# Clinical Significance of Peripheral Blood Lymphocyte Sensitivity to Glucocorticoids for the Differentiation of High-risk Patients With Decreased Allograft Function After Glucocorticoid Withdrawal in Renal Transplantation

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## ABSTRACT

**Purpose:** A reliable biomarker to differentiate high-risk recipients who will experience a decrease in allograft function after glucocorticoid withdrawal has not been established in renal transplantation. We examined the clinical significance of peripheral blood lymphocyte sensitivity to glucocorticoids in vitro for the differentiation of the high-risk patients after glucocorticoid reduction/withdrawal in renal transplant recipients.

**Methods:** The study included 44 renal transplant recipients with stable allograft function. Peripheral lymphocyte responses to suppressive effects of cortisol, methylprednisolone, cyclosporine, and tacrolimus in mitogen assay procedures in vitro were examined. Clinical outcome after glucocorticoid reduction/withdrawal was retrospectively compared between recipients with lymphocytes normally sensitive to the drugs and those with hyposensitivity. The receiver-operating characteristic (ROC) curve analysis was undertaken for setting the cutoff $IC_{50}$ values of the drugs against the T cell mitogen–induced lymphocyte proliferation to differentiate the high-risk recipients with decreased allograft function after glucocorticoid withdrawal.

**Findings:** The median (range) $IC_{50}$ value for cortisol in the recipients who showed decreased renal function due to glucocorticoid withdrawal was 10,000 (570.9–72,279.3) ng/mL (n = 9), which was significantly higher than the value of 351.6 (2.0–10,000) ng/mL in the recipients who had not experienced glucocorticoid withdrawal symptoms (n = 35) ($P < 0.001$). Similarly, the median (range) $IC_{50}$ value for methylprednisolone in the recipients who showed decreased renal function after glucocorticoid withdrawal was 69.1 (21.5–1442.7) ng/mL (n = 9), which was significantly higher than the value of 13.8 (0.7–1000) ng/mL in the recipients who had not experienced glucocorticoid withdrawal symptoms (n = 30) ($P < 0.003$). In contrast, there was no significant difference in the median $IC_{50}$ values of cyclosporine and tacrolimus between the 2 recipient subgroups. The ROC curve analyses for the $IC_{50}$ values of the immunosuppressive drugs estimated the cutoff value of cortisol and methylprednisolone to be 3580.0 and 21.5 ng/mL, respectively. The ROC AUCs for cortisol and methylprednisolone were 0.83 and 0.84, respectively. According to the cutoff $IC_{50}$ value, the incidence of decreased allograft function in the low cortisol sensitivity ($IC_{50}$ >3580.0 ng/mL) subgroup was 7 of 13 patients, which was significantly higher than that of the higher sensitivity subgroup of 2 of 31 ($P = 0.0012$). A similar case was observed using the cutoff $IC_{50}$ value of methylprednisolone ($P = 0.0012$), whereas recipient grouping according to the cutoff $IC_{50}$ values of cyclosporine and tacrolimus failed to

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differentiate the high-risk recipients with decreased allograft function after glucocorticoid withdrawal.

**Implications:** Glucocorticoid pharmacodynamics in lymphocytes of individual patient origin is a reliable biomarker for differentiation of renal transplant recipients who will experience a safe reduction/withdrawal of glucocorticoid. (*Clin Ther*. 2014;36:1264–1272) 

**Key words:** glucocorticoid withdrawal, immunosuppressive drugs, peripheral-blood mononuclear cells, pharmacodynamics, renal transplantation, T-cell mitogen.

## INTRODUCTION

The individual differences in the clinical efficacy of immunosuppressive drugs widely used for treatment of autoimmune diseases and organ transplantations are greater than expected.[1–4] Glucocorticoid reduction/withdrawal is one of the most important issues for successful long-term immunosuppressive therapy in renal transplantation.[5–8] However, glucocorticoids need to be restarted to prevent the worsening of renal function because of occurrence of acute rejection episodes in some patients or in patients who experience glucocorticoid withdrawal syndrome.[9–11] Increasing the dose of the immunosuppressive drugs could also result in a variety of side effects that plague the patients. Thus, it is necessary to establish reliable biomarkers to predict which patients will experience a safe reduction or withdrawal of glucocorticoids without experiencing glucocorticoid withdrawal syndrome.

We suggested in our previous study that in patients with peripheral blood mononuclear cells (PBMCs) exhibiting a good response to the suppressive efficacy of cortisol against T cell mitogen–stimulated PBMC proliferation in vitro, methylprednisolone administered for maintenance immunosuppressive therapy in renal transplant recipients can be withdrawn.[12] The clinical usefulness of the cellular pharmacodynamic approaches of immunosuppressive drugs using PBMCs of patient origin for patient-tailored drug therapy has also been demonstrated in the reports including those of our institutions.[13–19]

In the present study, we evaluated PBMC response to 4 immunosuppressive drugs: hydrocortisone (cortisol), methylprednisolone, cyclosporine, and tacrolimus in vitro in 44 consecutive renal transplant recipients who are undergoing a schedule of reduction/withdrawal of glucocorticoid treatment. PBMC responses to these drugs were retrospectively related to allograft function after reduction or withdrawal of glucocorticoid, and possible biomarkers of the safety glucocorticoid reduction were established.

## PATIENTS AND METHODS

### Patients

This study was approved by the Ethics Committee of Hachioji Medical Center, Tokyo Medical University and by the Ethics Committee of Tokyo University of Pharmacy and Life Sciences, and informed consent was obtained from all patients. The study was performed with 44 renal transplant recipients 50.3 (13.4) (mean [SD]) years of age (24 male and 20 female patients) who were receiving immunosuppressive therapy. These recipients were selected based on the following criteria: (1) stable allograft function with serum creatinine concentrations $<1.6$ mg/dL basically and (2) recovered adrenal function with serum cortisol concentration ranging from 40 to 180 ng/mL with no signs of infection. These recipients were treated with methylprednisolone at doses of 4 mg/d and either cyclosporine or tacrolimus at a dose of 105.2 (40.4) mg/d or 4.1 (2.2) mg/d, respectively. PBMC responses to hydrocortisone (cortisol), methylprednisolone, and cyclosporine (n = 24) and tacrolimus (n = 20) were examined, as described in the following.

### Isolation of PBMCs and Evaluation of Drug Effects in Vitro

Between 9:30 and 11:00 in the morning, 20 mL of venous blood were taken from the patients and heparinized. This 20-mL sample size was the smallest possible to carry out the drug sensitivity test. However, not all of the 44 patients could be examined for the PBMC response to all 4 immunosuppressive drugs because of the number of PBMCs obtained from the 20-mL blood sample (Table I). The heparinized blood was loaded on 6 mL of Ficoll-Hypaque (Nakarai Co, Tokyo, Japan), centrifuged at 1300*g* for 20 minutes, and PBMCs were separated as described previously.[1–4] For the evaluation of PBMC sensitivity to immunosuppressive drugs, cells were washed and resuspended in RPMI1640 medium containing 10% fetal calf serum, 100,000 IU/L penicillin, and 100 mg/L streptomycin to a final density of $1 \times 10^6$ cells/mL. Concanavalin A, as a T-cell mitogen, was added to each well to a final