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Therapeutic drug monitoring

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GUIDELINES

a. In renal allograft recipients receiving cyclosporin-based immunosuppression, clinicians can choose between monitoring by C_0 or by sparse sample-derived area under the curve (AUC) as there is no proven difference in predicting treatment failure at 3 months. (Level II evidence)

b. In renal allograft recipients being monitored by C_2 levels of cyclosporin (CSA), the following target levels are recommended after the first 3 months: 800–1000 ng/mL (3–6 months); 600–800 ng/mL (6–12 months). (Level II evidence)

c. No guidelines are possible for optimal therapeutic drug monitoring (TDM) of tacrolimus (TAC) as there is no Level I or II evidence available.

SUGGESTIONS FOR CLINICAL CARE

(Suggestions are based on Level III and IV evidence)

- **In both adults and children, C_2 monitoring of CSA is superior to C_0 as a predictor of AUC 0–4 and AUC 0–12. The C_3 level is marginally superior to C_2 for AUC 0–12.**
- **Targeting AUC 0–4 levels for CSA of greater than 4400 ng/mL or C_2 levels of greater than 1.5 by the end of the first week post transplant is associated with less acute rejection than targets lower than these levels.**
- **In maintenance renal transplant recipients with C_2 levels of >800 ng/mL, reduction to a level of 700–800 ng/mL allows CSA dose reduction without harm or proven benefit.**
- **In children and adults, C_0 (trough) levels of TAC correlate well with $AUC_{0-12\,h}$. Other time points (e.g. C_2, C_3 or C_4 levels) are better correlated, especially with $AUC_{0-4\,h}$ but as yet, there is no proven clinical advantage for routine transplants.**
- **For both calcineurin inhibitors (CNIs), two or three point limited sampling strategies (LSS) increase the predictive value for AUC 0–4 and AUC 0–12 over any single time point strategy.**
- **For both CNIs, two or three point sampling estimates of absorption profiles may be required in individuals with known variable absorption. This includes children, non-White races and those with concomitant disease such as liver dysfunction and diabetes.**

BACKGROUND

The introduction of CSA in the early 1980s was immediately associated with an enhanced 1 year renal allograft survival. Subsequently, there has been a protracted learning curve on how to optimally use the drug in renal transplant recipients to further enhance outcomes. Over the past two decades, there have been changes to recommended CSA dosing, changes in concomitant medications, and one major change to the oral drug formulation. Lately, there has also been the introduction of generic formulations of CSA.

In 1988, Kasiske *et al.* showed in a prospective study that although C_0 levels of CSA correlated poorly with dose, C_{max} was significantly correlated with dose, AUC and elimination half-life ($T_{1/2}$). Those who suffered acute rejection had a significantly lower C_{max} by 15–31%.[1] Since then, there have been many pharmacokinetic (PK) studies confirming other time point estimates (generally C_2 or C_3) or abbreviated AUC as better predictors of CSA exposure (as measured by full AUC 0–12) than C_0 levels.

Most of the inter-individual PK variability occurs in the first 4 h post dose and more recently, studies have concentrated on predicting the AUC 0–4 in what is now called 'absorption profiling'. Poor or variable absorbers of CSA have a worse outcome than good absorbers of the drug.[2] Thus, attempts have been made to set early and late target ranges for C_2 and AUC by comparing receiver operating characteristic curves of these PK parameters with observed rejection rates and toxicity parameters.

Tacrolimus was introduced into clinical practice in the mid-1990s and has a similar absorption profile to CSA but with a lower peak to trough ratio. Consequently, there has been an accepted dogma that trough monitoring reliably reflects TAC exposure and unlike CSA, there has been little pressure to adopt more precise methods of TDM.

Intuitively, one might expect that monitoring patients by these more precise measurements of CNI exposure would translate to better clinical outcomes. On the other hand, it can be argued that the ultimate guide to CNI efficacy is to measure calcineurin inhibition in recipient immune effector cells. The likely best predictor of nephrotoxicity is the drug uptake by the renal allograft itself. These assays are available but remain laboratory research tools.

