absorption, it also inhibits 1-alpha-hydroxylase and results in lower calcitriol synthesis.

CKD progression, the decrease in FGF 23's phosphaturic action and lower vitamin D rate produce a situation of resistance to FGF 23's phosphaturic action. As a result, the P's plasmatic rate increases, which upregulates the gene that codes for PTH through a "P sensor" in the parathyroid glands. Furthermore, in this hyperphosphataemia situation, extracellular P is transported by the co-transporter Pit-1 to the intracellular compartments, and once there, it acts as an indicator of an increase in mineral nucleation expression agents.[5]

Deficiency in vitamin D contributes to this process, given that Th1 lymphocyte action cannot be inhibited, which continue activating and perpetuating the ED process.

To conclude, there is a high prevalence of CKD in a non-selected and spontaneous population. Arteriosclerotic disease is not only related to kidney function, but also to age and pulse pressure. Pathogenic mechanisms are well known, meaning that a change is needed. Nephrology departments, in collaboration with primary care centres should create programmes for detecting CKD and incipient vascular lesions early, so as to reduce CKD progression and cardiovascular morbidity and mortality.

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C. Pereira Feijoo[1], V.E. Martínez Maestro[1], N. Bretaña Vilanova[1], L. Queija Martínez[1], A. Otero González[2]

[1] Nephrology Department. Íñigo Álvarez de Toledo Kidney Foundation. Ourense, Spain

[2] Nephrology Department. Ourense Hospital Complex.

Correspondence: A. Otero González
Servicio de Nefrología.
Complejo Hospitalario de Ourense. Spain.
alfonso.otero.gonzalez@sergas.es

Monitoring sirolimus levels: How does it affect the immunoassay used?

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To the Editor,

Sirolimus, an immunosuppressive agent used to prevent graft rejection, has a narrow therapeutic window and high interindivual and intraindividual variability. Its concentration in blood must be monitored to prevent graft rejection and some adverse effects.[1] To date, the microparticle immunoassay (MEIA, Abbott Laboratories®) on an IMx® analyser has been the most used method for measuring sirolimus concentrations in blood.[2-6] However, the 2010 Abbott Laboratories® stopped marketing the reagents for this technique, replacing them with a chemiluminescent microparticle immunoassay (CMIA) on the Architect® analyser. Different immunoassays do not always yield the same results, given that techniques can have different sample pretreatments, drug metabolite cross-reactivity, or quantification limits.

The aim of our study was to compare sirolimus limits in kidney transplant patients, obtained by analysing the same blood sample with the two immunoassays (IMx® and Architect®). The sirolimus concentration analysis included the samples received at Del Mar Hospital during the first half of 2010 (period in which both reagents were available). We analysed 21 samples from 13 kidney transplant patients (10 men, age: 57.5 years [SD=12.4], post-transplant time: 5.25 years [Q1-Q3=4.13-9.44]).

Average concentrations obtained were 4.98ng/ml (SD=2.14) for IMx® and 8.37ng/ml (SD=3.01) for Architect®. The mean absolute difference between the techniques was +3.39ng/ml (SD=1.76) for Architect® compared to IMx®.

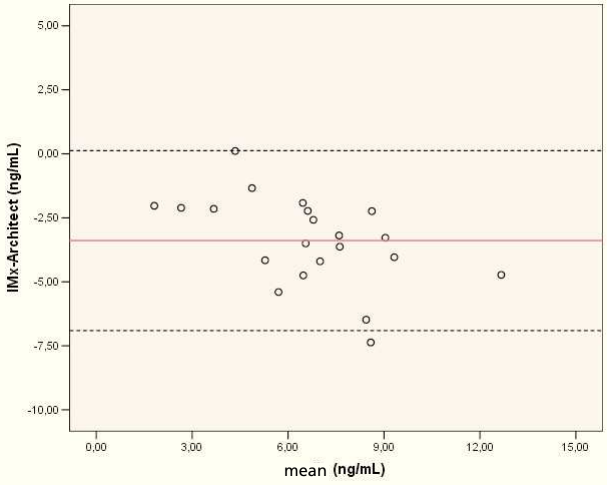
The Bland-Altman graph in Figure 1 shows the differences between the two techniques. Figure 2 shows the correlation of least squares between both techniques. The Pearson's correlation coefficient was r=0.819.

For 13 of the 21 samples, the difference between the two techniques was more than 50%, especially for samples less than 5ng/ml (9/11 compared to 4/10; *P*=.080).

Two of the samples analysed by IMx® (9.5%) were below their quantification limit (QL: 2.5ng/ml), while this was not found for the Architect®-analysed samples (QL: 0.7ng/ml).

For the IMx®-analysed samples, 47.6% (10/21) were within the therapeutic window (5-15ng/ml), the remaining 52.4% (11/21) were at an infra-therapeutic level. However, of the Architect®-analysed samples, 76.2% (16/21) were within the therapeutic window, 19.0% (4/21) were at an infra-therapeutic level and 4.8% (1/21) at a supra-therapeutic level.

Various immunoassays have been developed, making immunosuppressive drug monitoring easier.[7,8] Immunoassays use reagents with monoclonal antibodies against the drug analysed. Depending on the antibody's specificity, cross-reactivity may exist with the drug's metabolites. This cross-reactivity varies for each technique, giving rise



The continuous line shows the mean difference between the specific differences for each of the techniques. Broken lines shows ± 2 SD.

Figure 1. Bland-Altman graph showing the sirolimus concentration differences between IMx® and Architect® (n=21 samples)

to differences in the results from different immunoassays. This variance could cause conflict in deciding upon an immunosuppressive dose.

