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REVIEW

Use of Neoral C_2 monitoring: a European consensus

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Summary

Large-scale clinical trials using C_2 monitoring of cyclosporine (CsA) microemulsion (Neoral) in renal transplant recipients have demonstrated low acute rejection rates and good tolerability with a low adverse event profile in a variety of settings: with or without routine induction therapy; in combination with mycophenolate mofetil; with standard-exposure or low-exposure Neoral; and in patients with immediate or delayed graft function. In liver transplantation, C_2 monitoring significantly reduces the severity and incidence of acute rejection compared with C_0 monitoring, without adverse consequences in terms of renal function or tolerability. Different C_2 targets are appropriate depending on adjunctive immune suppression, level of immunologic risk, CsA tolerability, risk of renal toxicity and time since transplantation. CsA absorption may increase substantially in most patients during the first 1–2 weeks post-transplant, and this should be taken into account to avoid overshooting C_2 target range. A patient with a low C_2 value may be either a low or a delayed absorber of CsA, or be a normal absorber who is receiving too low a dose of Neoral. C_2 monitoring alone is insufficient to differentiate between these types of patients, and measurement of additional time-points is recommended. Adopting C_2 monitoring in maintenance transplant patients identifies those who are overexposed to CsA. In summary, randomized, prospective, multicenter studies and single-center trials have evaluated Neoral C_2 monitoring within a range of regimens in different organ types, providing a robust evidence base for the benefits of this sensitive monitoring technique.

Introduction

In September 2004, a panel of transplant specialists from nine European countries convened in Vienna, Austria, to analyze current evidence concerning the use of C_2 monitoring for Neoral (cyclosporine microemulsion). The objective of the meeting was to critically discuss existing data and develop evidence-based recommendations for the use

Table 1. Grading system for quality of evidence [1].

Quality of evidence	Definition
I	Evidence from ≥1 properly randomized, controlled trial
II	Evidence from ≥1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); from multiple time-series; or from dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees

of C_2 monitoring in renal and liver transplant recipients in the light of the growing experience with C_2 monitoring in clinical trials and in routine clinical practice.

Evidence is graded according to standards shown in Table 1 [1] and gradings are shown in the text in italics (i.e. *I*, *II* or *III*).

Rationale for monitoring Neoral using C_2

Cyclosporine (CsA) has a narrow therapeutic window [2] with variable absorption characteristics [3]. Even with the microemulsion formulation of CsA (Neoral) there is significant variation in absorption between and within patients [4]. Accordingly, CsA dosage must be individualized to reflect the particular absorption profile of each patient. Variability in absorption is greatest during the first 4 h after dosing (AUC_{0-4}) [5] (*II*), and is particularly prominent during the first weeks after transplantation; variability diminishes during the subsequent 3 months post-transplant [6]. Trough (C_0) CsA level, which has conventionally been used to determine Neoral dosing, does not correlate closely with AUC_{0-4} [7–14]. This has led to an examination of the relationship between Neoral pharmacokinetics and clinical outcomes [8], which has concluded that the clinical outcome in an individual patient at any time point after transplantation is related to CsA exposure (area under the curve, AUC). However, full area under the concentration–time curve during the first 12 h postdose (AUC_{0-12}) assessments are cumbersome and probably unnecessary because CsA absorption variability predominantly occurs during the first 4 h postdose. Accordingly, AUC_{0-4} probably offers the best combination of accuracy and convenience for assessing of variation in CsA absorption. A landmark study in renal transplant patients has shown that AUC_{0-4} values are predictive both for risk of acute rejection and risk of CsA-related nephrotoxicity [7]. Patients with AUC_{0-4} in the range 4400–5500 ng h/ml by day 5 post-transplant were found to have <10% risk of acute rejection and a low risk of CsA-related nephrotoxicity at 3 months [7]. In liver patients, a significant relationship has been demonstrated between risk of graft rejection and AUC_{0-6} [15].

These results reflect an important temporal relationship between CsA pharmacokinetics and the immunosuppressive effect of the drug. *In vitro* data from peripheral blood leukocytes have shown that inhibition of calcineurin peaks at approximately 2 h after CsA administration [16]. Similarly, maximal calcineurin inhibition, along with the suppression of interleukin-2 (IL-2), also occurs during the first 4-h phase post-transplant in patients who absorb Neoral normally. Furthermore, the proportion of CD4+ lymphocytes expressing IL-2 is also lowest at around 2 h postdose in normal absorbers [17].

The first 4 h after receiving a dose of Neoral is clearly an important phase during which the success or failure of treatment may be determined, and effective monitoring of absorption in this period is necessary for making accurate dosing decisions. However, AUC_{0-4} measurement is impractical in routine clinical practice. An acceptable single time-point marker for AUC_{0-4} has been found to be CsA concentration 2 h postdose (C_2). The correlation between C_2 and AUC has now been validated in a number of studies and centers [6,7,9–15].