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The main aim in researching this guideline was to review the quality of evidence to date that TDM of CNI blood levels by any other method than trough monitoring is advantageous.

SEARCH STRATEGY

Databases searched: Medline (1966 to August Week 4, 2004). MeSH terms and text words for kidney transplantation were combined with MeSH terms and text words for therapeutic monitoring and the CNI of interest (CSA and TAC). The results were then limited to randomized controlled trials (RCT) and/or cohort studies.
Date of searches: 6 September 2004.

WHAT IS THE EVIDENCE?

1. For CNIs, which single time point (and which limited sampling-derived AUC) correlates best with a full 4 or 12 h AUC?

Table 1 summarizes key papers in the CSA-microemulsion era which give coefficients of determination (r^2) between single time point concentrations and measured AUC in renal transplant patients only. The most predictive LSS is also listed for interest. There are fewer papers dealing with the PK of TAC and these are summarized in Table 2 (in renal transplantation only).

David and Johnston[3] systematically reviewed the published literature on LSS for CSA monitoring in 2001. They reviewed 38 references (30 full papers, 7 abstracts and 1 personal communication) and included predominantly de novo and stable renal transplant recipients, both children and adults, but also some liver, heart and bone marrow recipients. They also included one paper with some patients taking Sandimmun and another using SangCya. Two papers were about end-stage renal disease patients.

Only a minority (11 papers) validated algorithms predicted on a training set of patients by using a second (testing) set of results and deriving a percentage predictive error.

Results:

- For CSA, all studies demonstrate a much better correlation between full AUC and C_2 or C_3 (range 0.75–0.96) than C_0 (range 0.13–0.65).
- The r^2 values are further enhanced by combining two or three time points from within the first 4 h (range 0.73–0.99, 77% greater than 0.90).
- Some studies chose certain time points as mandatory and others sought to validate time points suggested by previous studies. Many LSS algorithms include the closest time point to t_{max} (i.e. C_2 or C_3) as this point showed the highest single time point prediction of AUC and is a starting point for forward multiple linear regression. There is willingness to include C_0 as this gives a measure of patient compliance outside the clinic. A gap of 2 h between time points was considered compulsory for good predictions. A further time point outside the 0–4 h range is often deemed impractical.
- For TAC, there are fewer studies but the findings are similar except for the fact that C_0 r^2 values (range 0.81–0.98) are not much inferior to C_2 or C_3 values (and are similar to the range of correlations seen with C_2 monitoring of CSA).

Comments on CNI blood level variability

Calcineurin inhibitors are notorious for intra- and particularly, inter-subject variability in bioavailability. Full AUC can vary up to fivefold between individuals with the same trough levels. Some studies have attempted to define the important clinical variables associated with this diversity. Those consistently defined are age, gender, race, time since transplantation, presence or post-prandial administration, renal allograft function, liver function, diabetes, diarrhoea and concomitant medication. Part of the time-related increase in bioavailability that continues for most of the first year is the effect of steroid reduction or withdrawal which raises CNI levels, thereby somewhat diminishing the goal of reducing immunosuppression and possibly enhancing nephrotoxicity. Some medications may be used to enhance CNI levels (e.g. diltiazem). There may be differences between CSA and TAC with regard to some of these variables (e.g. infectious diarrhoea can dramatically enhance TAC absorption but less so CSA).

In clinical situations where the above variables are operating (e.g. diabetics, non-White races, children), many advocate the use of the most reliable means of TDM which may be C_2 (or later time points) and/or abbreviated AUC for both CSA and TAC.

2. In de novo renal transplants, does monitoring of CNI by C_2 (2 h) blood levels (or by sparse sampling AUC) improve graft outcomes compared with monitoring by C_0 (trough) blood levels?

Cyclosporin

Randomized controlled trials. Only one RCT exists that compares two equivalent groups of renal transplant recipients receiving CSA-based immunosuppression, prospectively randomized to be monitored by C_0 or by sparse sampling-predicted AUC levels from the day of transplantation (see Tables 5–8).

