous meta-analyses have performed comparative analysis of the two CNIs and found tacrolimus to be superior to ciclosporin by preventing early graft loss and episodes of rejection, but at the expense of more NODAT.[66,67] Our intention was not to compare these two agents to each other but to compare standard CNI *versus* any CNI sparing strategy. The results demonstrate that irrespective of the comparator CNI, there is a reduction in NODAT incidence if the CNI is omitted or minimized. The absolute risk reduction will depend on the CNI used, but the lack of heterogeneity in this analysis suggests the relative reduction is similar between compounds.

Importantly, renal function and acute rejection performed poorly as surrogate end points in clinical trials. For example, despite increased graft failure in mTORI/mycophenolate-based CNI avoidance protocols, no increase in acute rejection was demonstrable. Similarly, despite an increase in graft failure renal function was preserved in those patients surviving with graft function. The limitations of these surrogates has recently been discussed by Schold and Kaplan[68] as they pertain to observational data, and the current study demonstrates this in the context of clinical trial interventions.

The limitations of this analysis, as with most meta-analyses, include lack of patient-centered endpoints (such as quality of life) and inclusion of trials which were heterogeneous in design. The lack of heterogeneity for the most important analyses in this report suggests that the results are consistent across immunosuppression regimens and individual agents. Indeed this is consistent with data showing comparable outcomes for the calcineurin inhibitors ciclosporin and tacrolimus (when used at comparable levels of exposure) in terms of histologic damage,[69,70] graft survival in clinical trials[17] and registry analyses.[71] Another limitation is the inability to clearly distinguish drug concentrations between comparator groups, especially important in the context of CNI minimization study comparisons. While the studies are clear on target levels, very few actually report achieved levels; therefore, looking at overlap between groups as a confounder in the results is not possible. The likelihood is that more overlap than intended was observed by study investigators and there are certainly proven examples of this in the literature (most notably the SYMPHONY study drug level data[17]). This is, unfortunately, the "nature of the beast" in randomized controlled trials of this kind, but these nevertheless still represent the best level of evidence available. We believe this is a widely acknowledged issue among transplant clinicians and accordingly interpretation of such studies (and therefore this meta-analysis) is in the context of such limitations. Indeed our intention with this study was not to identify the optimal target level for CNIs. The results of the meta-regression analysis did not support the notion that induction agents drive any difference in graft survival or acute rejection. Although evidence in the literature would suggest such induction improves graft survival in recipients at high immunological risk,[72,73] no such evidence exists for standard risk patients. Our studies comprised such standard-risk patients and this could explain the lack of any significant effect. Therefore, there is no evidence to suggest difference in outcome between study limbs is "driven" by differential use of induction.

In summary, our intention was not to define the single most effective immunosuppressive regimen for renal transplant recipients but rather to logically identify the risks and benefits of reduced CNI exposure to guide future development and refinement. To ensure transparency and robustness, our studies spanned over three decades of published material. It is the very nature of randomized controlled trials that intrastudy confounders should be minimized; interstudy differences due to effects of era, protocol changes and evolution of practice are likely evident, however the cotemporaneous nature of the individual randomized trials should also account for this phenomenon. Similarly the categorization of studies into CNI delay/minimization/avoidance has successfully minimized (or eliminated) interstudy heterogeneity for important analyses such as graft failure. Meta-analyses are considered the gold standard of clinical evidence by deriving results from appropriate pooling of empirical data but they can also be hypothesis generating and help direct future research and development. We believe this meta-analysis achieves both these aims and addresses one of the most topical and challenging aspects of transplantation.

To conclude, this large meta analysis suggests significant clinical advantage can result from reducing CNI exposure immediately post transplantation and beyond. This sets the stage for further studies to assess the durability and generalization of these findings.

CONCISE METHODS

Search Strategy

Medline, Embase and the Cochrane Central Register of Controlled Trials were searched from 1966 to 2010 for randomized controlled trials with the following MeSH words: calcineurin inhibitor, CNI, ciclosporin/cyclosporine, Neoral, Sandimmune, tacrolimus. Prograf, Rapamune, sirolimus, everolimus, FK506, belatacept, sotrastaurin,

AEB071, JAK-3, CP-690550, tofacitinib, FTY720, alemtuzumab, Campath and kidney/renal transplantation. Reference lists of identified papers were searched for relevant studies. The abstracts of relevant conferences for the last three yr were searched for randomized controlled trials not available in published format.