Clinical benefits of Neoral C_2 monitoring

Renal transplantation

Large-scale clinical trials using Neoral C_2 monitoring have demonstrated low acute rejection rates and good tolerability with a low adverse event profile to at least 1 year post-transplant (*I*). The MO2ART (Monitoring of 2 h Absorption in Renal Transplantation) study was a 12-month, prospective, randomized, multicenter, open-label study in which 296 *de novo* renal transplant patients were managed using Neoral C_2 monitoring with steroids and mycophenolate mofetil (MMF) or azathioprine [18,19]. Patients were randomized at day 3 into higher-C_2 or lower-C_2 groups, effective from the start of month 4 post-transplant (Table 2). The overall incidence of biopsy-proven acute rejection (BPAR) was 11.5% at 3 months. Mean glomerular filtration rate (GFR, Nankivell) was 59 ml/min and mean serum creatinine was 132 μmol/l across all patients. In the multicenter, open-label CONCERTO study, 119 *de novo* renal transplant patients were managed by Neoral C_2 monitoring in combination with basiliximab, MMF and steroids [20]. Patients were randomized at the end of month 2 to higher-C_2 or lower-C_2 groups (Table 2). At 6 months, the incidence of BPAR was 9.3%. Mean GFR (Cockcroft-Gault) was 68 ml/min/1.73 m^2 and mean serum creatinine was 141 μmol/l.

Two studies have assessed the use of Neoral C_2 monitoring (using low-exposure Neoral) in combination with everolimus and steroids, with or without basiliximab

Table 2. Examples of published C_2 target levels and outcomes in *de novo* renal transplant recipients.

Trial (immunosuppressive regimen)	C_2 targets (ng/ml)	Mean C_2 achieved (ng/ml)	Incidence of BPAR (% patients)	Median serum creatinine	GFR
MO2ART (Neoral, MMF/aza, steroids, no routine induction) [18,19]	M1: 1800 M2: 1500 M3: 1300 M4–6: 900 or 1100 M7–12: 700 or 900	D7: 1600 D28: 1700 M2: 1400 M3: 1400 M6: 900 M12: 800	M3: 11.5% M12:13.7%	M3: 132 μmol/l M12: 122 μmol/l	M3: 59 ml/min M12: 65.5 ml/min
CONCERTO (Neoral, MMF/aza, steroids, basiliximab) [20]	M1: 1700 M2: 1500 M3–6: 800 or 1000	D14: 1600 D28: 1600 M2: 1200 M3: 1200 M6: 900	M6: 9.3%	M6: 141 μmol/l*	M6: 68 ml/min/ 1.73 m^2
RAD A2306 Neoral, everolimus, steroids, no induction) [21]	M1: 1200 M2: 800 M3: 600 M4–6: 400	M1:1100† 1200‡ M2: 900† 900‡ M3: 700† 800‡ M4: 600† 600‡ M6: 500† 500‡	M6:25.0%† M6:15.2%‡	M6: 133 μmol/l† M6: 132 μmol/l‡	M6: 68 ml/min† M6: 62 ml/min‡
RAD A2307 (Neoral, everolimus, steroids, basiliximab) [21]	M1–2: 600 M3–6: 400	M1: 700† 700‡ M2: 600† 600‡ M3: 600† 600‡ M4: 500† 500‡ M6: 400† 500‡	M6:13.7%† M6:15.1%‡	M6: 130 μmol/l† M6: 130 μmol/l‡	M6: 66 ml/min† M6: 67 ml/min‡
DE01 (Neoral, enteric-coated mycophenolate sodium, steroids, basiliximab) [22]	M1: 1500§¶ M2–3:1300§ 1100¶ M4–6 1100§ 850¶ M7–12: 900§ 625¶	M1: 1300§¶ M2–3:1000–1200§¶ M6: 900§ 850¶ M12: 800§ 700¶	M12: 18%§ M12: 16%¶	M12: 153 μmol/l§* M12: 160 μmol/l¶*	M12: 53 ml/min§** M12: 54 ml/min¶**

*Mean serum creatinine.
†1.5 mg/day Certican.
‡3 mg/day Certican.
§Standard-dose Neoral.
¶Low-dose Neoral.
**Mean creatinine clearance.

induction therapy (RAD A2306 and RAD A2307) [21]. Among patients receiving everolimus 1.5 mg/day, the 6-month incidence of BPAR was 25% (no induction) and 13.7% (with basiliximab); with 3 mg/day these values were 15.2% and 15.2% respectively. Median GFR (Nankivell) was 65 ml/min and 66 ml/min respectively.