- The International Neoral Renal Transplantation Study Group[4] conducted a study in 21 centres in eight countries that randomized cadaveric or live donor renal recipients to be monitored by C_0 levels ($n = 95$) or by sparse sampling-derived AUC ($n = 109$). Initial immunosuppression was with CSA, prednisone and basiliximab. Primary outcome measures were the feasibility, functionality, accuracy and precision of the two forms of monitoring but also analysed was the cumulative incidence of treatment failure (acute rejection, graft loss and death) at 3 months. By the latter endpoint, there was no difference between the two groups ($P = 0.80$) nor was there any difference in biopsy-proven rejection or graft function. A subsequent Cox regression analysis across both groups showed a significant relationship

between CSA exposure (by C_2, $AUC_{(0–4)}$ or $AUC_{(0–12)}$) and probability of acute graft rejection when interaction with absorber status (exposure variable corrected for dose and weight) is taken into account. C_0 just failed to reach significance in this analysis ($P = 0.064$).

This study is frequently misquoted as an example of an RCT demonstrating the clinical superiority of AUC or C_2 over C_0 monitoring when in fact it failed to find any difference in clinical outcomes between the two groups. The two arms were designed to provide equivalent CSA exposure and then look at predictors of clinical events across both groups. It does, however, provide evidence (Level III) that as early as day 3 when using C_2 levels, one can discriminate between good and poor absorbers with the latter having doubled the risk of acute rejection. A C_2 level of >1500 ng/mL by day 7 was associated with the lowest risk profile for acute rejection.

Comment on RCT in non-renal transplants. In a RCT of 307 d novo liver transplants monitored by either C_2 or C_0 levels there were less patients with acute cellular rejection in the C_2 monitored group. Rejection episodes were also less severe.[5]

Cohort studies with an historical control group: two prospective cohort studies using C_2 monitoring have compared outcomes with similar cohorts of recipients previously managed using C_0 monitoring:

- In a recent single centre study, Birsan *et al.*[6] compared acute rejection rates in 89 C_2-monitored patients with 88 C_0-monitored renal transplant recipients from the previous year. The C_2 target was 1500 ± 200 ng/mL and was achieved by day 4 on average; the C_0 target was 250 ± 50 ng/mL. All patients received mycophenolate and prednisolone. In the intention-to-treat analysis, 40 (15.4%) patients in the C_0 group and 25 (28.1%) patients in the C_2 group received treatment for rejection ($P = 0.013$). Mean CSA-MEF doses were 1.7–2 times higher in the C_2 group than in the C_0 group throughout follow up ($P = 0.019$). However, there was no significant difference in C_2 levels between patients who rejected and patients who did not reject.
- In a multicentre study of the efficacy and safety of C_2 monitoring of CSA, the current cohort was compared with a C_0-monitored cohort from a previous registration study.[7] The C_2-monitored group at 3 months had significantly less biopsy-proven acute rejection (11.3% *vs* 21.3%, $P < 0.03$) and clinical acute rejection (14.6% *vs* 24.5%, $P = 0.04$) compared with the C_0 group. Glomerular filtration rate (GFR) was similar at 2 weeks but was higher in the C_2 patients at 3 months (59 *vs* 53 mL/min, $P < 0.01$) even though the weight-adjusted dose of CSA was higher in the C_2 group at all time points. Systolic blood pressure (134/82 *vs* 139/82 mmHg, $P = 0.03$) and cholesterol (5.7 *vs* 6.0 mmol/L, $P = 0.02$) were lower in the C_2 group at 3 months.

Despite using historical controls, these two studies do seem to be comparing similar populations managed in much the same era and provide the strongest evidence to date that C_2 monitoring has clinical advantages over C_0. Both, however, used significantly higher doses of CSA to achieve the C_2 targets whereas the above multicentre RCT used only marginally more CSA (P = ns) in the AUC group in the first few weeks.

