

A Novel Approach in Clinical Immunosuppression Monitoring: Drug Lymphocyte Level

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Like others, we have shown a weak correlation between drug blood levels and clinical outcome and immune response. We recently established that in contrast to blood levels, drug lymphocyte levels are strongly associated with therapeutic efficacy. The discordance between the 2 methodologies regarding clinical outcome and immune response is related mainly to the weak association between drug blood level and target-cell content. This weak association explains the intra- and interindividual variabilities of the therapeutic response. These variations are related mainly to genetic and environmental factors including age, sex, body mass index, organ function, food and subsequent drug absorption, drug interactions, and the availability of extra-target-cell binding sites. These factors seem to influence the modes of action of immunosuppressive agents including drug absorption, metabolism, elimination, transport, extra-target-cell sites, as well as target-cell receptor expression and its binding affinity and specific enzyme baseline activity. Therefore, the cellular fraction of a drug at a fixed dosage is the result of the integration of out-fluxing and in-fluxing forces that are affected by genetic and environmental factors. Any redistribution of the drug between the different binding sites will ultimately affect its bioactivity with no change to its extracellular bioavailability (which is currently determined by pharmacokinetic measurements). Compared with whole-blood or plasma-level measurements, monitoring immunosuppression therapy at the site of action appears to be not only more clinically and

immunologically relevant (since it measures the free fraction of the drug at its effector site), but this method also bypasses the potentially complex extracellular factors that affect bioactivity.

Since the intracellular content of a drug strongly correlates with its biological effect, monitoring immunosuppression therapy at the site of action is comparable to pharmacodynamic monitoring. It is cost effective and easy to perform in clinical practice and could be used for all immunosuppressive drugs. Since it allows maximal reduction of adverse effects through dosage reduction while maintaining an optimal level of immunosuppression, it should offer a new alternative for immunosuppressive therapy monitoring and tailoring to the individual patient.

Key words: *Immunity, Bioavailability, Bioactivity, Pharmacokinetic, Pharmacodynamic*

The lack of a reliable immune response assay has been a significant hurdle for clinicians in their attempts to monitor the immune state. Despite the enormous amount of literature published over the last 2 decades, the clinical relevance of most monitoring techniques for cyclosporine therapy and other immunosuppressive agents in solid organ transplant remains controversial (1-5). Unfortunately, short of graft biopsies, all currently used noninvasive pharmacokinetic therapeutic monitoring techniques have proven unreliable (6-9). Monitoring the efficacy of immunosuppressive therapy in most trials is accomplished mainly by measuring target-drug blood or plasma levels, which differ among trials and among immunosuppressive protocols within each trial. Additionally, surveillance of renal function is accomplished by performing graft biopsies according to clinical indications.

We and others have shown a weak correlation between drug blood dosages and levels and clinical outcome and immune response (3-11). We established that in contrast to drug blood levels, drug lymphocyte

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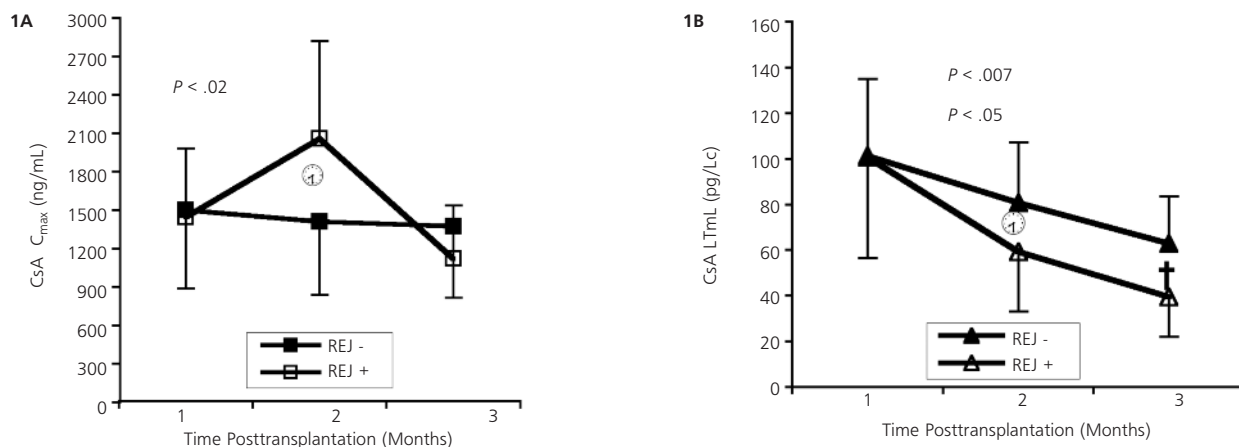