Good outcomes with C_2 monitoring have been demonstrated in a variety of protocols:

1 With or without routine induction therapy (*I*). Low rejection rates and good renal function have been demonstrated in prospective multicenter trials of Neoral C_2 monitoring in which *de novo* renal transplants routinely received induction therapy [20,21], or in which the protocol specified that induction was to be given only in cases of delayed graft function (DGF) [18] (Table 2).

2 In combination with MMF, enteric-coated mycophenolate sodium or everolimus (*I*). Prospective trials have shown good clinical outcomes in *de novo* renal transplant patients receiving adjunctive therapy with MMF (MO2ART [18], CONCERTO [20]) or enteric-coated mycophenolic acid (DE01) [22] (Table 2). Where Neoral C_2 monitoring (with low-exposure Neoral) has been used in combination with everolimus and induction therapy, the incidence of BPAR has been low with good renal function (Table 2) [20].

3 In patients receiving standard-exposure or low-exposure Neoral (*I*). Protocols involving standard-exposure Neoral were used in the MO2ART [18] and CONCERTO [20] studies. Low-exposure Neoral was employed in two studies using everolimus [21], and a comparative study of standard-exposure and low-exposure has been carried out in patients receiving enteric-coated mycophenolate sodium, steroids and basiliximab [22].

4 In patients with immediate or DGF (*I*). In the MO2ART study, 188 patients had immediate graft function and 108 had DGF [18]. Among patients with immediate graft function, the incidence of BPAR was 10.4% at 3 months and median serum creatinine was 130 μmol/l; for patients with DGF, the BPAR rate was 13.3% and medium serum creatinine was 140 μmol/l.

Table 3. Examples of published C_2 target levels and outcomes in liver transplant recipients.

Trial (protocol)	C_2 targets (ng/ml)	Mean C_2 achieved (ng/ml)	BPAR	Creatinine
INT 06 (Neoral ± azathioprine ± steroids) [23]	M1–3: 1100	M1: 1300 M3: 1000	M3: 21.6%	M3: 121 μmol/l
LIS2T (Neoral ± azathioprine ± steroids) [25,26]	M1–3: 1000 M4–6: 800	D7: 900 D28: 1000 M3: 900 M6: 800	M3: 26% M6: 28%	M3: 104 μmol/l M6: 106 μmol/l

Liver transplantation

A large-scale clinical trial has shown that Neoral C_2 monitoring significantly reduces the severity and incidence of acute rejection compared with C_0 monitoring, without adverse consequences in terms of renal function or tolerability (*I*). This 3-month, prospective, multicenter, open-label study assessed clinical outcomes using Neoral C_2 monitoring or conventional C_0 monitoring in 307 *de novo* liver transplant patients receiving steroids with or without azathioprine [23] (Table 3). At 3 months, 21.6% of C_2-monitored patients and 30.4% of C_0-monitored patients had experienced BPAR ($P = 0.07$). Moderate or severe BPAR had occurred in 10.1% of patients in the C_2 monitoring group and 19.1% of those in the C_0 monitoring group ($P = 0.004$). The incidence of acute rejection was significantly lower with C_2 monitoring versus C_0 monitoring in patients with hepatitis C virus (HCV) infection (21.2% versus 33.0%, $P < 0.05$), while rates were similar in HCV-negative patients (26.1% versus 28.6% respectively). Safety profiles were similar between the C_0 and C_2 groups at 3 months [23] and at 12-month follow-up [24]. There was no difference in calculated creatinine clearance between the groups for the duration of the study [24].

A further large-scale study (LIS2T) has shown that Neoral C_2 monitoring results in equivalent efficacy versus tacrolimus C_0 monitoring to 6 months post-transplant (*I*).

LIS2T was a 6-month, prospective, multicenter, open-label randomized trial comparing the efficacy and safety of Neoral (using C_2 monitoring) versus tacrolimus (using C_0 monitoring) in 495 *de novo* liver transplant recipients [25]. There were no significant differences in the incidence of BPAR, severity of rejection grade, graft loss or death in the Neoral C_2 or tacrolimus cohorts at 6 months [25] or at 12-month follow-up [26] across the total patient population. Among HCV-positive patients, the incidence of graft loss or death at 6 months was significantly lower with Neoral C_2 (5/88, 6%) than with tacrolimus (13/85, 15%, $P < 0.05$) [20]; at 12 months this difference was no longer statistically significant [10/88 (11%) with Neoral C_2 versus 16/85 (19%) with tacrolimus] [26]. Renal function and the incidence of adverse events were similar in both treatment groups at 6 months. However, the incidence of new-onset diabetes mellitus was significantly lower with Neoral C_2 compared with tacrolimus (7% versus 14%, $P < 0.02$) and more patients reported diarrhea with tacrolimus (29% versus 14% with Neoral C_2, $P < 0.0001$) [25].