Other cohort studies of CSA monitoring in de novo recipients. Table 3 summarizes a further six single or multicentre cohort studies, in both paediatric and adult renal transplant recipients receiving CSA. These all confirm the superiority of C_2 (or abbreviated AUC) levels over C_0 in predicting acute rejection in the early months post transplant. Only two studies have used receiver operating characteristic curves to relate target levels to risk of acute rejection. Target C_2 levels of above 1500 ng/mL in the first week post transplant seem best to predict low rejection rates over the next 3–6 months. The relationship with toxicity is not as clear, with no study in this group defining a limit above which nephrotoxicity is statistically more likely to occur.

Comment re lack of uniform targets. There is a lack of intercentre uniformity in trough target ranges for CSA, particularly in North America, which restricts comparisons between cohort studies.[8] In five of the above studies that began by targeting C_0, four used different target ranges. In the two controlled studies, the former chose an AUC target that resulted in a lesser (weight-adjusted) dose of CSA being given compared with the C_0 group, while in the latter, a higher dose was necessary in the C_2 group, to achieve chosen targets.

Tacrolimus

Randomized controlled trials. There are no RCT comparing outcomes in de novo recipients between groups of patients on TAC-based immunosuppression monitored using different therapeutic targets.

Cohort studies. Balbontin *et al.*[9] monitored 28 renal transplant recipients with C_0 levels but measured TAC concentrations at 0, 1, 2, 3 and 4 h post dose. C_0 was not significantly different in six patients with acute rejection (AR) and 23 without. There was a trend towards lower TAC C_3 levels in patients with acute rejection (AR) ($P = 0.06$).

3. Does targeting of a particular maintenance CNI level compared with any other target level improve outcomes?

Cyclosporin

Table 4 summarizes one RCT and seven cohort studies attempting to define target ranges for CSA (predominantly C_2 levels) in maintenance adult and paediatric renal transplant recipients.

Randomized controlled trials. One RCT (MO2ART study) randomized 306 patients from 13 centres into two groups who were targeted to achieve different C_2 blood levels of CSA from 3 months to 1 year post transplant. A total of 250 patients entered phase 2. Group 1 targets at 3–6

months were 1000–1200 ng/mL and at 6–12 months were 600–800 ng/mL, 200 ng/mL lower than group 2 (and over the 9 month follow up with the primary endpoint being GFR at 12 months). Late acute rejection events were rare in both groups. Neither group had superior GFR at 12 months. When patients were regrouped into three strata according to C_2 level from 8 to 12 months, those with the lowest C_2 levels had the worst function at 3 months but improved the most over the subsequent 9 months, to be no different at 12 months.

Comment on RCT in non-renal transplants: An RCT of C_2 versus C_0 monitoring in stable liver and heart transplant recipients have shown clinical benefit in the C_2-monitored groups.[10]

Cohort studies. Five out of seven selected cohort studies examined the relationship between C_0 and C_2 monitoring in stable renal recipients. Two of these identified subgroups (44% and 49%) in which CSA dose could be reduced to achieve target C_2 levels of 600–800 ng/mL. This did not cause a significant fall in serum creatinine in either group as a whole. In one of the studies, this subgroup did achieve a better diastolic blood pressure and total cholesterol level.

In Denmark, a national study of 1032 stable recipients (>12 months post transplant) had C_0 and C_2 levels compared with serum creatinine. The largest group of recipients (666) had C_0 levels within the target range (75–125 ng/mL) and they had the best serum creatinines. A linear regression of this group's C_2 levels showed a significant relationship with serum creatinine. The optimum serum creatinine fell in the C_2 range to 700–800 ng/mL; 29% were above this level and could theoretically have had their dose reduced; 15% were below 500 ng/mL.

