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*Original Article*

# No rise in renal Doppler resistance indices at peak serum levels of cyclosporin A in stable kidney transplant patients

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## Abstract

**Background.** The measurement of intrarenal resistance indices by duplex ultrasound plays an important role in the follow-up care of renal transplant patients. Increasing resistance indices indicate rejection episodes, but may also occur e.g. in parenchymal renal diseases. As calcineurin inhibitors induce vasoconstriction both *in vivo* and *in vitro*, we studied whether peak serum levels of cyclosporin A led to an acute rise in renal resistance indices via the induction of intrarenal vasoconstriction.

**Methods.** The acute impact of peak serum levels of cyclosporin A on intrarenal resistance indices was studied in 36 patients after allogeneic renal transplantation. All patients were transplanted for $> 6$ months and received an immunosuppressive treatment comprising cyclosporin A. Intrarenal resistance indices were measured by duplex ultrasound immediately before (trough serum level) and 2 h after (peak serum level) the oral intake of cyclosporin A at the individual maintenance dose.

**Results.** Compared with renal resistance indices measured at trough serum levels [resistive index (RI) $0.72 \pm 0.07$; pulsatility index (PI) $1.40 \pm 0.27$], values remained unchanged at peak serum levels of cyclosporin A (RI $0.72 \pm 0.08$; PI $1.43 \pm 0.31$). Renal resistance indices correlated with the age of the patients, but not with mean arterial pressure or time since transplantation.

**Conclusions.** The oral intake of cyclosporin A does not induce an acute rise in intrarenal resistance indices in stable transplanted patients. Thus, timing of duplex ultrasound examinations with regard to the intake of cyclosporin A is not necessary in these patients.

**Keywords:** cyclosporin; Doppler; duplex; immunosuppressive agents; kidney transplantation; ultrasonography

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## Introduction

Despite the introduction of new immunosuppressive agents, the incidence of acute allograft rejection within 1 year after renal transplantation still ranges at 24% among all patients receiving a cadaveric kidney [1]. Acute allograft rejections impair graft survival, predisposing to early graft loss and to chronic allograft dysfunction. Therefore, a rapid and reliable detection of an acute rejection episode is mandatory.

The introduction of renal duplex sonography established a non-invasive diagnostic tool in transplanted patients, allowing early detection of an acute rejection episode, which induces an increase in predefined resistance indices [2,3]. However, the sensitivity and specificity of duplex resistance indices for the diagnosis of acute rejection episodes remains controversial [4–6], as these indices may rise in a variety of causes of renal dysfunction.

In addition, renal resistance indices may also reflect vasomotoric changes induced by pharmacological therapy, the relative importance of which has not been thoroughly investigated. As calcineurin-inhibitors induce intrarenal vasoconstriction both *in vivo* and *in vitro* [7–9], we studied whether peak serum levels of cyclosporin A (CyA) lead to an acute rise in renal resistance indices via the induction of intrarenal vasoconstriction. If the intake of CyA increased renal resistance indices, timing of duplex ultrasound examinations should take this into account and should be standardized with regard to the time of day, e.g. before the morning intake of CyA or at peak serum levels of CyA, at about 2 h after the intake of CyA.

## Subjects and methods

### *Subjects*

Thirty-six patients (24 male, 12 female) after allogeneic renal transplantation, who had been transplanted for $> 6$ months (mean: $82.6 \pm 53.4$ months; range: 7–188 months), were included in a prospective study. All patients received

an immunosuppressive drug regimen comprising the calcineurin-inhibitor CyA (Neoral®; Novartis, Basel, Switzerland). As co-immunosuppressive medication, patients received azathioprine ($n=6$), methylprednisolone ($n=17$) or a combination of azathioprine and methylprednisolone ($n=9$). Four patients had an immunosuppressive monotherapy with CyA. Concomitant medication comprised calcium-channel blockers (CCB; $n=22$, of whom 20 had a morning intake of CCB), angiotensin-converting enzyme inhibitors ($n=14$), $\beta$-blockers ($n=13$), statins ($n = 12$), diuretics ($n=12$), central $\alpha(2)$-adrenoceptor agonists ($n=10$), angiotensin II receptor antagonist ($n=7$), adrenergic $\alpha$-antagonists ($n=2$), digitalis ($n = 2$), nitrates ($n=2$) and theophylline ($n=1$). CyA was administered twice daily in all patients, at a 12 h interval. The dosing was adjusted to achieve trough serum levels of 100–150 ng/ml. The mean age of the patients studied was 54.4 ± 12.8 years and the mean serum creatinine was 1.6 ± 0.7 mg/dl (141.4 ± 61.9 µmol/l). All patients gave informed consent.