Neoral C_2 targets

Growing experience suggests that the target levels initially proposed for Neoral C_2 monitoring do not reflect current European practice (*III*).

The faculty reviewed a range of local experiences in terms of C_2 targets and efficacy and safety outcomes using Neoral C_2 monitoring in *de novo* renal transplant patients (Table 4) and *de novo* liver transplant patients (Table 5). Current C_2 targets vary between countries and between centers. Monitoring strategies also vary; in Germany many centers measure both C_0 and C_2 and adjust Neoral dose based on interpretation of both values [27]. Leiden University Medical Center uses abbreviated AUC measurements before relying solely on C_2 values; Neoral dose is calculated by a population-based model with Bayesian fitting [28]. This approach has led to a 15% incidence of acute rejection at 6 months post-transplant, with a mean GFR of 62 ml/min at both 6 and 12 months post-transplant.

C_2 target levels have been evaluated in large-scale randomized studies in renal and liver transplant patients using a variety of immunosuppressive regimens. These provide general guidance for target levels in different settings (*I*).

Tables 2 and 3 summarize the Neoral C_2 targets and the C_2 levels achieved in large-scale randomized studies of Neoral C_2 monitoring in *de novo* renal and liver transplant recipients respectively. Different C_2 targets have been adopted in different trials depending, for example, on use of concomitant immunosuppressive agents or induction therapy.

The C_2 target level in an individual patient should take into account (*III*): (i) Adjunctive immunosuppression; (ii) increased risk of rejection due to the presence of well-recognized risk factors (e.g. HLA mismatch, PRA status, retransplantation); (iii) risk of reduced tolerability (donor and recipient factors); (iv) relative risk of renal toxicity (DGF, donor and recipient factors); (v) time after transplantation.

The outcomes of studies shown in Tables 4 and 5 may provide useful guidance on appropriate C_2 targets in different settings.

Table 4. Examples of C_2 target levels and outcomes in renal transplant recipients used in European centers. Unless stated otherwise, C_2 targets are unchanged in patients with delayed graft function.

Center	Regimen	C_2 targets (ng/ml)	Incidence of acute rejection (% patients)	Mean serum creatinine
Universitair Ziekenhuis Antwerpen, Edegem, Belgium	Neoral, MPA therapy, steroids ± induction therapy (basiliximab)	M1: 1300–1500 M2–3: 900–1300 M4–6: 750–950 >M6: 700	M6: 10%	M6: 146 μmol/l M12: 151 μmol/l
Rikshospitalet, Oslo, Norway*	Neoral, MPA therapy, steroids	M1: 1500–2000 M2: 1400–1600 M3: 1000–1200	Week 10: approximately 30%	Week 10: 123 μmol/l
Campus Charité Mitte, Berlin, Germany	Neoral, MPA therapy, steroids	M1: 1200–1400* M2–6: 1100–800* >M6: 450–700*	not available	not available
	Neoral, MPA therapy, steroids + induction (basiliximab)	M1: 1000–1100* M2–6: 700–900* >M6: 450–700*	M12: 15%	M1: 161 μmol/l M3: 154 μmol/l M9: 142 μmol/l
	Neoral, sirolimus or everolimus, steroids	M1: 500–700* M2–6: 400–600* M7–12: 300–500 >M12: 200–400	not available	not available
Hospital Universitario Dr Peset, Valencia, Spain	Neoral, MPA therapy, steroids	W1–2:1400–1600 W3–4:1200–1400 M2–3:1000–1200 M4–6: 800–1000 >M6: 600–800	M12: 22%	M1: 148 μmol/l M6: 134 μmol/l M12: 134 μmol/l
	Neoral, MPA therapy, steroids + induction, including older recipients and patients with DGF	W1–2:1000–1200 W3-M6:800–1000 >M6: 600–800	M12: 12%	M1: 188 μmol/l M6: 172 μmol/l M12: 168 μmol/l
	Neoral, sirolimus or everolimus, steroids	W1–2: 1000–1200 W3–M3: 600–800 >M3: 400–600	not available	not available
Freeman Hospital, Newcastle, UK	Neoral, azathioprine, steroids, induction (anti-CD25)	M1–3: 1000–1500† M4–6: 800–1200 >M6: 600–1000	M12: 25%	M1: 162 μmol/l M3: 140 μmol/l M6: 143 μmol/l M12: 148 μmol/l
Center Hôpital Necker, Paris France	Neoral, MPA therapy, steroids, induction (anti-CD25)	M1–3:1000–1200 M4–6: 800–1000 >M6: 600–800	M6: 13%	M6: 155 μmol/l M12: 160 μmol/l

*In combination with C_0. †In patients with delayed graft function, C_2 target is 600–1000 ng/ml until graft function.

