Trends in Transplant. 2008;2:107-16

Impact of Pharmacogenetics and Pharmacodynamics on Transplantation

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Abstract

In solid organ transplantation, new approaches able to reflect individual responses are required to monitor immunosuppressive therapy and to improve its safety and efficacy. In the last few years, preliminary studies about the clinical impact of the measurement of new specific biomarkers of exposure (pharmacogenetic biomarkers) and the biological effects (pharmacodynamic biomarkers) of immunosuppressive drugs have been carried out. Pharmacogenetics, by the association between some polymorphisms and drug dose requirement, combined with pharmacokinetic drug monitoring allows therapeutic concentrations to be achieved a few days after transplantation. However, there is also a need for pharmacodynamic monitoring that may reflect the degree of immunosuppression achieved in each patient. This pharmacodynamic measurement is based on the analysis of specific biomarkers strongly related with the mechanism of action of every immunosuppressive agent, and shows its immunomodulatory effect on T-cell response. The present article reviews some pharmacogenetic and pharmacodynamic biomarkers that have been proposed in solid organ transplant monitoring. **(Trends in Transplant. 2008;2:107-16)**

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Key words

Immunosuppression. Biomarkers. Pharmacodynamics. Polymorphism. Pharmacogenetics. Transplantation.

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Introduction

The strategy used to prevent the toxicity associated with immunosuppressive therapy in solid organ transplant recipients is based on measuring drug blood levels to ensure that concentrations within the established therapeutic range are reached. However, this strategy is insufficient to determine individual responses or to tailor the dose to real requirements. Consequently, new approaches able to reflect individual responses are required to monitor immunosuppressive therapy and to improve safety and efficacy. In the last few years, preliminary studies of new specific biomarkers of exposure (pharmacogenetic biomarkers) and the biological effects of (pharmacodynamic biomarkers) immunosuppressive drugs have been carried out.

In 1959, Vogel proposed the term "pharmacogenetics" to describe the discipline that studies the genetically determined variations that explain interindividual differences in drug response. Idiosyncrasy and pharmacologic intolerance have a genetic origin and can also be influenced by environmental factors.

Pharmacogenomics and pharmacogenetics are areas of clinical research that are under constant development and are highly topical. Pharmacogenetic studies allow the possible genetic causes of differences in individual responses to a drug to be identified, since these studies investigate the association between the degree of response to a drug and the polymorphisms of genes involved in drug metabolism and transport.

Moreover, some genetic polymorphisms can affect important organic functions, such as the metabolism and regulation of drug transport and drug distribution in the body, that is, some polymorphisms are closely associated with drug exposure and biological effect.

This article focuses on the study of the genes codifying for proteins that act as metabolizing enzymes or as transport proteins for immunosuppressive drugs, as these genes influence the pharmacokinetics of these drugs. Immunosuppressive drugs, which include calcineurin activity inhibitors, mammalian target of rapamycin (mTOR) activity inhibitors and inosine monophosphate dehydrogenase (IMPDH) activity inhibitors, regulate the immune response against the allograft and are strongly able to reduce the incidence of acute rejection. However, there is wide variability in individual response to these drugs, indicating that genetic factors may play an important role in this phenomenon and may also reflect different needs in individual patients.

In transplantation, pharmacogenetics is a helpful tool for the choice of starting dose at treatment initiation. Combined with pharmacokinetic analysis, this tool could allow therapeutic concentrations to be reached as quickly as possible. During the early posttransplant period, optimal drug exposure in the shortest time possible is required to prevent acute rejection of the transplanted organ. Preliminary studies have identified several genetic polymorphisms related to metabolizing enzymes and transport regulatory proteins, which are relevant to interindividual variability in pharmacokinetics and pharmacodynamics in response to immunosuppressive drugs.

Previous studies of immunosuppressive drugs have demonstrated the association between the CYP3A5*1 and CYP3A*1B polymorphisms and dose requirement of tacrolimus[1], as well as the association between the UGTA9*3, UGT1A9 -275T>A and UGT1A9 -2152C>T polymorphisms and mycophenolic acid (MPA) exposure in transplant recipients[1,2]. Moreover, other studies have associated the transport regulatory protein, multidrug resistance (MDR)-1, with the risk of toxicity[3,4].