End-stage renal disease was due to glomerulonephritis (12 patients), autosomal dominant polycystic kidney disease (six patients), hereditary nephritis (four patients), diabetic nephropathy (one patient) or other/unknown renal diseases (13 patients).

Patients with clinical signs of acute rejection, with rapid deterioration of renal function for any other reason, or with evidence of urinary tract obstruction were excluded.

Trough serum levels and peak serum levels of CyA were measured before the intake of the calcineurin-inhibitor and 2 h thereafter, using an enzyme immunoassay (Roche, Grenzach, Germany).

### *Ultrasound measurement*

Colour Doppler examinations were performed with a phased-array transducer (Acuson Sequoia, Mountainview, CA, USA; B-mode frequency: 5 MHz; Doppler frequency: 2.5 MHz) in supine position.

Doppler spectra were obtained from the segmental arteries at five separate locations. For each spectrum obtained, the resistive index (RI) and pulsatility index (PI) were calculated using the system's software from the Doppler wave form according to the following formulae:

$$\mathrm{RI} = (\text{peak systolic frequency shift} - \text{minimum diastolic frequency shift}) / \text{peak systolic frequency shift}$$

$$\mathrm{PI} = (\text{peak systolic frequency shift} - \text{minimum diastolic frequency shift}) / \text{mean frequency shift}$$

The average RI and PI were computed to yield an overall RI and PI for the renal transplant, respectively. Intrarenal resistance indices were measured by duplex ultrasound immediately before (trough serum level, 12 h after the preceding intake of CyA) and 2 h after (peak serum level) the oral intake of CyA at the individual maintenance dose.

In addition, immediately before and 2 h after the oral intake of CyA, systolic blood pressure ($RR_{sys}$), diastolic blood pressure ($RR_{dia}$) and heart rate were registered and blood was taken by venopuncture in order to determine serum levels of CyA, respectively. Mean arterial blood pressure ($RR_{mean}$) was calculated as:

$$RR_{dia} + (RR_{sys} - RR_{dia})/3$$

Additional ultrasound measurements were performed in four patients 4 and 6 h after the intake of CyA, in order to rule out any haemodynamic effect that might occur with a latency of 2–4 h after peak serum levels are reached [8,9].

### *Statistics*

Data management and statistical analysis were performed with the Prism V3.0 statistical software (Graphpad, San Diego, CA, USA). All data are expressed as means ± SD unless indicated otherwise.

Significance of differences in renal resistance indices, arterial blood pressure, heart rate and serum levels of CyA before and 2 h after the intake of CyA was calculated using the Wilcoxon signed rank test for paired, not normally distributed samples. The non-parametric Friedman test for repeated measurements was applied in patients who underwent additional ultrasound studies 4 and 6 h after the intake of CyA. Spearman's linear regression analysis was performed to determine the association between Doppler indices and each of the different parameters listed below. The level of significance was set at $P < 0.05$.

## Results

Serum levels of CyA increased significantly after the intake of the calcineurin-inhibitor (pre-CyA 105.1 ± 7.0 ng/ml, post-CyA 481.7 ± 143.8 ng/ml; $P < 0.001$).

Mean arterial blood pressure and heart rate did not differ significantly at trough serum levels and at peak serum levels [$RR_{mean}$: pre-CyA 116.6 ± 11.4 mmHg, post-CyA 114.2 ± 11.9 mmHg; heart rate: pre-CyA 65.8 ± 9.9 beats per minute (BPM), post-CyA 66.6 ± 8.3 BPM; Figure 1].