Table 5. Examples of C_2 target levels and outcomes in liver transplant recipients used in European centers. MPA, mycophenolic acid.

Center	Regimen	C_2 targets (ng/ml)	Incidence of acute rejection (% patients)	Mean serum creatinine
Hospital Juan Canalejo, Coruña, Spain	Neoral, MPA therapy, steroids	M1: 800–1000 M2–6: 600–800 >M6: 600	M6: 22%	Week 1: 100 μmol/l M1: 110 μmol/l
	Neoral, MPA therapy, induction (anti-CD25)	M1: 600–800 >M1: ~600	M6: 15%	Week 1: 90 μmol/l M1: 105 μmol/l
Fédération de Chirurgie Viscérale et Digestive, Caen, France	Neoral, MPA therapy + steroids	M1: 800–1200 M2–3: 800–1000 M4–6: 600–800 >M6: 400–600	M6: 14%	M2: 112 μmol/l M6: 120 μmol/l M12: 133 μmol/l M24: 140 μmol/l
Medizinische Hochschule Hannover, Hannover, Germany	Neoral, steroids ± induction	M1: 800–1000 M2–6: 600–800 >M6: 600	100 days: 15%	M1: 95 μmol/l* M1: 140 μmol/l†
	Neoral, MPA therapy, steroids	M1: 600–800 >M1: ~600		
Liver Transplantation Centre, Molinette Hospital, Turin, Italy	Neoral, MPA therapy, steroids	M1: 800–1000 M2–6: 600–800 >M6: 400–600	M12: 15%	M1: 150 μmol/l M6: 129 μmol/l M12: 126 μmol/l

*Patients with normal renal function pretransplant.
†Patients with impaired renal function pretransplant.

Use of adjunctive agents may mean that lower C_2 target levels are appropriate. Patients receiving everolimus benefit from reduced Neoral C_2 target levels [21,29]. Individual patients are at varying risks of rejection or adverse events (e.g. renal function impairment), and C_2 target should be adjusted accordingly. In patients with DGF, some centers elect to reduce C_2 targets until renal function recovers: in the MO2ART study C_2 levels were initially lower in the DGF cohort (mean dose at day 5 was 7.6 mg/kg/day compared with 11.0 mg/kg/day among patients with immediate graft function) and induction therapy was initiated in 38 of 108 DGF patients. At 3 months, the incidence of BPAR was 13.3% in the DGF group [18]. Of the 38 patients with DGF who received induction therapy, only one experienced BPAR [18].

C_2 targets should be reduced in renal transplant recipients if Neoral is administered concomitantly with sirolimus/everolimus (*I*). In a multicenter, open-label trial, 156 *de novo* renal transplant patients receiving everolimus, steroids and basiliximab were randomized to standard-exposure or reduced-exposure Neoral [27]. Results showed that reduced-exposure Neoral with everolimus is associated with at least equivalent efficacy to full-exposure Neoral. Renal function was significantly improved with reduced-exposure Neoral at 12 months post-transplant (creatinine clearance 62 ml/min compared with 51 ml/min with full-exposure Neoral, $P = 0.001$). Two large studies in which reduced C_2 targets were used to facilitate low-exposure Neoral with everolimus and steroids, with or without basiliximab induction therapy, have reported good rejection rates and renal function [21].

Clinical trials of Neoral C_2 monitoring have generally used declining C_2 targets over time post-transplant, in line with experience using C_0 monitoring.

Achieving C_2 target

Cyclosporine absorption may increase substantially in most patients during the first 1–2 weeks post-transplant (i.e. C_2 increases for the same dose of Neoral) (*II*). Absorption of CsA increases steadily over time as gut motility, diet and other factors improve after transplantation. In renal transplant patients, absorption stabilizes by approximately the end of the first month, whereas in liver transplant recipients, absorption reaches a plateau by approximately the end of month 2 [30]. Data from the MO2ART study suggest that the mean C_2 level achieved per 1 mg/kg/day Neoral increases from approximately 100 ng/ml on day 2 to 160 ng/ml on day 7, reaching 200 ng/ml on day 14 and 250 ng/ml at month 1 [30]. In liver transplants, data from LIS2T indicate that C_2 level per 1 mg/kg/day rises from 30 ng/ml to 60 ng/ml during the first week, reaching 140 ng/ml by month 1 and 190 ng/ml by month 2 [31].