Figure 1: Correlation between cyclosporine (CsA) monitoring of whole blood maximum concentrations (C_{max}) (A), and their corresponding lymphocyte maximum levels (LTmL) (B) with clinical outcome (acute rejection) in de novo kidney transplant patients during the first 3 months after transplant. CsA dosage was adjusted according to CsA lymphocyte content. Patients with acute rejection (REJ +) were compared to those without rejection (REJ -) (Reference No. 13).

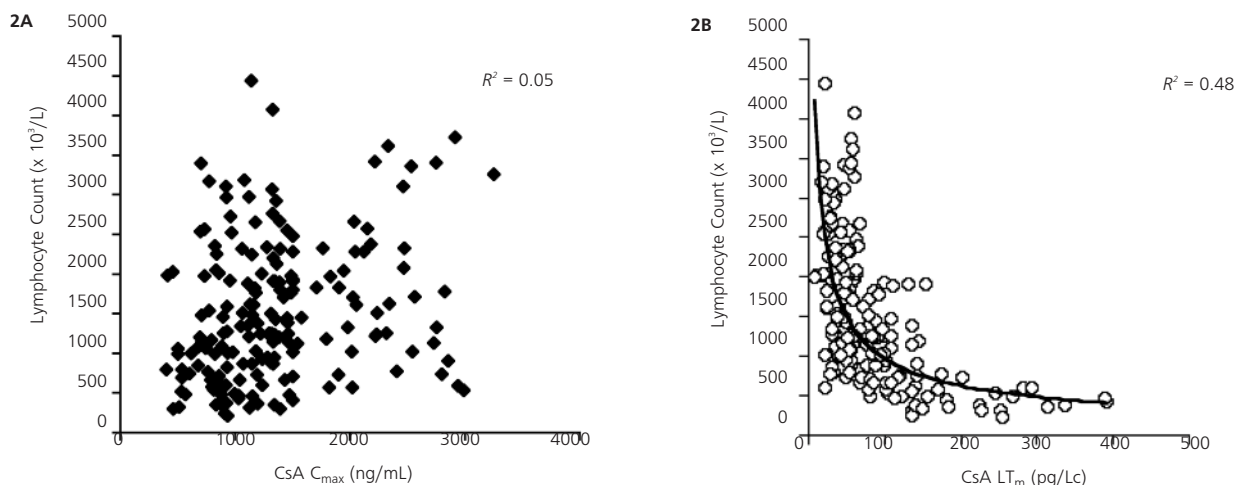


Figure 2: Relationship of the cyclosporine (CsA) monitoring parameters: (A) whole blood concentrations and (B) their respective lymphocyte levels with the total lymphocyte count. CsA maximum lymphocyte level (LTmL) exhibits a strong exponential relationship with lymphocyte count, a rough indicator of the immune state. In contrast to LTmL, CsA whole blood maximum level (C_{max}) correlates poorly with the total lymphocyte count (Reference No. 15).

levels are strongly associated with therapeutic efficacy as demonstrated by clinical outcome (mainly acute rejection [Figure 1] and immune responsiveness [11-17] [Figure 2]). These differences are due to the significant discordance between bioavailability and bioactivity in relation to immune sensitivity (6, 7, 8, 11, 12, 13, 18), acute rejection, and adverse effects.

Bioavailability, as measured by pharmacokinetic assay, represents the fraction of an administered amount of a drug that reaches the peripheral circulation; it reflects the effect of the body on the drug. In contrast, bioactivity, which is a major pharmacodynamic marker that reflects the effect of the drug on the body, correlates strongly with the amount of the active ingredient of a drug that is available at the site of its effector (18, 19). As we and

others have demonstrated (11-16, 20), the discordance between bioavailability (drug levels in blood and plasma) and bioactivity (biological effect) is related mainly to the weak association between drug levels in blood or plasma and target-cell content and tissue concentration (Figure 3). The poor correlation between drug blood level and drug lymphocyte level is explained by the striking interindividual variability that we have shown recently regarding drug lymphocyte levels for cyclosporine, tacrolimus, and sirolimus within the same ranges of drug blood levels and dosages (12, 14, 17) (Figure 4). These observations are in agreement with the works of Londono (21) and Hartmann (22) regarding the poor relationship between tacrolimus and sirolimus blood levels and target enzyme (ie, calcineurin and p70 S6 kinase) activities, respectively.