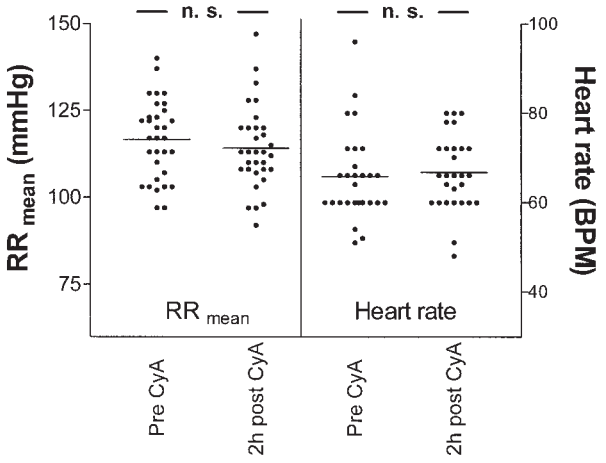


**Fig. 1.** The intake of CyA does not induce a significant rise in mean blood pressure ($RR_{mean}$) or in heart rate.

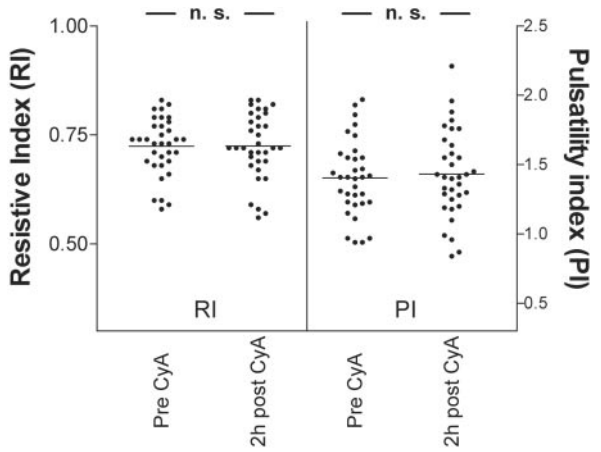


**Fig. 2.** The intake of CyA does not induce an acute increase in renal resistance indices.

The intake of CyA did not induce a significant increase in renal resistance indices: mean RI was 0.72 ± 0.07 before the intake of CyA and 0.72 ± 0.08 at 2 h afterwards. Mean PI was 1.40 ± 0.27 before and 1.43 ± 0.31 after the intake of CyA (Figure 2).

To rule out any haemodynamic effect that might occur with a latency of 2–4 h after peak serum levels are reached, a subgroup of four patients underwent additional ultrasound studies 4 and 6 h after the intake of CyA. There was no increase in renal resistance indices at these additional time points (RI: pre-CyA 0.79 ± 0.05, 2 h post-CyA 0.78 ± 0.03, 4 h post-CyA 0.78 ± 0.04, 6 h post-CyA 0.79 ± 0.05; PI: pre-CyA 1.72 ± 0.37, 2 h ± 0.09, 4 h post-CyA 1.63 ± 0.18, 6 h post-CyA 1.72 ± 0.17).

Resistance indices measured at trough levels and at peak levels of CyA correlated significantly with the age of the patients [RI at trough levels: $r = 0.62$ ($P < 0.0001$), RI at peak levels: $r = 0.62$ ($P = 0.0001$); PI at trough levels: $r = 0.65$ ($P < 0.0001$), PI at peak levels: $r = 0.60$ ($P = 0.0002$); Figure 3A], but not with the age of the donors at the time point of transplantation (Figure 3B) or with the age of the kidney (age of the donor + time since transplantation). There was no significant correlation between resistance indices and body weight of patients, the time since transplantation or mean arterial blood pressure. In addition, resistance indices did not correlate with trough serum levels or with peak serum levels of CyA (Figure 4). Finally, there was neither a significant difference in renal resistance indices between male and female patients nor between recipients of a kidney from a male or female donor.

When studying an additional group of seven patients who had an immunosuppressive drug regimen which comprised tacrolimus instead of CyA, tacrolimus did not induce an increase in renal resistance indices within 2 h after the drug intake either (data not shown).

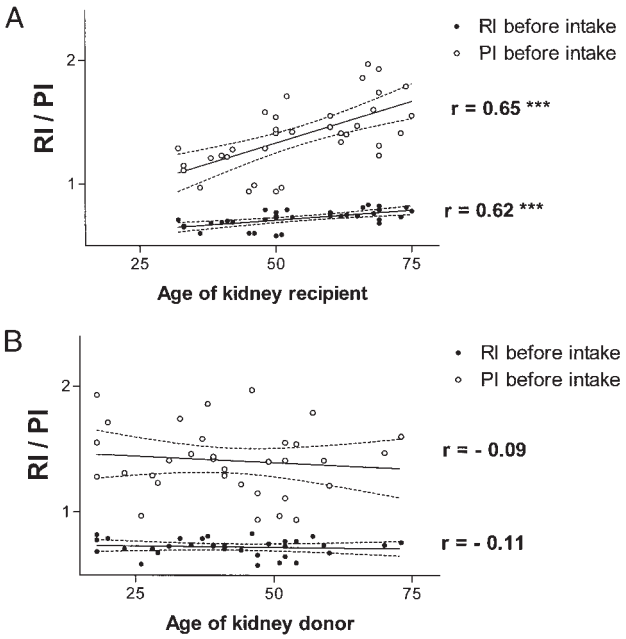


**Fig. 3.** Renal resistance indices show a significant correlation with the age of the kidney recipient (**A**), but not with the age of the kidney donor (**B**). (A) ***$P < 0.0001$.