In a recent report by Einecke *et al.* of a single centre experience with switching from C_0, the mean C_2 level was only 564 ng/mL, but there were only two minor acute rejections in the next 13 months. On the other hand, Citterio *et al.*[12] in a retrospective analysis of 79 patients over 43 months found that a C_2 of 1000 ng/mL was significantly associated with the development of chronic renal allograft dysfunction (rising creatinine and proteinuria). In children, Pape *et al.*[13] also found a low C_2 (<750 ng/mL) was associated with a higher serum creatinine and <500 ng/mL was associated with acute rejection.

Also in a paediatric population, David-Neto *et al.*[14] found that C_2, C_4 or AUC 0–4 monitoring was predictive of hypertrichosis and tremor but not gingival hyperplasia whereas C_0 did not correlate with any of these adverse effects.

Tacrolimus

Randomized controlled trials. There are no RCT comparing outcomes in de novo recipients between groups of patients on TAC-based immunosuppression monitored using different therapeutic targets.

Cohort studies. There are no cohort studies looking at the monitoring of TAC in stable long-term renal transplant recipients by any time points other than C_0.

WHAT DO THE OTHER GUIDELINES SAY?

Kidney Disease Outcomes Quality Initiative: No recommendation.
UK Renal Association: No recommendation.
Canadian Society of Nephrology: No recommendation.
European Best Practice Guidelines: No recommendation.
International Guidelines:
National Institute for Clinical Excellence (UK): No recommendation.
Cardio Renal Anemia: No recommendation.

IMPLEMENTATION AND AUDIT

No recommendation.

SUGGESTIONS FOR FUTURE RESEARCH

Randomized controlled trials examining long-term outcomes of TDM of CNI in renal transplantation focusing on late graft loss, GFR and development of chronic allograft nephropathy.

CONFLICT OF INTEREST

Paul Trevillian has a Level II a conflict of interest according to the conflict of interest statement set down by CARI.

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APPENDICES

Table 1 Cyclosporin pharmacokinetic studies in early and stable renal transplant recipients

Study ID (author, year)	*n*	Days post transplant	Predicted AUC	Best 2 or 3 point predictor	Best 1 point predictor	Best 1 point r^2 value	C_0 r^2 value
Brunet *et al.*, 2004[15]	15	Stable	$AUC_{(0-4)}$		C_2	0.94	0.53
Trompeter *et al.*, 2003[16]	22	28	$AUC_{(0-4)}$	N/A	C_2	0.79	0.32
			$AUC_{(0-12)}$	N/A	C_2	0.82	0.59
Jaiswal *et al.*, 2003[17]	28	Stable	$AUC_{(0-4)}$		C_3	0.96	0.65
Felipe *et al.*, 2003[18]	72	<180	$AUC_{(0-12)}$		C_3	0.88	0.24
David-Neto *et al.*, 2002a[19]	34†	Stable > 540	$AUC_{(0-4)}$	$C_1 + C_2$	C_2	0.81	
David-Neto *et al.*, 2002b[20]	74	13	$AUC_{(0-4)}$	$C_1 + C_2$	C_2	0.87	
International Neoral Renal	24	14	$AUC_{(0-4)}$	$C_1 + C_2 + C_3$	C_2	0.80	0.13
Transplantation Study Group, 2002[4]			$AUC_{(0-12)}$	$C_1 + C_2 + C_3$	C_3	0.75	0.24
Canadian Neoral Renal	16	14	$AUC_{(0-4)}$	$C_1 + C_2 + C$	C	0.82	0.47
Transplantation Study Group, 2001[21]			$AUC_{(0-12)}$	$C_1 + C_2 + C_3$	C_3	0.84	0.55
Wacke *et al.*, 2000[22]	40		$AUC_{(0-12)}$	$C_1 + C_3 + C_6$	C_3	0.79	0.20
Tam *et al.*, 2000[23]	14†	Stable > 540	$AUC_{(0-12)}$		C_{max}	0.96	
Kelles *et al.*, 1999[24]	25	Stable > 180	$AUC_{(0-12)}$	$C_1 + C_{\ldots} + C_4$	C_2	0.91	0.64
Meier-Kriesche *et al.*, 1998[25]	23†	268	$AUC_{(0-8)}$	$C_{\ldots} + C_4$	C_4	0.87	0.52
Primmett *et al.*, 1998[26]	55	Stable	$AUC_{(0-12}$	$C_0 + C_1 + C_2$	N/A	N/A	N/A
Gaspari *et al.*, 1997[27]	20		$AUC_{(0-12)}$	$C_0 + C_1 + C_3$	N/A	N/A	N/A

†Paediatric. AUC, area under the curve.