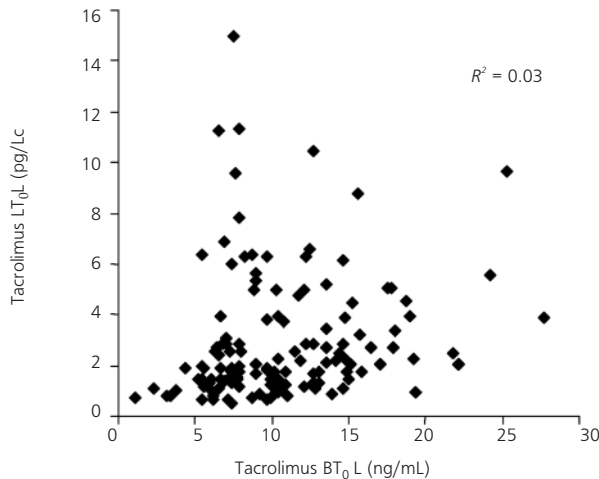


Figure 3: Relationship between tacrolimus blood trough concentrations (BT₀L) and their respective simultaneous lymphocyte trough levels (LT₀L) (Reference No. 14).

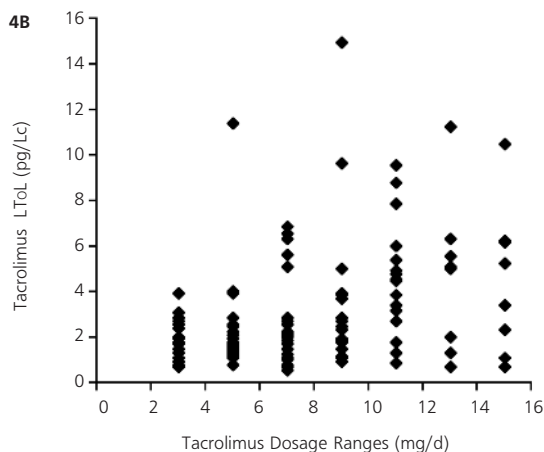
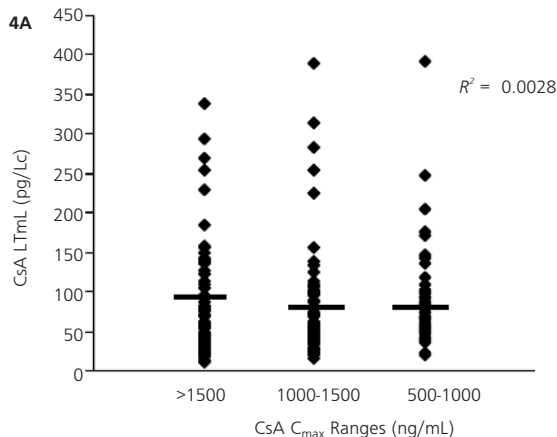


Figure 4: Significant interpatient variability in (A) cyclosporine (CsA) lymphocyte maximum level (LTmL) within each CsA blood maximum concentration (C_{max}) range and in (B) tacrolimus (TAC) lymphocyte trough level (LT₀L) within each TAC dosage. These observations explain the intra- and interindividual variability in therapeutic response and the lack of correlation between drug dosage and blood levels and lymphocyte content and therefore, between bioavailability (blood or plasma levels) and immune responsiveness (bioactivity). The bar (—) in Figure 4A represents the mean CsA LTmL within each CsA C_{max} range. These values were comparable irrespective of C_{max} ranges (Reference Nos. 14, 15).