To achieve personalized therapy, immunosuppression monitoring is required. Pharmacogenetics and pharmacokinetics allow

therapeutic concentrations to be achieved a few days after transplantation, but there is also a need for pharmacodynamic monitoring based on analysis of specific biomarkers of the effect of immunosuppressive drugs on T-cell response.

Several strategies can be used to evaluate the biological impact of immunosuppressive drugs on the immune system, depending on their mechanism of action. An initial approach, probably the most specific, is based on target enzyme activity measurement for each immunosuppressive drug (calcineurin activity for cyclosporine and tacrolimus, IMPDH activity for mofetil mycophenolate, and P70S6 kinase activity for mTOR inhibitors, sirolimus and everolimus). Another approach is to evaluate an intermediate involved in the drug's mechanism of action (e.g. interleukin synthesis, lymphocyte proliferation, T-cell activity). Finally, biomarkers that reflect the collateral effects induced by immunosuppressive drugs, but which are unrelated to their mechanism of action (e.g. lymphocyte surface antigens expression) can be evaluated.

The optimal biological matrix to determine pharmacodynamic biomarkers depends on the methodology used; for example, the enzyme-linked immunosorbent spot (ELISPOT) assay requires peripheral blood lymphocytes, not whole blood. Additionally, there is a tendency to use whole blood instead of peripheral blood lymphocytes, especially since the introduction of techniques based on flow cytometry. These whole blood assays are faster, require less sample manipulation, and need a smaller sample volume than methods requiring lymphocyte purification. Furthermore, immunosuppressive drug level is currently evaluated in whole blood, except in MPA monitoring which is assessed in plasma. Monitoring in whole blood is the optimal situation to establish correlations between a drug's concentration and its effect.

In the last few years, different immunologic parameters have been evaluated as biological biomarkers of the immunosuppressant effect of these drugs. Research is being carried out to correlate pharmacokinetics and pharmacodynamics, as well as to identify the biomarkers that most effectively predict clinical events (rejection, infection, etc.).

Pharmacogenetic biomarkers

Influence of pharmacogenetics on immunosuppressant metabolic disposition and dose requirement

CYP3A polymorphisms

The promising role of pharmacogenetics in the discovery of gene polymorphisms of metabolizing enzymes and transporters of immunosuppressive drugs may help to better select the different drugs involved in the treatment and their appropriate initial dose.

Results from some studies in kidney transplant recipients demonstrate that there is a strong association between CYP3A5*1/*3 and *1/*1 genotypes and tacrolimus pharmacokinetics and dose requirement[5-8]. Knowledge from these studies shows that patients with CYP3A5*3/*3 genotype require low tacrolimus dose to achieve target concentrations compared to CYP3A5*1 allele carriers.

Concerning CYP3A4 polymorphisms, there is a notable controversy. The CYP3A4*1B carriers require more tacrolimus to reach therapeutic concentrations compared to patients carrying CYP3A4*1/*1 genotype[5], but several studies point out that there is no association between CYP3A4*1B/*1 genotype and tacrolimus requirement[9,10].

Results from our study[2] in 123 kidney transplant recipients demonstrate that patients

carrying the CYP3A4*1B allele required higher tacrolimus doses to achieve target levels, and showed significantly reduced minimum concentration (C_0) dose-adjusted or area under the curve (AUC) dose-adjusted values from months 6-12 after transplantation. With reference to CYP3A5 genotype, our results completely agree with previous studies and demonstrate the significant association between CYP3A5 *1/*1 or CYP3A5 *1/*3 genotypes and higher tacrolimus dose requirement during all the period of treatment (Fig. 1).

With respect to cyclosporine (CsA), most of the previous studies have shown a lack of correlation between the CsA dose requirement or CsA trough dose-adjusted concentrations and the presence of the CYP3A4*1B or CYP3A5*3 allele in kidney transplant patients[5,11,12]. These studies concluded that genotyping of transplant recipients for CYP3A4 or CYP3A5 is thus unlikely to assist in selecting the best initial CsA dose.

The discrepancy observed between the role played by CYP3As polymorphisms in the effects on tacrolimus and CsA metabolism and disposition is not well understood, but may be explained by the more complex pharmacokinetic profile of CsA, which needs to achieve higher concentrations (100-250 ng/ml) and may interact with many other drugs.