Our results show that Architect® shows 3ng/ml more than IMx®. Courtais et al obtained similar results with slightly lower difference (2.28±1.28ng/ml). However, only 4 out of the 53 patients studied had undergone a kidney transplant.[9] Furthermore, the difference was only calculated for 51 out of the 100 samples analysed, meaning that the infra-therapeutic or the supra-therapeutic ones were not considered.

According to the HPLC data provided at that time by the United Kingdom External Quality Assessment Service (UK NEQAS) for Clinical Laboratories, IMx® presents a bias of around -10%, and Architect® of +15%-20%.[10]

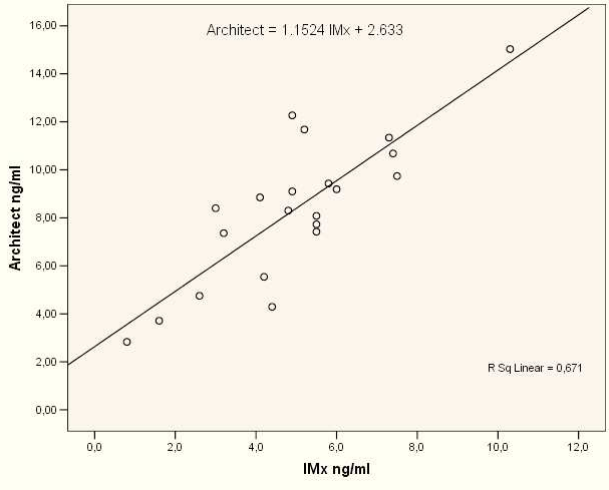


Figure 2. Linear correlation between sirolimus concentrations of IMx® and Architect® (n=21)

These differences can be due to different causes. Firstly, the two techniques use different methods for extracting the drug from the protein FKBP12. Dimethyl sulfoxide (DMSO) is used in Architect® pretreatment to heat the sample so that more sirolimus can be extracted.[11] Secondly, Architect® has better metabolite cross-reactivity. This cross-reactivity is always positive with metabolites F2 (8.7%), F3 (4.1%), F4 (36.8%) and F5 (20.3%) (data provided by Abbott Laboratories®). For IMx®, these interferences are lower: F2 (2.8%), F4 (26.2%) and F5 (6.8%), but higher with F3, and, also, negative (-22%). This difference was extended when we directly compare IMx® and Architect®.

The decrease in QL from 2.5ng/ml (IMx®) to 0.7ng/ml (Architect®) allows for regimen adjustment when lower levels are required.[1]

Recently, the laboratory that markets sirolimus sent a communication to health care professionals warning of the changes made to immunoassays and the consequences that this has on monitoring levels.[12] This communication especially emphasised the need for doctors to contact the laboratory to find out which assay is used, given that changes between different immunoassays or between one immunoassay and HPLC could produce clinically significant differences in results. These differences could provoke inadequate dosage adjustments, possibly causing adverse consequences. In our study, IMx® had more infra-therapeutic results than Architect® (52% vs. 19%), which could mean that there more patients' doses would be increased than with Architect®.

To date, therapeutic windows have not been standardised for each measurement technique. Recently, the

University of Colorado Hospital tried to adapt this therapeutic windows.[13] Given that the levels obtained by Architect® are higher, the window has increased from 3-8ng/ml (with HPLC) to 4.5-13ng/ml (with Architect®).

Our study's most significant limitation is that we have included a small amount of measurements in the sample, which could not have been increased as Abbott Laboratories® stopped marketing the IMx® reagent. Furthermore, our study includes the most kidney transplant patients to date.

It confirms that the laboratories that determine the sirolimus levels should inform doctors when they make changes to the immunoassay employed, and the consequences that could arise. This information is of vital importance so that appropriate dose adjustments can be made. Furthermore, this information should be considered when conducting clinical studies or comparisons between different hospitals. Similarly, sirolimus therapeutic windows should be standardised for each of the techniques in use.

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M. Marín-Casino[1], M. Crespo[2], J. Mateu-de Antonio[1], J. Pascual[2]

[1] Hospital Pharmacy Department. Del Mar Hospital. Parc de Salut Mar. Barcelona, Spain.

[2] Nephrology Department. del Mar Hospital. Parc de Salut Mar. Barcelona, Spain.

Correspondence: M. Marín-Casino
Servicio de Farmacia Hospitalaria. Hospital del Mar. Parc de Salut Mar. Passeig Marítim, 25-29. 08025. Barcelona, Spain.
mmarinc@hospitaldelmar.cat

Good practice guidelines on the use of erythropoiesis-stimulating agents in 2011

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To the Editor,

As coordinator of the Kidney, Dialysis and Transplant Programme in Cuba, I would be extremely grateful if you could publish this letter. I would like to highlight my opinions regarding the safe use of erythropoiesis-stimulating agents (ESA), and give my contributions on its optimal use, which is currently subject to debate.[1]

For me, introducing recombinant human erythropoietin (rhEPO) and ESA to clinical practice following replacement dialysis has been one of the most important advances in stage 5 chronic kidney disease (CKD) treatment. These techniques are the best example of how biotechnology has been successfully applied as a clinical treatment as it is used to correct severe anaemia linked with CKD, despite the adverse results highlighted by the most recent prospective and controlled studies.[2] Furthermore, we must remember that to do so we have to use supraphysiological doses of erythropoietin, justified by its non-haematopoietic effects.[3]

The reason why these studies report a greater risk to negative events, mortality and cancer makes us reflect upon important questions that are yet to be completely resolved:

1. Would the population with the greatest haemoglobin levels and worst results show other rhEPO effects and be likely to have to a homogeneous analysis?
2. Is the maximum rhEPO dose to be employed for each haemoglobin level clear?
3. Have we considered that rhEPO dose does not have to be increased to reach any haemoglobin level?
4. Are patients with adverse effects