A patient with a low C_2 value may be either a low or a delayed absorber of CsA, or be a normal absorber who is receiving too low a dose of Neoral. C_2 monitoring alone is insufficient to differentiate between these types of patients. Abbreviated AUC or measurement of CsA level at a second time point is useful to determine if a patient

is a normal absorber who requires an increase in Neoral dose, or to differentiate low and delayed absorbers (*III*). Low and delayed absorbers may be defined as follows:

Low absorber = C_2 remains low and C_2 >later time points (e.g. C_4 or C_6); delayed absorber = C_2 is low and C_2 <later time points (e.g. C_4 and C_6).

The recommended strategy for C_2 monitoring in *de novo* transplant recipients is as given below (*III*):

1 Start with 8–15 mg/kg/day (for standard therapy) or consider use of a short intravenous infusion of CsA in liver transplant patients.

2 Measure C_2 frequently (several times a week). Routine use of additional monitoring in all patients during the first week post-transplant is relatively convenient as patients are likely to be still in hospital.

3 Measure additional time points (e.g. C_0, C_4, C_6 or abbreviated AUC) during the first week after transplantation in order to differentiate low and delayed absorbers.

4 Observe changes in C_2 to assess any trend in variation before changing the dose, in order to allow for increasing absorption of CsA.

If the C_2 value remains low, or if additional time points suggest that a patient is a low or delayed absorber of CsA, further monitoring may be required.

Use of a linear formula to calculate changes to Neoral dose based on CsA levels may lead to overdosing while absorption is improving. In the MO2ART study [18], an analysis of Neoral dose adjustments showed that patients in whom dose was adjusted according to a linear formula during the first week post-transplant had a 30% risk of overshooting the C_2 target by day 7 compared with only a 10% risk among patients in whom dose was increased by less than that indicated by a linear formula.

Identifying low and delayed absorbers of CsA early post-transplant

In the early post-transplant phase, a sizeable proportion of patients may be low or delayed absorbers of CsA (*II*/*III*). Figure 1 illustrates typical CsA absorption patterns for individual patients with normal/good, low or delayed absorption. A patient with low absorption shows a normal time course (i.e. peak CsA concentration occurs at approximately C_2), but both the peak and AUC are low. A delayed absorber may have adequate absorption despite a delayed peak concentration, but may alternatively be a low absorber and be at risk of rejection.

The proportion of patients who are low or delayed absorbers of CsA is not well defined. Anecdotal evidence indicates that between 10% and 20% of renal transplant patients may be either delayed or low absorbers of CsA during the early post-transplant period. A higher proportion of liver transplant patients demonstrate either low or

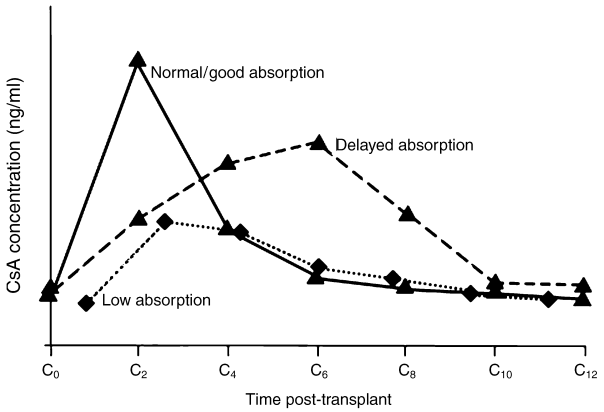

Figure 1 Typical absorption pattern of a normal/good absorber, a low absorber and a delayed absorber of CsA (R. Lück, personal communication).

delayed absorption early post-transplant. Many patients with delayed absorption will revert to a normal pattern of absorption over time.

Risk factors for low or delayed absorption of CsA are not fully elucidated. In renal transplant patients, the presence of diabetes [32], hepatic impairment [33], acute tubular necrosis with dialysis [33] and systemic diseases that affect gut motility may predispose patients to delayed absorption (*II*). Black race [32] and impaired gut motility may increase risk of low CsA absorption (*II*). In liver transplantation, delayed liver function, duct-to-jejunum anastomosis, cholestasis, external biliary drainage and the reduced gut motility are believed to heighten risk for low or delayed absorption of CsA (*III*).

It is important to differentiate between low and delayed absorbers because low absorbers are underexposed to CsA and at risk of rejection, such that additional immunosuppression is required, whereas delayed absorbers may achieve adequate CsA exposure despite low C_2 values and therefore increasing the Neoral dose places these patients at risk of overexposure. Measurement of additional time points (e.g. C_0, C_4, C_6 or abbreviated AUC) are recommended in order to differentiate low and delayed absorbers (*III*). Additional time points should be recorded: (i) in all patients during the first week after transplantation; (ii) if the ratio of C_2:C_0 is <3–4 and (iii) if the dose of Neoral is escalating to an inappropriately high level (e.g. >15 mg/kg/day) without approaching C_2 target.