Regarding sirolimus, results from different studies have demonstrate that there is a significant association between sirolimus concentration/dose ratio and CYP3As polymorphisms[13,14]. A lower sirolimus concentration/dose ratio was observed in the CYP3A5*1 carriers (*1/*3 or *1/*1) than in the CYP3A5*3/*3 carriers, suggesting that CYP3A5 non-expressors require lower sirolimus dose to achieve therapeutic concentrations. There is also an association between the CYP3A4*1B polymorphism and higher sirolimus requirement[15-17].

Concerning the frequency of CYP3A5 genotypes, results from different studies in kidney and pediatric and adult liver transplant recipients are summarized in table 1[18-21].

UGT1A9 polymorphisms

Previous studies about the impact of UGT1A9 polymorphisms on MPA exposure in *de novo* renal allograft recipients demonstrate that carriers of UGT1A9*3 allele had higher MPA exposure[1,22], whereas the -275T>A and -2152C>T single-nucleotide polymorphisms (SNP) of the UGT1A9 gene promoter region are associated with significantly lower MPA exposure in renal transplant recipients receiving 1 g twice daily of mycophenolate mofetil (MMF)[23]. Part of this effect may be explained by the interruption of enterohepatic recirculation of MPA. On the other hand, it has recently been shown that the UGT2B7 -840G>A SNP is associated with an increase in acyl mycophenolic acid glucuronide metabolite concentrations[1,23].

In our study in kidney transplanted patients[2], the -275T>A and -2152C>T SNP of the UGT1A9 gene promoter region were associated with significantly lower MPA exposure that was statistically significant only for those patients receiving 1 g/day of MMF during the first month after transplantation, whereas MPA exposure was similar in carriers and non-carriers from months 3-12 after transplantation. A possible explanation for that is to consider the significant increase of MPA concentration (about 35% from months 1-3) which may saturate UGT1A9 enzymes in carriers and non-carriers.

Table 2 summarizes the frequency of CYP3A and UGT1A9 genotypes in Caucasian transplanted patients.

P-glycoprotein (MDR1)

Significant interindividual variations in the expression and function of P-glycoprotein (P-gp) may be the result of genetic polymorphisms.