Table 2 Tacrolimus pharmacokinetic studies in early and stable renal transplant recipients

Study ID (author, year)	*n*	Days post transplant	Predicted AUC	Best 2 or 3 point predictor	Best 1 point predictor	Best 1 point r^2 value	C_0 r^2 value
Balbontin *et al.*, 2003[9]	28		$AUC_{(0-4)}$		C_2	0.96	0.86
Felipe *et al.*, 2002[28]	22	<1[illegible]	$AUC_{(0-12)}$	$C_0 + C_{1.5} + C_4$	C_{max}	0.91	0.81
Jorgensen *et al.*, 2002[29]	21		$AUC_{(0-12)}$		C_3	0.95	0.84
		[illegible]4	$AUC_{(0-12)}$		C_3	0.95	0.94
Filler *et al.*, 2002[30]	53	stable	$AUC_{(0-12)}$	$C_1 + C_2 + C_6$	C_4	0.91	0.55
Wong *et al.*, 2000[31]	18	>90	$AUC_{(0-12)}$	$C_2 + C_4$			0.34
Ihara *et al.*, 1995[32]	10		$AUC_{(0-12)}$		C_0	0.98	0.98

AUC, area under the curve.

Table 3 Outcome studies of CSA TDM in de novo renal transplant recipients

Study ID (author, year)	*n*	Target AUC (ng ×h/mL) or C_2 (ng/mL)	Target C_0 (ng/mL)	Endpoint(s)	Finding(s)	Evidence level
Birsan *et al.*, 2004[7] Historical control group	89 *vs* 88	C_2 = 1500–2000	250–500	Acute rejection	Less AR in C_2 group (P = 0.017) 28.1% *vs* 45.4% but CSA dose 1.7–2.0 ×higher in C_2 group	III
Thervet *et al.*, 2003[33] MO2ART – historical control group	151 *vs* 155	C_2 = 1600–2000 for 1 month then taper	200–400	1. Acute rejection at 3 months 2. GFR 3. HTN 4. Cholesterol	1. Less AR in C_2 group (P < 0.04) 2. Better GFR 3. Lower systolic BP 4. Lower chol.	III
Trompeter *et al.*, 2003[16]		32†	15[illegible]–25	Acute rejection at 6 months	Less AR if C_2 > 1500 (P < 0.05)	III
Felipe *et al.*, 2003[18]	72		200[illegible]400 (d [illegible]–30) 150–[illegible] (d 30–90) 100–200 (d 90+)	Acute rejection at 6 months	C_0 at d3 does not predict AR (C_2 not analysed)	III
Clase *et al.*, 2002[34]	98	$AUC_{(0–4)}$ = 4400–5500 in the first 5 days		Acute rejection at 6 [illegible]hs	$AUC_{(0–4)}$ > 4400 & C_2 > 1700 neg. predict AR (92%)	III
Pescovitz *et al.*, 2002[35]	135	C_2 not targeted	Not specified	Acute r[illegible]ection	C_2 but not C_0 predicts AR C_2 > 1500 = 15% AR C_2 < 1500 = 45% AR	III
International Neoral Renal Transplantation Study Group, 2002[4]	109 *vs* 95	Group 1: $AUC_{(0–12)}$ = 6000–12 500 (wk 1) 6000–10 400(wk 2–4) 4400–9900 (wk 5–12) algorithm $C_0 + C_2 + C_3$ for 2 weeks then $C_0 + C_2$	Group 2: C_0 = 200–450 (wk 1) 200–400 (wk 2–4) 150–300(wk 5–12)	1. Fea[illegible]lit[illegible] of sparse sample monitoring CSA 2. 3/12 risk of treatm[illegible]t failure	1. AUC slightly less practical, better accuracy & precision 2. No difference (P = 0.8)	II RCT
International Neoral Renal Transplantation Study Group, 2002[4]	204	As above	As above	Acute rejection (combined risk of AR in both groups)	Ab[illegible]orber status + $AUC_{(0–4)}$ or $AUC_{(0–12)}$ C_2 is a[illegible]ociated with AR. [illegible]ess AR ([illegible]%) if C_2 > 1500 (P < 0.001)	III
Morris *et al.*, 2002[36]	55	$AUC_{(0–4)}$ ($C_0 + C_2 + C_4$) C_2		Acute rejection	No [illegible]R if C_2 [illegible]120[illegible]	III
Mahalati *et al.*, 2001[37]	59	$AUC_{(0–4)}$ = 4400–5500 in the first 5 days		1. Acute rejection 2. CSA toxicity 3. [Cr] at 3/12	1. Less AR (7% *vs* 34%) if target AUC met <d[illegible] (P = 0.0002) 2. More CyANT if AUC > 5500 (P = ns) 3. Lower Cr (LD) 143 *vs* 114 (P = 0.04)	III
Canadian Neoral Renal Transplantation Study Group, 2001[21]	38		250–450	1. Acute rejection 2. CSA toxicity	Less AR if: $AUC_{(0–4)}$ > 4500 (P = 0.04) or C_2 > 1500 (P < 0.001) 2. No difference	III