If a patient is identified as a low or delayed absorber of CsA, the following monitoring options are appropriate: (i) perform an abbreviated AUC (e.g. C_2, C_4 and C_0); (ii) monitor C_4 or C_6 in addition to C_2 and (iii) monitor AUC_{0-4} or AUC_{0-12}.

Monitoring should be repeated during the early post-transplant period as absorption patterns often normalize.

Where absorption normalizes, use of C_2 monitoring is likely to be adequate.

Management of low and delayed absorbers of CsA early post-transplant

Management options in low and delayed absorbers have not yet been evaluated in clinical trials. However, clinical experience suggests a number of possible strategies. Improvements may also be seen over time in those patients in whom the absorption pattern normalizes, regardless of intervention.

Low absorbers with low C_2 values are underexposed to CsA and at risk of rejection (*III*).

In low absorbers of CsA, consider: (i) increasing Neoral dose until adequate absorption is achieved, with frequent monitoring of C_2 and another time point to avoid overexposure if absorption improves; (ii) adding an additional immunosuppressive agent, or increasing the dose of existing adjuvant agents; (iii) using intravenous CsA. There is evidence that use of 4-h intravenous infusions of CsA may be beneficial in *de novo* liver transplant patients [9]; and (iv) changing the immunosuppressive regimen (e.g. tacrolimus, sirolimus, everolimus). It is not known whether poor absorbers of CsA may also be poor absorbers of tacrolimus.

Delayed absorbers may achieve adequate CsA exposure despite low C_2 values, and therefore increasing the Neoral dose could potentially place these patients at risk of overexposure (*III*). In delayed absorbers of CsA who have adequate exposure, increasing Neoral dose may induce overexposure. The patient should be monitored to detect changes in the CsA absorption pattern over time; the absorption pattern may normalize with time, such that C_2 monitoring becomes adequate.

If CsA exposure is considered inadequate in a patient with delayed absorption, consider: (i) cautiously increasing the Neoral dose, taking into account C_2 values and another time point or abbreviated AUC; (ii) adding an additional immunosuppressive agent, or increasing the dose of existing adjuvant agents; (iii) using intravenous CsA [9]; and (iv) changing the immunosuppressive regimen.

Neoral C_2 monitoring in the maintenance patient

Adopting C_2 monitoring in maintenance transplant patients identifies those who are overexposed to CsA (*II*). Consideration should be given to measuring C_2 at least annually in maintenance patients. Using C_2 levels to detect over-immunosuppression may help to improve long-term allograft survival by reducing CyA nephrotoxicity. Clinicians should also be aware that additional monitoring (C_2 and/or abbreviated AUC) may be required when changes are made to medications which affect CsA metabolism and thereby change the relationship between C_2 and AUC [34], particularly if such therapies are taken for a prolonged period.

Renal transplantation

Three single-center studies have measured C_2 levels in previously C_0-monitored maintenance renal transplant patients. Cole *et al.* [35] have reported that 77/161 (48%) patients more than 1 year post-transplant exceeded the predefined upper C_2 target of 800 ng/ml. In a separate study of 188 renal patients, all of whom had received their transplant more than 1 year previously (mean 8 years), annual C_2 monitoring showed that 31 (26%) had C_2 values above 800 ng/ml [36]. Loss of GFR showed a U-shaped dependence on C_2 level, with the smallest loss seen in patients with C_2 levels between 400–600 ng/ml ($P < 0.05$ for percentage change in GFR). Midtvedt *et al.* [37] found that 29% of patients (296/1032) more than 1-year post-transplant had C_2 values >800 ng/ml, and that 9% (94/1032) had values >950 ng/ml. Serum creatinine values were significantly higher in patients with C_2 >950 ng/ml (152 μmol/l) compared with those with C_2 in the range 700–800 ng/ml (136 μmol/l, $P < 0.02$) or those with $C_2 < 450$ ng/ml (141 μmol/l, $P < 0.05$). In another study, which investigated C_2 levels in 127 maintenance patients on a variety of CsA-based regimens with a follow up of approximately 14 months [38], the authors concluded that C_2 concentrations as low as 500–600 ng/ml were well-tolerated and provide effective and safe rejection prophylaxis in the maintenance population.

Consequent dose reductions in overexposed patients can improve renal function and reduce adverse events (*II*). In 85 patients who received a Neoral dose reduction because they exceeded C_2 target by >10%, 46 showed a fall in serum creatinine (153–132 μmol/l, $P < 0.05$) and a reduction in mean blood pressure (135/82–131/77 mmHg, $P < 0.05$ for diastolic blood pressure) [35]. A general improvement in well-being, tremor or gum hyperplasia was reported by 75% of the patients.