This poor relationship clearly indicates that bioavailability and bioactivity are not bioequivalent. Variations between these 2 factors are related mainly to genetic, ethnic, and environmental parameters such as age, sex, body mass index, organ function, food and subsequent drug absorption, drug interactions, and the availability of extra-target-cell binding sites (23-25). These parameters seem to influence all the steps involved in the mode of action of any immunosuppressive agent (including drug absorption, metabolism, elimination, transport, extra-target-cell binding sites) as well as target-cell P-glycoprotein efflux pump expression, receptor affinity, and specific enzyme inhibition (Figure 5), which explains the existence of several recipient profiles with regard to drug absorption, metabolism, transport, and cellular binding. Therefore, the intracellular fraction of a drug depends not only on the amount of free drug available in the blood (extracellular compartment) but also on the availability of extra-lymphocytic binding sites

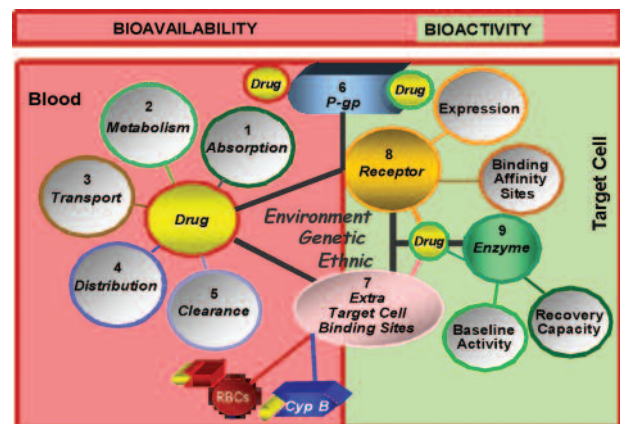


Figure 5: Genetic, ethnic, and environmental factors such as age, sex, body mass index, organ function, food and subsequent drug absorption, drugs interaction, target cell P-glycoprotein (P-gp) efflux pump activity, and availability of extra-target-cell binding sites such as red blood cell mass (RBC) and cyclophilin B (CyP B) (in the case of cyclosporine) seem to influence all of the modes of action of any immunosuppressive drug ranging from its oral absorption to its entry inside the cell and its binding to the specific enzyme (1 to 9). Lymphocyte P-gp activity (6), extra-target-cell binding site such as CyP B level (7), and receptor binding site expression and affinity (8), in addition to the specific enzyme baseline activity and its capacity for recovery (9), are genetically controlled. Intestinal absorption (1), metabolism (2), transport (3), tissue distribution (4), and hepatic and/or renal elimination (5) of a drug are major determinants of its bioavailability routinely assessed by pharmacokinetic measurements of either whole blood or plasma levels. Conversely, bioactivity (biological effect of a drug) depends mainly on intracellular concentration and the target enzyme baseline activity (9). The cellular fraction of a free drug (target cell concentration) is determined not only by the amount of free drug present in the extralymphocytic compartment in the blood (1-5), but also by the P-gp activity (6), the availability of extra-target-cell binding sites (7), and receptor binding site expression and affinity (8). This complex interaction between these different parameters results in an obvious discordance between bioavailability and bioactivity and hence, in their lack of bioequivalence.

(cyclophilin B and red blood cell mass for cyclosporine), the activity of the lymphocyte P-glycoprotein pump, and receptor expression and its drug-binding affinity, which are all affected by genetic and environmental factors (Figure 5). Any variation in lymphocyte P-glycoprotein activity and/or redistribution of the drug between the different binding sites (eg, red blood cell mass [Figure 6] and cyclophilin B and lymphocyte mass) will ultimately affect the cellular concentration of the drug and hence, its bioactivity, with no change in its extracellular bioavailability (12, 26-29).

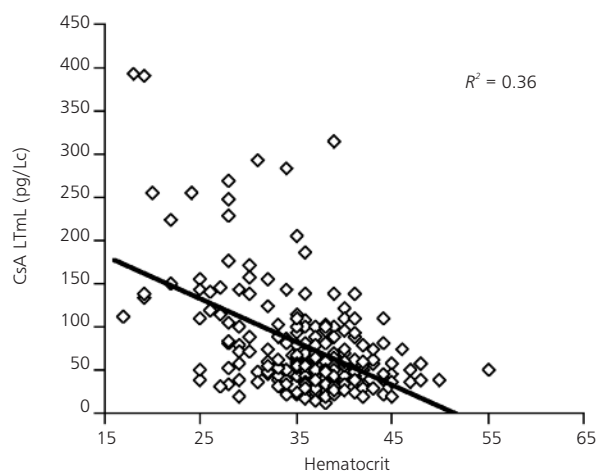


Figure 6: Relationship between hematocrit and cyclosporine (CsA) lymphocyte maximum levels (LTmL) in CsA-treated patients. Low RBC mass (hematocrit) seems to adversely affect the cellular content of CsA by facilitating its extrusion from the lymphocyte (Reference No. 29).