†Paediatric. AR, acute rejection; AUC, area under the curve; BP, blood pressure; CSA, cyclosporin; GFR, glomerular filtration rate; HTN, hypertension; TDM, therapeutic drug monitoring.

Table 4 Studies of cyclosporin TDM in stable renal transplant recipients

Study ID (author, year)	*n*	Target CSA level (ng/mL)	Comparison CSA level (ng/mL)	Endpoint(s)	Finding(s)	Evidence level
Sandrini *et al.*, 2004[38] (Switch C_0–C_2)	62	C_0: 130–190	C_2: 700–900	1. CSA dose 2. Serum [Cr]	40% decrease 30% increase No change in any group	III
Einecke *et al.*, 2004[11] (Switch C_0–C_2)	127	C_0: 90–120	C_2 Mean = 564 ± 186	1. Acute rejection	2 minor AR in 13/12 follow up despite low C_2	III
Thervet *et al.*, 2003[33] MO2ART (Randomized at d3 to different C_2 targets from 3/12)	151 *vs* 155	C_2: 1000–1200 (mo 4–6) 800–1000 (mo 6–12)	C_2 800–1000 (mo 4–6) 600–800 (mo 6–12)	1. GFR – 12/12 2. Acute rejection 3. BP 4. Cholesterol	1. No difference 2. No difference 3. No difference 4. Less cholesterol medications ($P = 0.02$)	II (RCT)
Citterio *et al.*, 2003[12]	79	C_0: 100–200	C_2	CRAD (chronic renal allograft dysfunction – rising creatinine with proteinuria)	Low AUC and low C_2 <900 ng/mL predicts CRAD ($P < 0.0001$)	III
Midtvedt *et al.*, 2003[39]	1032	C_0: 75–125	C_2 Mean = 697 ± 211	Serum [Cr]	C_0 = no association C_2 = 700–800 and C_2 <450 lower Cr. than C_2 >950	III
Cole *et al.*, 2003[40] (Switch stable patients (>3/12) from C_0 to C_2 monitoring)	175	C_0: Mean = 214 (3–6 mo) 197 (6–12 mo) 154 (> 12 mo)	C_2: Target = 1200 (3–6 mo) 1000 (6–12 mo) 800 (>12 mo)	1. Serum [Cr] in 85 patients found to be over C_2 target + 10% 2. BP 3. Cholesterol	1. No difference (46% improved, 49% stable, 5% worse) 2. DBP fell 81- >77 3. Cholesterol fell 5.5- >5.2	III
Pape *et al.*, 2003[13]	101†	C_2 > 750	C_2 < 750	1. GFR at 6/12 2. Acute rejection	1. Lower GFR if C_2 < 750 ($P < 0.05$) 2. More AR if C_2 < 500 ($P < 0.05$)	III
Sitland *et al.*, 2002[41] (Reduce dose in stable patients if C_2 > 1000)	167	C_2: 800–1000	reduce if C_2 > 1000	Serum [Cr] in 65 patients (44%) found to be over C_2 target	No difference in mean [Cr]	III
David-Neto *et al.*, 2000[14]	46†	C_0: NA C_2, C_4, $AUC_{(0-4)}$	AUC (0–4)	Correlate with adverse effects	Hypertrichosis & tremor but not gingival hyperplasia	III

