Reference Change Value Concept Combining Two Delta Values to Predict Crises in Renal Posttransplantation, Carmen Biosca,1 Carmen Ricoś,2 Ricardo Lauzurica,1 Romań Galimany,1 and Per Hyltoft Petersen3 (1 Hospital Universitari “Germans Trias i Pujol”, 08916 Barcelona, Spain; 2 Hospital “Vall d’Hebron”, 08035 Barcelona, Spain; 3 Odense University Hospital, 5000 Odense, Denmark; *address correspondence to this author at: Department of Biochemistry, Hospital “Germans Trias i Pujol”, Ctra. de Canyet s/n, 08916 Barcelona, Spain. Fax 34-34-978-843, e-mail cbiosca@ns.hugtip.scs.es)

The concept of reference change value (RCV) was developed by Harris and Yasaka (1, 2) to identify significant changes in the state of patients when monitoring their pathology. This concept can be applied to the laboratory data routinely acquired for renal posttransplantation patients to detect potential crises before clinical indications are manifested. The RCV takes into account the within-subject biological variation as well as the analytical variation, when considering serial laboratory results. The distinction between pathologic change and laboratory “noise” can be improved to provide better information about the patient’s status if the RCV is calculated (3–7) when:

- The clinical situation is well defined and managed through a strict protocol
- The reference group studied is homogeneous regarding the disease and treatment and is within a demonstrated period of stability
- The event to predict is the same for all patients
- The analytical procedure used is well controlled

In previous work using data from kidney transplantation patients (6, 8), we noted that the analytes most suitable for detecting significant changes during posttransplantation follow-up are serum creatinine, urate, and urea. We hypothesized that the predictive power of these analytes might greatly increase if more than one showed simultaneous and independent changes before the clinical manifestations of reduced kidney function became apparent. We developed an objective analytical indicator (using these analytes) to detect potential subclinical crises in renal transplant recipients based on the RCV concept. To make the model relevant for use in daily practice, we attempted to include nephrologists’ criteria in the final values proposed by determining the factors they consider crucial when monitoring their transplant patients (9, 10). The underlying goal of this effort was to extract the most information possible from routine laboratory data and offer the clinician an improved tool for patient care.

To test whether the concentrations of creatinine, urea, and urate were independent, pairs of data from the three quantities in the same individuals and the same samples during the stable period obtained in the previous study (8), were associated using Pearson’s correlation coefficient ($r$). The pairs, creatinine/urate and urea/urate, gave $r$ values >0.50 in the majority of patients, whereas the pair creatinine/urate gave $r$ values symmetrically distributed around zero for all patients. This fact suggested independence between serum creatinine and urate in renal recipients during the stable period condition.

We established the diagnostic validity of significant changes in these quantities according to the evolution of a population of transplant patients.

Among the 75 renal transplant recipients included, two subgroups were established: (a) 57 patients who were clinically stable and showed no evidence of crisis for more than 3 years after transplantation (nonrejection group); (b) 18 patients who suffered acute rejection after a short period of clinical stability lasting 2–5 weeks (rejection group). Rejection had been confirmed by clinical observation, analytical profile, Doppler ultrasound study in 7 patients and by histologic study in 11 patients.

Permission for enrollment in the study was obtained from all patients, as required by the Helsinki II protocol. Serum specimens were collected according to the standard hospital follow-up protocol designed by nephrologists for renal transplant recipients (6, 8). Under this protocol the samples studied in the present work were collected at different intervals of time, ranging from once per week to once per month (for the nonrejection group (6)).

The RCVs for creatinine and urate, at various probabilities and applied to a single quantity or the two quantities combined, were calculated according to the formula:

$$RCV = z_p(2)^{1/2} \times (CV_{w+a})$$

where $z_p$ is the covering factor for a certain probability, and $CV_{w+a}$ is the within-subject biological plus analytical CV.

If there is no correlation between two quantities in a subject, then under stable conditions, each quantity has less than a given probability for error when detecting changes, depending on the $z_p$ selected (e.g., if the probability for one quantity to exceed the reference change is 5%, the probability of two quantities combined is $1/20 \times 1/20 = 1/400$ (0.25%).

The criteria commonly used to judge the diagnostic performance of a biochemical test are diagnostic sensitivity, diagnostic specificity, and predictive values of the positive and negative tests (11, 12). The diagnostic performance of the RCV for creatinine and urate combined was calculated from the group of 75 patients studied. The sensitivity is the fraction of all true-positive test values from posttransplantation patients that preceded an acute rejection, and the specificity is the fraction of all true-negative test values for all tests of all stable posttransplantation patients (without any results above the RCV assigned). The prevalence of acute rejection ($n = 18$) in the patients deemed eligible for the study in our hospital ($n = 75$) was 0.24.

The RCVs between creatinine and urate combined were calculated using the RCV formula described above. The application of this formula to the $CV_{w+a}$ values for creatinine of 12.3% and urate of 13.5% (6), using $z_p = 1.04$...
which yields a 2.25% false-positive (FP) probability for two independent quantities combined gives RCVs of 18.1% for creatinine and 19.8% for urate.