Any increase in cyclophilin B and/or a decrease in hematocrit and/or lymphocyte P-glycoprotein activity will enhance cyclosporine entry into the lymphocyte and hence, its bioactivity, with no change in its extracellular bioavailability. Conversely, any decrease in cyclophilin B and/or increase in red blood cell mass and/or lymphocyte P-glycoprotein activity will facilitate extrusion of cyclosporine from the lymphocyte and hence, will reduce its bioactivity with no change in its extracellular bioavailability. Other genetic and environmental combinations are possible as well and may influence the intracellular concentration of a drug in one way or another. Therefore, the ultimate net intracellular amount of a drug at a fixed dosage is the net result of the integration of both out-fluxing and in-fluxing forces that are influenced by genetic (50%) and environmental factors (50%).

We recently suggested that cyclosporine-induced nephrotoxicity is predominantly donor dependent with a partial recipient influence related to drug bioavailability and exposure (8, 12, 13, 30). Hauser

and associates recently confirmed this hypothesis by establishing that the ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine nephrotoxicity after renal transplant (7). Expression of the TT genotype in the donor at the ABCB1 3435C→T polymorphism, which is associated with decreased expression of the P-glycoprotein pump in renal tissue that extrudes cyclosporine from renal cells, becomes a major risk factor for developing cyclosporine nephrotoxicity. Evidently, this nephrotoxicity is amplified and exaggerated by higher drug exposure and hence, a greater bioavailability that is solely recipient dependent. Interestingly, the effect of this specific genotype expression on cyclosporine lymphocyte content and hence, on immune responsiveness in the recipient, remains unknown. These findings are in agreement with our own observations and those of others (3, 7, 8, 12, 13, 16) on the poor correlation between cyclosporine nephrotoxicity, which is donor dependent, and cyclosporine monitoring tools (ie, blood and lymphocyte levels), which are recipient related. These interesting observations explain the intra- and interindividual variabilities in therapeutic response and adverse reactions and hence, the poor correlation between pharmacogenetics assessing recipient bioavailability and chronic allograft nephropathy, which is both donor and recipient related.

It is currently estimated that genetic factors account for nearly half of the individual variations in the efficacy and toxicity of drugs (7, 31-33). These genetic factors could explain the differences (despite their similar designs) between the 2- and 3-continent trials, in which calcineurin inhibitors were withdrawn and sirolimus was added (34, 35), in the reported incidences of biopsy-proven acute rejection in the calcineurin-inhibitor-sparing groups. These genetic factors also may explain the higher incidence of hyperlipidemia and the use of lipid-lowering agents in sirolimus-maintenance groups observed mainly in 2 other calcineurin-inhibitor-sparing studies conducted in Spain and Italy (36, 37). These findings are in agreement with our own observations (38) on the relatively high rate of adverse events (mainly mixed hyperlipidemia) in our sirolimus-treated patients. Given the importance of these genetic and environmental factors and their significant potential impact on clinical outcome, using the newly emerging disciplines of pharmacogenetics and pharmacogenomics, it is essential to account for them when designing future trials (39) (31-33). This also should caution against extrapolating universal recommendations based on

studies conducted in a specific geographic area or in a population of a particular ethnic background. Conducting similar studies in different areas of the world is crucial in today's era of applied clinical practice according to evidence-based medicine. Given the strong correlation between drug lymphocyte content and clinical outcome (mainly acute rejection and immune responsiveness and the potential impact of extra-target-binding sites and the P-glycoprotein pump expression on drug lymphocyte content in the recipient that could significantly affect bioactivity without changing extracellular bioavailability), monitoring immunosuppressive drugs at their target cells becomes that much more clinically relevant and hence, more appropriate than monitoring the drugs in blood or plasma.

This type of specific monitoring at the site of action is easily applicable in clinical practice, cost-effective, and could be used for all immunosuppressant medications. Since it allows maximal reduction of adverse effects through dosage reduction, while maintaining an optimal level of immunosuppression, it offers a new alternative for immunosuppressive therapy monitoring and tailoring to the individual patient. The validity of this novel approach should be confirmed through larger, multicentered, multinational trials.

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