To summarize, single-center experience suggests that C_2 levels >800 ng/ml in renal transplant patients >1 year post-transplant receiving triple therapy regimens should be avoided (*II*). The results of single-center studies suggest that an upper C_2 limit of 800 ng/ml is appropriate in renal transplant patients more than 1-year post-transplant who are receiving triple therapy [35–37]. Until the results of prospective trials are available, reduction of Neoral dose is recommended in these patients if C_2 exceeds 800 ng/ml.

To date, there is a scarcity of data assessing the effect of increasing Neoral dose in patients who have low C_2 values and stable graft function. A minimum C_2 target level has not yet been defined.

Liver transplantation

C_2 monitoring has been assessed prospectively in 35 stable liver transplant recipients more than 1-year post-transplant [10]. Patients were randomized to continue C_0 monitoring (target 100–200 ng/ml) or to switch to C_2 monitoring using either a higher C_2 target range (700–1000 ng/ml) or a lower range (300–600 ng/ml). The lower C_2 target range resulted in lower Neoral dose and greater clinical benefit than the other two monitoring strategies (defined as the absence of rejection and no increase in serum creatinine at the 7-month follow-up). Additionally, two studies have measured C_2 levels in maintenance liver transplant patients with stable graft function in whom Neoral dose was adjusted according to C_0 level, and found mean C_2 level to be 500–600 ng/ml [39,40].

These findings suggest that an upper target of 600 ng/ml may be appropriate in the maintenance liver transplant population.

Implementing C_2 monitoring

The optimal sampling time for C_2 is 2 h ± 15 min (*III*). If the C_2 blood sample is taken within 15 min of the C_2 time point, the sampling error is reduced to <10% [41]. Measurements of C_2 are largely assay independent, i.e. values are congruent regardless of the type of assay used [42]. A stepwise procedural approach facilitates smooth implementation of Neoral C_2 monitoring, and has been described in detail elsewhere [43].

Several participants reported their experience with Neoral C_2 monitoring. At the Hôpital Necker in Paris, France, C_2 monitoring is used routinely in all *de novo* and maintenance patients; a survey undertaken after 1 year reported a positive impact on patients, nursing and medical staff. The Universitair Ziekenhuis Antwerpen in Edegem, Belgium, has adopted dose adjustment based on C_2 monitoring for all Neoral-treated *de novo* patients, in addition to the existing C_0 monitoring, and is progressively switching from C_0 to C_2 monitoring for maintenance patients. Two centers, the Hospital Universitario Dr Peset in Valencia, Spain, and Rikshospitalet in Oslo, Norway, employ C_2 monitoring in all *de novo* renal transplant patients until 6 and 3 months post-transplant, respectively, then use C_0 monitoring with regular C_2 measurements at least once a year or if the patient's clinical course is unsatisfactory. Similarly, the Kantonsspital Aarau in Switzerland uses annual C_2 monitoring in all renal maintenance patients to complement C_0 monitoring at regular visits; CsA dose reductions are considered in patients with C_2 levels >800 ng/ml [36]. In Germany, the Campus Charité Mitte in Berlin uses C_2 monitoring in *de novo* renal transplant patients considered to be at risk of rejection or DGF, and periodically in maintenance patients or in any patient in whom CsA overexposure is suspected in order to help avoid toxicity. At the Medizinische Hochschule Hannover in Germany, C_2 monitoring is standard in kidney and pancreas transplantation for at least the first 3 months post-transplant with abbreviated AUC measurements undertaken every 2 weeks, or immediately in any case of suspected CsA malabsorption. A similar approach is used for liver transplant recipients at the Medizinische Hochschule Hannover. Neoral C_2 monitoring is used in all *de novo* and maintenance liver transplant patients at the Fédération de Chirurgie Viscérale et Digestive in Caen, France, the Liver Transplantation Centre at the Molinette Hospital in Turin, Italy, and at the Hospital Juan Canalejo in Coruña, Spain. At the Hospital Juan Canalejo this has led to lower CsA doses in 45% of maintenance patients with no rejection and with a fall in serum creatinine levels.

Future research needs

Many aspects of Neoral C_2 monitoring, using a variety of regimens in different organ types, have now been assessed in randomized, prospective, multicenter studies, with additional data available from single-center trials. Future research priorities include investigation of the effect of increasing the Neoral dose in patients with low C_2 values (e.g. <400 ng/ml), the long-term effects of C_2 monitoring on graft outcome and controlled studies to evaluate C_2 monitoring in heart and lung transplantation.

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