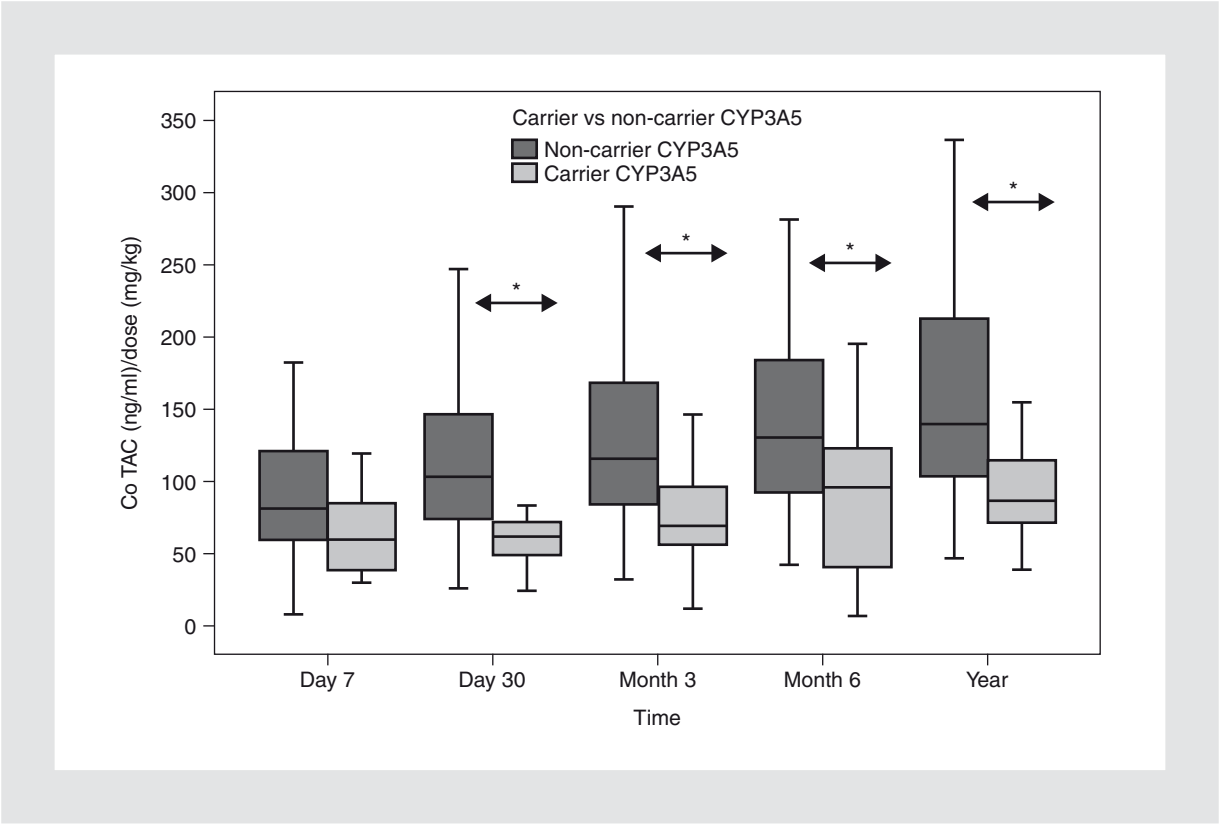

Figure 1. *Follow-up from week 1 to month 12 posttransplantation of Dose-adjusted TAC trough concentration (C0/Dose) and CYP3A5*1 polymorphism. A significant difference ($p < 0.05$) in C_0/dose was observed between CYP3A5*1 carriers and non-carriers from months 1 to 12 after transplantation[2].*

Cyclosporin A is a substrate of P-gp that, when it achieves therapeutic concentrations, acts as a potent inhibitor of the P-gp function. This pharmacologic inhibition may limit the potential impact of the genetic variations in CsA disposition. In fact, the results from most studies demonstrate that there is a lack of strong correlation between cyclosporine oral bioavailability and MDR1 3435C>T polymorphism[5,11]. The results from Thervet, et al.[15] show, in stable Caucasian renal transplant recipients, a weak association between CsA exposure and MDR1 1236C>T SNP.

Concerning tacrolimus and P-gp, some studies found that tacrolimus dose requirement was lower in patients who had one or two mutant alleles in their exon 12, 21, and 26 SNP of MDR1[15,24]. More interestingly, results from liver transplant recipients receiving tacrolimus demonstrated that there was a strong correlation between a higher intestinal mRNA expression of the MDR1 gene and the incidence of acute rejection[19,20,25]. In these studies in pediatric and adult liver transplant recipients, patients who strongly expressed MDR1 showed a high incidence of tacrolimus < 7 ng/ml four days after transplantation. These patients required higher tacrolimus doses to achieve, as soon as possible, adequate tacrolimus concentrations (> 7 ng/ml) in the first days after transplantation.

Regarding the clinical impact of the MDR1 gene, the role of the MDR1 donor genotype has been evaluated with reference to the incidence of nephrotoxicity. The results demonstrate that the 3435T>T polymorphism of kidney donors was significantly overexpressed in those patients with CsA nephrotoxicity[3].

Table 1. Summary of results obtained in different studies about incidence of CYP3A5 polymorphisms in liver and kidney transplant recipients

Reference	Solid Organ Transplant	Ethnic Background	n	CYP3A5 (%)		
				*1/*1	*1/*3	3/3
Wei-lin W, et al., Liver Transplant. 2006;12(5):775	Adult liver	Chinese	50	13	50	37
Yu S et al., Transplantation. 2006;81:46	Adult liver	Chinese	100	11,3	50,9	37,8
Goto M, et al., Pharmacogenetics. 2004;14(7):471	Adult liver	Japanese	70	3	38	59
Fukudo M, et al., Clin Pharmacol Ther. 2006;80:331	Paediatric liver	Japanese	130	3	38	58
Hesselink DA, et al., Clin Pharmacol Ther. 2003;74:245	Adult Kidney	Caucasian	100	1,6	17,5	81
Bach, et al., Pharmacogenetics. (Submitted)	Adult Kidney	Caucasian	125	2	18	80
Mourad M, et al., Clin Chem Lab Med. 2006;44(10):1192	Adult Kidney	Caucasian	90	1	19	80

Pharmacodynamic biomarkers

Biomarkers of T-cell response

Lymphocyte proliferation and T-cell surface antigens

Since all currently administered immunosuppressive drugs can inhibit lymphocyte proliferation, pharmacodynamic assays to evaluate this parameter are frequently used as a general biomarker of the degree of immunosuppression achieved. Moreover, surface lymphocyte antigens are currently being evaluated as possible biomarkers. These actively participate in the different signals produced in the activation and clonal expansion of T-lymphocytes, especially in co-stimulation, adhesion, and apoptosis.