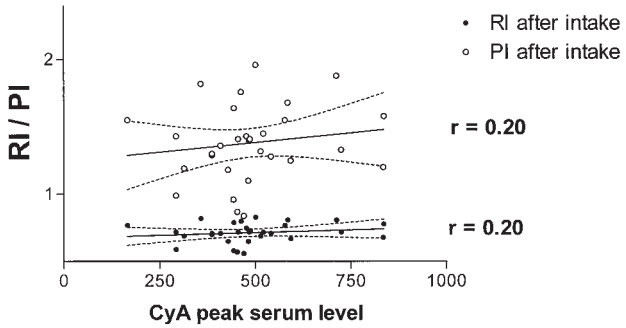


**Fig. 4.** Renal resistance indices measured 2 h after the intake of CyA do not correlate with peak serum levels of cyclosporin.

## Discussion

The diagnosis of acute allograft rejection in renal transplant patients has become more difficult in the CyA era, since clinical signs and symptoms of rejection became more subtle. Non-invasive techniques to diagnose episodes of renal dysfunction can help avoid biopsies, which carry some risk of complication and cannot be easily carried out frequently.

Therefore, duplex Doppler ultrasound has become an important diagnostic tool for the early diagnosis of acute allograft rejection [2,3]. In acute rejection, an interstitial oedema results in an increase in interstitial pressure, compromising blood flow in intrarenal arteries with decreased diastolic perfusion. When measuring velocity of blood flow during systolic and diastolic perfusion in intrarenal arteries by means of intrarenal duplex ultrasound, the determination of RI

and PI allows the estimation of diastolic perfusion in relation to systolic perfusion. An intraindividual increase in RI and PI signifies an impaired diastolic perfusion, which, in patients suspected clinically to have allograft rejection, supports this diagnosis [6].

However, an increase in resistance indices is not specific to any one clinical entity. Resistance indices may rise whenever peripheral vascular resistance is increased due to constriction of peripheral arteries. This may occur either indirectly when renal parenchyma is compressed, e.g. by increased intrarenal interstitial pressure as in acute rejection, but also e.g. in acute tubular necrosis [3], or by direct vasoconstriction.

CyA induces constriction of the afferent arteriole proximal to the glomerulus [7,10], which results in an acute drop in renal plasma flow and in glomerular filtration rate [11]. Many mediators have been suggested to mediate this cyclosporin-induced vasoconstriction, including thromboxane A2, reduction of vasodilator prostaglandins, endothelin, angiotensin II, platelet-derived growth factor and changes in adrenergic nerve traffic and sensitivity [12,13].

Due to these vasoconstrictive properties, one might suggest that the acute intake of CyA induces a rise in resistance indices, which may complicate the interpretation of repetitive duplex ultrasound measurement in one patient performed at different times during the day.

In contrast to these theoretical considerations, we found that the vasoconstrictive properties of cyclosporin do not result in a clinically significant increase in resistance indices, at least in stable renal transplant patients. Compared with the resistance indices measured before the intake of CyA, indices did neither increase at peak serum levels of CyA nor when a latency of 4 h after peak serum levels had been reached, the time point of the maximum haemodynamic effect of CyA [8,9].

The intake of CCB may counteract the haemodynamic effects of CyA. However, in the 14 patients who had no CCB medication, we also did not find an increase in renal resistance indices (data not shown).

Apparently, a localized, cyclosporin-induced constriction of the afferent arteriole does not lead to a relevant change in flow patterns measured more proximately in the segmental arteries, whereas more diffuse alterations, such as an increase in intrarenal interstitial pressure due to e.g. acute rejection, does influence flow patterns in the segmental arteries significantly.

To our best knowledge, there is only one study published on the effect of CyA on renal resistances measured by colour Doppler flowmetry of renal grafts [14], in which di Palo and coworkers report a significant rise in resistance indices after the intake of CyA. However, the authors studied a preparation of cyclosporin that is characterized by a highly variable bioavailability and pharmacokinetics after oral administration and which has been replaced in many transplantation centres by a new, microemulsion formulation (Neoral®) of CyA with a more predictable pharmacokinetic profile [15]. In addition, the amount of CyA administered by di Palo and coworkers ($4.5 \pm 0.5$ mg CyA/kg/day) was well beyond the dosage currently used by most transplantation centres and would be considered rather toxic for long-term immunosuppression nowadays. In comparison, we used only a moderate dosage of cyclosporin ($2.3 \pm 0.1$ mg CyA/kg/day). We, thus, believe that the findings of di Palo and coworkers are no longer applicable to the current clinical practice.

In addition, we could confirm that renal resistance indices correlate with the age of the recipient, but not with the age of the donor after kidney transplantation, which is in accordance with data by other groups [16].

Taken together, the oral intake of cyclosporin, when used for long-term immunosuppression in a dosage that aims to achieve a trough serum level of 100–150 ng/ml, does not induce an acute rise in intrarenal resistance indices. Thus, timing of duplex ultrasound examinations with regard to the intake of CyA is not necessary, at least in stable transplant patients with therapeutic plasma levels of CyA.

*Conflict of interest statement*. None declared.

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