Selection Criteria and Data Sources

Randomized controlled trials were selected, with control arms receiving "standard" CNI-based regimens and experimental arms receiving reduced CNI exposure from the first post operative day. Reduced exposure consisted of either complete avoidance of CNI (henceforth known as "CNI avoidance"), reduction in the dose of CNI followed by therapeutic drug monitoring to target CNI levels lower than in the control arm (henceforth known as "CNI minimization"), or omission of CNI in the immediate post operative period followed by CNI introduction later during the first post operative week, thereby avoiding the immediate and additive deleterious effects of ischemia-reperfusion injury and CNI toxicity (henceforth known as "CNI delay").

Two investigators (AS and RB) examined each study independently and recorded eligibility, quality and outcome measures, with disagreement resolved by discussion. In instances of publication duplication the index paper was utilized, with additional data from subsequent reports included where appropriate.

Studies where reduced dose CNI in combination with an mTORI were compared with full dose CNI in combination with a non-mTORI adjunctive immunosuppressant were specifically excluded. The rationale for this was that mTORIs increase tissue concentrations of CNI (thereby potentiating their toxicity), and therefore the experimental arms of these studies does not truly represent reduced tissue exposure to CNI.[72]

For completeness and transparency of data collection and presentation all other studies were evaluated. Therefore, this analysis included studies of azathioprine and mycophenolate monotherapy that are seldom used in current clinical practice but have historical relevance. Similarly studies involving newer agents that await approval by regulatory authorities (*e.g.* tofacitinib) are included. FTY720 is no longer in development as a transplant immunosuppressant, although this "pipeline" ceased largely due to side effects rather than purely lack of efficacy, and so trial inclusion was deemed relevant.

Meta-Analysis and Outcome Measures

The primary outcome measure investigated was overall graft failure (composite of death-censored graft loss and death with graft function) at the main study endpoint (most commonly one yr). This was chosen as the most robust hard outcome in renal transplantation. Additional outcomes were as follows: death-censored graft failure (total, and specifically due to acute rejection); mortality; delayed graft function (DGF); acute rejection (biopsy proven where available); graft function (estimated GFR or creatinine clearance $\pm$ SD); new onset diabetes after transplantation (NODAT) with particular attention to those studies specifically utilizing current diagnostic guidelines for NODAT;[63] infection rates (total infections, cytomegalovirus and polyoma virus); study withdrawal rate.

Authors and pharmaceutical companies were contacted to request additional information not contained within manuscripts. Of 25 such approaches made, responses were obtained from 18 sources.

Statistical Analysis

The Review Manager 5 program was utilized for the execution of the meta-analysis. For dichotomous data (graft failure, mortality, DGF, acute rejection, NODAT, infections, withdrawals) the odds ratio (OR) was calculated. For continuous data (graft function) results are expressed as the weighted mean difference (WMD).

Statistical heterogeneity between trials was assessed with the I^2 statistic, which provides a measure of overall variation attributable to between-trial heterogeneity. It scores heterogeneity on a score between 0% and 100%, with $I^2 < 30\%$ generally accepted as low heterogeneity. When primary analyses revealed significant heterogeneity subgroup analyses were performed by categorizing studies into the following biologically plausible, clinically intuitive and historically relevant experimental groups:

i) CNI avoidance with concomitant azathioprine or mycophenolate monotherapy

ii) CNI avoidance with concomitant mTORI and mycophenolate co-therapy

iii) CNI avoidance with mycophenolate in combination with either of the newer immunosuppressants, belatacept or tofacitinib

iv) CNI minimization (see definition above)

v) CNI delayed (see above)

Fixed-effect (assumption of similar treatment effect across studies) models were chosen for analyses not displaying heterogeneity ($I^2 < 30\%$). In analyses where heterogeneity persisted ($I^2 \geq 30\%$), results from random-effect (assumption that treatment effect varies across studies) models are reported.

To explore the effect of induction agents as a confounder, a meta-regression analysis was performed in subgroups with differential induction for the outcomes of overall graft failure, death-censored graft failure and acute rejection. The aim was to examine if the difference between CNI and CNI sparing regimens varied dependent on whether an induction agent was used in the CNI sparing arm. This effect is summarized using the odds ratio of relative risk between groups.

ACKNOWLEDGMENT

The authors wish to acknowledge the support of the Birmingham Clinical Trials Unit for their statistical support and advice. There is no funding source for this work to acknowledge.

DISCLOSURES

None.

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