Stalder, et al.[26] compared lymphocyte proliferation and T-cell activation antigen expression in stable renal transplant recipients at six months posttransplantation and in healthy normal controls. All transplanted patients were treated with CsA, MMF and prednisone. Lymphocyte proliferation, evaluated by proliferating cell nuclear antigen (PCNA) expression, was significantly inhibited in transplanted patients in comparison with that in healthy normal controls. Similarly, the different surface antigens evaluated were decreased in the group of transplant recipients in comparison with the control group. The most affected surface antigens were CD11a and CD154, whose expression in transplanted patients was 25% of control group expression. The CD25, CD71 and CD95 expression in transplanted patients represented approximately 50% of the expression obtained in the control group.

Table 2. Frequency distribution of CYP3A and UGT1A9 polymorphisms studied in renal allograft recipients[2]

n = 123	Single-nucleotide polymorphism					
	CYP3A4	CYP3A5	UGT1A9*3	T-275A	C-2152T	T-275 and C-2152T
Noncarriers	115 (93,5%)	1 (<1%)	120 (97,5%)	105 (85,4%)	107 (87%)	NA
Total No. Of carriers	8 (6,5)	122 (99,1%)	3 (2,4%)	18 (14,6%)	16 (13%)	14
Heterozygous carriers (1/2)	7 (5,7%)	18 (14,6%)	3 (2,4%)	18 (14,6%)	16 (13%)	14
Homozygous carriers (2/2)	1 (<1%)	104 (84,5%)	0	0	0	0

Barten, et al.[27] recently published a study where the analysis of biomarkers of the immunosuppressive effect, in a population of cardiac transplant recipients, revealed that a combination of very low concentrations of calcineurin activity inhibitors (CsA or tacrolimus) and very low sirolimus concentrations can produce synergism of action for T-cell proliferation inhibition (measured by PCNA expression) or interleukin 2 (IL-2)- specific receptor expression (CD25). The potential of surface lymphocyte antigen analysis was shown in a study by Chang, et al.[28] in cardiac transplant recipients in which $CD4^+CD25^+$ T-cell expression, in contrast to cytokine expression, was related to the degree of rejection.

Our group[29] evaluated inhibition of lymphocyte proliferation, among other biomarkers, in a stable transplant recipient population treated with sirolimus monotherapy that showed low intrapatient variability for sirolimus C_0. Although all patients had sirolimus concentrations inside the therapeutic range (8-12 ng/ml), the results obtained for this biomarker demonstrated wide interindividual variability, with an average inhibition value of 60% (range 31-96%). This finding, which is common in other biomarkers, revealed the importance of pharmacokinetic and pharmacodynamic monitoring of transplanted patients in order to tailor the dose to each individual patient.

Soluble cytokines

Several research groups have evaluated cytokine synthesis as a biomarker, even though monitoring of cytokine production is difficult, especially that of circulating cytokines. These substances have different half lives and their gene expression is regulated through the cell cycle phases.

The pattern of T-helper Th1/Th2 cytokine expression has been studied by several groups. Barten, et al.[30] found that Th1/Th2 cytokine levels measured by cytometric bead array differed significantly in cardiac transplant recipients treated with cyclosporine and MMF in comparison with pretransplantation levels. Moreover, cytokine levels were significantly reduced at two hours after immunosuppressive drug administration. Interleukin-2 and IL-4, which are highly synthesized in the early phases of T-cell activation and during acute rejection, were significantly reduced. After cardiac transplantation, inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) or IL-6, which regulate T-cell clonal expansion and activation during immunologic response, were increased in comparison with pretransplantation levels. For interferon gamma (IFNγ) and IL-10, no differences were found before and after transplantation.

In a study carried out by our group[31] in renal transplant recipients treated with a combination of tacrolimus and MMF or CsA and MMF, pharmacodynamic monitoring showed synergism in the immunosuppressive action of MPA combined with calcineurin inhibitors, since the decrease in IL-2 was significantly more important in patients treated with both drugs than in those treated with monotherapy.