†Paediatric. AR, acute rejection; AUC, area under the curve; BP, blood pressure; CSA, cyclosporin; DBP, diastolic blood pressure; GFR, glomerular filtration rate; NA, not available; RCT, randomized controlled trial; TDM, therapeutic drug monitoring.

Table 5 Characteristics of included RCT studies

Study ID (author, year)	*n*	Study design	Setting	Participants	Intervention (experimental group)	Intervention (control group)	Follow up (months)
International Neoral Renal Transplantation Study Group, 2002[4]	207	Randomized controlled clinical trial	Multicentre, international 21 renal transplant centres in 8 countries	Primary or secondary renal allograft recipients	Cyclosporin microemulsion absorption profiling	Conventional trough-level drug monitoring	3
Stefoni *et al.*, 2005[42]	296	Randomized controlled clinical trial	Multicentre, 31 transplant centres in 10 countries	De novo renal transplant recipients, first or second renal transplant from deceased or living donor	High C_2 target value	Lower C_2 target values	12

Table 6 Quality of randomized trials

Study ID (author, year)	Method of allocation concealment	Blinding			Intention-to-treat analysis	Loss to follow up (%)
		Participants	Investigators	Outcome assessors		
International Neoral Renal Transplantation Study Group, 2002[4]	Not specified	No	No	No	No	0.0
Stefoni *et al.*, 2005[42]	Not specified	No	No	No	Yes	0.9

Table 7 Results for continuous outcomes

Study ID (author, year)	Outcomes	Intervention group mean (SD)	Control group mean (SD)	Difference in means (95% CI)
Stefoni *et al.*, 2005[42]	Glomerular filtration rate at 12 months	*n* = 116 65 (17)	*n* = 103 66 (14)	–1.00 (–5.11, 3.11)

CI, confidence interval; SD, standard deviation

Table 8 Results for dichotomous outcomes

Study ID (author, year)	Outcomes	Intervention group (number of patients with events/number of patients exposed)	Control group (number of patients with events/number of patients not exposed)	Relative risk (95% CI)	Risk difference (95% CI)
International Neoral Renal Transplantation Study Group, 2002[4]	Acute rejection, graft loss or death	33/109	31/95	0.93 (0.62, 1.39)	–0.02 (–0.15, 0.10)
	Clinical or biopsy-proven acute rejection	29/109	27/95	0.94 (0.60, 1.46)	–0.02 (–0.14, 0.10)
	Graft loss	8/109	6/95	1.16 (0.42, 3.23)	0.01 (–0.06, 0.08)
	Deterioration in renal function (increased serum creatinine > 30% above baseline)	55/109	31/95	1.55 (1.10, 2.18)	0.18 (0.05, 0.31)
	Infection	47/109	42/95	0.98 (0.71, 1.33)	–0.01 (–0.15, 0.13)
	Malignancy	0/109	1/95	0.29 (0.01, 7.06)	–0.01 (–0.04, 0.02)
Stefoni *et al.*, 2005[42]	Renal impairment starting during phase 2	25/131	18/119	1.26 (0.73, 2.19)	0.04 (–0.05, 0.13)

CI, confidence interval