In a recently published study of a renal transplanted population in the maintenance phase treated with sirolimus monotherapy, our group[29] found that patients with a higher risk of infection had significantly lower IL-10 levels than those free from infection and healthy controls. Inhibition of IL-10 in patients treated with sirolimus has been associated with a higher risk of bacterial infection, hyper-inflammation and sepsis, as well as with a decrease in cytolytic activity and, consequently, a lower incidence of rejection[32].

Intra-lymphocyte cytokines

Interest in determining which types of cell subpopulations synthesize specific cytokines is growing because the cell type can determine the immune response.

A study by Ahmed, et al.[33] demonstrated that the frequency of $CD8^+$ and $CD8^-$ cells that synthesize IL-2 and IFNγ correlates with the biological effect of tacrolimus. These authors found that the biological matrix chosen to analyze these biomarkers was whole blood (previously, peripheral blood mononuclear cells were mainly used) because whole blood allows the environment in which the drug usually acts to be preserved.

Results from a study of Boleslawski, et al.[34] that evaluated 21 liver transplant recipients treated with CsA or tacrolimus showed that, unlike blood concentration of tacrolimus, the biomarker that reflects the percentage of $CD3^+CD8^+$ T-cells that synthesize IL-2 is closely related to the onset of acute rejection, especially in patients treated with tacrolimus and prednisone. Furthermore, determination of this biomarker before transplantation showed that patients with a higher percentage $CD8^+IL\text{-}2^+$ were those who later developed acute rejection. This finding suggests that IL-2 production in these patients could be constitutively higher.

The lack of correlation between drug levels and these intra-lymphocyte biomarkers might confirm the finding that immunosuppressive drugs produce different effects in different patients, even when levels and doses are similar.

Biomarkers of tolerance

Some transplant recipients achieve a state of immune tolerance spontaneously after reducing immunosuppressive treatment until total suppression (spontaneous operational tolerance). This situation has been more frequently observed in liver transplant recipients (about 20% would be tolerant) than in other solid organ transplants.

Identification of biomarkers could allow strategies to induce tolerance to be developed, which would allow immunosuppressive treatment and associated toxicity to be reduced safely in these patients. Regulatory lymphocytes co-expressing the surface antigens CD4 and CD25 are the best-known regulatory subtype. Regulatory T-cells (T_{reg}) suppress immune activation and play a major role in maintaining tolerance. Forkhead box protein 3 (FoxP3), a transcription factor highly expressed in this cell population, is decisive in the development of these cells.

Consequently, some studies have evaluated the frequency of $CD4^+CD25^{high}FoxP3^+$ as a tolerance biomarker in transplanted patients after gradually withdrawing immunosuppressive treatment. Braudeau, et al.[35] identified operational tolerance in a population in which this tolerance is hard to find: renal transplant recipients free of immunosuppressive treatment who were compared with a group of stable renal transplant recipients. The results obtained showed a reduction in FoxP3 transcripts and $CD4^+CD25^+$ T-cells in patients with chronic rejection in comparison with patients free of immunosuppressive treatment.

Similar results have been found in liver transplant recipients. San Segundo, et al.[36] evaluated the effect of different immunosuppressive drugs on the $CD4^+CD25^{high}FoxP3^+$ T-cell percentage in a population of stable renal transplant recipients. These authors observed that in contrast to sirolimus, anti-calcineurin drugs (CsA, tacrolimus) reduce the number of circulating T_{reg} cells. Therefore, these authors suggested that quantification of T_{reg} blood levels could be useful to identify transplanted patients susceptible to immunosuppression reduction.

Conclusions

It is currently known that immunosuppressive treatment in solid organ transplant recipients must be individually tailored to each patient. Consequently, monitoring of this treatment in the near future should not only be based on pharmacokinetics, which is highly useful to ensure that concentrations are within the established therapeutic range for each drug, but should also be based on the patient's pharmacogenetic characteristics (the presence of polymorphisms in the metabolizing enzymes of these drugs), which would be useful in selecting drugs and their initial doses. Besides, evaluation of biomarkers reflecting the real effect of immunosuppressive drugs and tolerance biomarkers would allow candidates for reduction or even withdrawal of immunosuppressive treatment to be identified.

Based on the preliminary studies performed to date on biomarkers, no conclusions can be drawn on which biomarkers are the most appropriate to prevent rejection or adverse effects, for three main reasons: (i) the studies included a small number of patients; (ii) there were no standardized analytic protocols to analyze biomarkers, thus hampering comparison of the results obtained in the different centers; and (iii) these studies were usually performed at specific time-points which did not allow changes in these biomarkers throughout treatment to be evaluated (sequential pharmacodynamic monitoring). For all these reasons, multicenter trials with a large number of patients are required. Such trials would allow the results of previous studies to be confirmed and the most effective biomarkers to reflect the effect of immunosuppressive drugs on the immune system to be chosen. Furthermore, the most important clinical events that can occur after transplantation (acute rejection, chronic nephropathy, infection, toxicity) could be predicted.

Acknowledgments

CIBEREHD is funded by the Instituto de Salud Carlos III.

References

1. Thervet E, Anglicheau D, King B, et al. Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. Transplantation. 2003;76:1233-5.
2. Bach V, Campistol JM, Arnedo T, et al. Impact of CYP3A5, CYP3A4, and UGT1A9 polymorphisms on tacrolimus and mycophenolic acid exposure in *de novo* renal transplant recipients: correlation with incidence of acute rejection and nephrotoxicity. Pharmacogenet Genomics. [Submitted].
3. Hauser IA, Schaeffeler E, Gauer S, et al. ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. J Am Soc Nephrol. 2005;16:1501-11.
4. Anglicheau D, Pallet N, Rabant M, et al. Role of P-glycoprotein in cyclosporine cytotoxicity in the cyclosporine-sirolimus interaction. Kidney Int. 2006;70:1019-25.
5. Hesselink DA, van Schaik RH, van der Heiden I, et al. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. Clin Pharmacol Ther. 2003;74:245-54.
6. Tsuchiya N, Satoh S, Tada H, et al. Influence of CYP3A5 and MDR1 (ABCB1) polymorphisms on the pharmacokinetics of tacrolimus in renal transplant recipients. Transplantation. 2004;78:1182-7.
7. Zhang, X., Liu, Z. H., Zheng, J. M. et al. Influence of CYP3A5 and MDR1 polymorphisms on tacrolimus concentration in the early stage after renal transplantation. Clin Transplant. 2005;19:638-43.
8. Mourad M, Wallemacq P, De Meyer M, et al. The influence of genetic polymorphisms of cytochrome P450 3A5 and ABCB1 on starting dose- and weight-standardized tacrolimus trough concentrations after kidney transplantation in relation to renal function. Clin Chem Lab Med. 2006;44:1192-8.
9. Roy JN, Barama A, Poirier C, Vinet B, Roger M. Cyp3A4, Cyp3A5, and MDR-1 genetic influences on tacrolimus pharmacokinetics in renal transplant recipients. Pharmacogenet Genomics. 2006;16:659-65.
10. Op den Buijsch RA, Christiaans MH, Stolk LM, et al. Tacrolimus pharmacokinetics and pharmacogenetics: influence of adenosine triphosphate-binding cassette B1 (ABCB1) and cytochrome (CYP) 3A polymorphisms. Fundam Clin Pharmacol. 2007;21:427-35.
11. Yates CR, Zhang W, Song P, et al. The effect of CYP3A5 and MDR1 polymorphic expression on cyclosporine oral

disposition in renal transplant patients. J Clin Pharmacol. 2003;43:555-64.
12. Min DI, Ellingrod VL. Association of the CYP3A4**1B 5'-flanking region polymorphism with cyclosporine pharmacokinetics in healthy subjects. Ther Drug Monit. 2003;25:305-9.*
13. Lampen A, Zhang Y, Hackbarth I, Benet LZ, Sewing KF, Christians U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. J Pharmacol Exp Ther. 1998;285:1104-12.
14. Crowe A, Lemaire M. In vitro and in situ absorption of SDZ-RAD using a human intestinal cell line (Caco-2) and a single pass perfusion model in rats: comparison with rapamycin. Pharm Res. 1998;15:1666-72.
15. Thervet E, Anglicheau D, Legendre C, Beaune P. Role of pharmacogenetics of immunosuppressive drugs in organ transplantation. Ther Drug Monit. 2008;30:143-50. ***This is a revision of many pharmacogenetic studies of immunosuppressive drugs.*
16. Marquet P, Djebli N, Picard N. Pharmacogenetics and immunosuppressor drugs: impact and clinical interest in transplantation. Ann Pharm Fr. 2007;65:382-9.
17. Mourad M, Mourad G, Wallemacq P, et al. Sirolimus and tacrolimus trough concentrations and dose requirements after kidney transplantation in relation to CYP3A5 and MDR1 polymorphisms and steroids. Transplantation. 2005; 80:977-84.
18. Wei-lin W, Jing J, Shu-sen Z, et al. Tacrolimus dose requirement in relation to donor and recipient ABCB1 and CYP3A5 gene polymorphisms in Chinese liver transplant patients. Liver Transpl. 2006;12:775-80.
19. Goto M, Masuda S, Kiuchi T, et al. CYP3A5**1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. Pharmacogenetics. 2004;14:471-8.*
20. Fukudo M, Yano I, Masuda S. et al. Population pharmacokinetic and pharmacogenomic analysis of tacrolimus in pediatric living-donor liver transplant recipients. Clin Pharmacol Ther. 2006;80:331-45.
21. Yu S, Wu L, Jin J, et al. Influence of CYP3A5 gene polymorphisms of donor rather than recipient to tacrolimus individual dose requirement in liver transplantation. Transplantation. 2006;81:46-51.
22. Levesque E, Delage R, Benoit-Biancamano MO, et al. The impact of UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in healthy volunteers. Clin Pharmacol Ther. 2007;81:392-400.
23. Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early MPA dose-interval exposure in de novo renal allograft recipients. Clin Pharmacol Ther. 2005;78:351-61.
24. Macphee IA, Fredericks S, Tai T, et al. Tacrolimus pharmacogenetics: polymorphisms associated with expression of cytochrome P4503A5 and P-glycoprotein correlate with dose requirement. Transplantation. 2002;74:1486-9.
25. Masuda S, Goto M, Fukatsu S, et al. Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients. Clin Pharmacol Ther. 2006;79:90-102.
26. Stalder M, Birsan T, Holm B, Haririfar M, Scandling J, Morris RE. Quantification of immunosuppression by flow cytometry in stable renal transplant recipients. Ther Drug Monit. 2003; 25:22-7.
27. Barten MJ, Tarnok A, Garbade J. et al. Pharmacodynamics of T-cell function for monitoring immunosuppression. Cell Prolif. 2007;40:50-63. ***This article reflects the clinical impact of biomarkers of immunosuppression monitoring and describes some methodologies of interest.*
28. Chang DM, Ding YA, Kuo SY, Chang ML, Wei J. Cytokines and cell surface markers in prediction of cardiac allograft rejection. Immunol Invest. 1996;25:13-21.
29. Brunet M, Campistol JM, Diekmann F, Guillen D, Millan O. T-cell function monitoring in stable renal transplant patients treated with sirolimus monotherapy. Mol Diagn Ther. 2007; 11:247-56.
30. Barten MJ, Rahmel A, Bocsi J, et al. Cytokine analysis to predict immunosuppression. Cytometry A. 2006;69:155-7.
31. Millan O, Brunet M, Campistol JM, et al. Pharmacodynamic approach to immunosuppressive therapies using CNI and MMF. Clin Chem. 2003;49:1891-9.
32. Jorgensen PF, Wang JE, Almlof M, et al. Sirolimus interferes with the innate response to bacterial products in human whole blood by attenuation of IL-10 production. Scand J Immunol. 2001;53:184-91.
33. Ahmed M, Venkataraman R, Logar AJ, et al. Quantitation of immunosuppression by tacrolimus using flow cytometric analysis of IL-2 and IFNγ inhibition in CD8(-) and CD8(+) peripheral blood T-cells. Ther Drug Monit. 2001;23:354-62.
34. Boleslawski E, Conti F, Sanquer S, et al. Defective inhibition of peripheral CD8+ T cell IL-2 production by anti-calcineurin drugs during acute liver allograft rejection. Transplantation. 2004;77:1815-20.
35. Braudeau C, Racape M, Giral M, et al. Variation in numbers of $CD4^+CD25^{high}FOXP3^+$ T-cells with normal immuno-regulatory properties in long-term graft outcome. Transpl Int. 2007;20:845-55.
36. Segundo DS, Ruiz JC, Izquierdo M, et al. Calcineurin inhibitors, but not rapamycin, reduce percentages of $CD4^+CD25^+FOXP3^+$ regulatory T-cells in renal transplant recipients. Transplantation. 2006;82:550-7.