The percentages of difference between consecutive results for creatinine and urate found in the 57 patients from the nonrejection group and in the 18 patients from the rejection group are shown in Fig. 1. The horizontal and vertical boundaries represent the RCVs for creatinine and urate, respectively, based on the predetermined theoretic FP probability of 2.25% (85% covering interval for each). The inverted triangles represent consecutive combined differences higher than the respective RCVs, indicating FP and true-positive results, respectively. The filled circles indicate true-negative and false-negative results in Fig. 1, A and B, respectively. In Fig. 1A, only one randomly chosen consecutive combined difference for each patient was plotted to simplify the value. In Fig. 1B, the open circles correspond to all remaining consecutive combined differences available for the rejection group of patients.

Table 1 shows the sensitivity, specificity, and predictive values for the prevalence of kidney rejection in the patients studied with a short period of stability in this study (0.24) at the 95%, 85%, and 80% covering intervals.

According to the questionnaire directed to nephrologists (10), a >25% difference between consecutive creatinine determinations when creatinine concentrations were outside the reference interval was the relevant signal for impending crisis. The clinicians requested urate analysis to evaluate immunosuppressant concentration but did not use it as an indicator of rejection.

When the specialists were asked for their opinions on the consequences of false-negative prediction of crises, their answers were related to patient safety and the cost of treatment. Regarding the patient, graft loss means a return to dialysis, and this implies a high cost, both in discomfort to the patient and in the expense of dialysis.

The consequences of FP prediction of crisis were related to excessive immunosuppression, which has repercussions for the patient as physiologic stress and susceptibility to opportunistic infections. Cost considerations depend on the immunosuppressant used and possible complications from infection. The clinicians emphasized that false positives have to be minimized.

We found that RCVs of 18.1% for creatinine together with 19.8% for urate at an 85% covering interval showed the best combination of sensitivity, specificity, and positive and negative predictive values for the purpose of detecting potential rejections. When we compared the 25% criterion used by the nephrologists with the combined RCV criterion in the population of 75 patients studied, we found that the number of rejections detected and false negatives were the same (sensitivity = 0.722; confidence interval, 0.433–0.905; see Table 1). However, eight more FP predictions were obtained with the 25% criterion than with the combined RCV criterion. In the nonrejection group (n = 57), 10 patients were falsely classified as experiencing rejection with the >25% crite-

4 For $z_p = 1.04$, the tail area of distribution for one quantity is 15%. Thus, 3 false positives would be expected on average for each 20 results. For two quantities combined, the probability is $3/20 \times 3/20 = 9/400 = 0.0225$, or 2.25%.

Table 1. Diagnostic validity for the patients (based on our prevalence of kidney rejection) for three covering intervals.

<table>
<thead>
<tr>
<th>Prevalence</th>
<th>Coverage Interval</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence = 0.24 at 95% covering interval</td>
<td>0.278</td>
<td>1.000</td>
<td>1.000</td>
<td>0.814</td>
<td></td>
</tr>
<tr>
<td>Prevalence = 0.24 at 85% covering interval</td>
<td>0.722</td>
<td>0.965</td>
<td>0.867</td>
<td>0.917</td>
<td></td>
</tr>
<tr>
<td>Prevalence = 0.24 at 80% covering interval</td>
<td>0.778</td>
<td>0.877</td>
<td>0.667</td>
<td>0.926</td>
<td></td>
</tr>
</tbody>
</table>
tion and 2 with the combined RCV criterion, giving specificities of 0.825 (confidence interval, 0.677–0.916) and 0.965 (0.851–0.995), respectively.

We found that when RCVs from creatinine and urate combined were used to predict crises, the FP probability was reduced and there was a considerable increase in diagnostic specificity.

The methodology used in this work pointed out the need to improve laboratory reports (13) as follows:

• Include the RCV calculations for the combination of significant analytes for the pathology studied in the laboratory data processing system
• Mark the test results showing a significant RCV with respect to the previous result
• Include in the report a plot showing evolution of the analytes with a critical role in detecting changes in the pathology monitored

A limitation of our RCV model to detect changes in the evolution of kidney graft recipients during monitoring is that it can only be applied to patients who have experienced a certain period of favorable clinical evolution. Regarding the usefulness of the model, we mention a few points. First, although the model can benefit only patients who have achieved an interval of clinical stability, these are precisely the ones in whom surveillance may be more relaxed (the patient has recovered from the operation and feels better; analyses are less frequent), and a specific, objective biochemical marker could be of greatest value. Second, the constituents providing an early indicator of rejection are among those analyzed in the standard protocol and at exactly the same frequency. Thus, no additional cost, effort, or discomfort to the patient is implied by the use of this approach (14).

References


Supraregional Interlaboratory Quality-Control Survey for an Immunoradiometric Renin Assay, Adriano Piffanelli,1 Alberto Morganti,2 Franco Mantero,3 Antonio Cianetti,4 Gian Carlo Zucchelli,5 Gloria Giovannini,1 and Dario Pelizzola1 (1 Department of Experimental and Clinical Medicine, Section of Nuclear Medicine University, 44100 Ferrara, Italy; 2 Hypertension and Clinical Physiology Center, Institute of Clinical Medicine University, 20100 Milan, Italy; 3 Endocrinology Section University, 60100 Ancona, Italy; 4 Central Laboratory RIA Section, S. Camillo Hospital, 00100 Rome, Italy; 5 Institute of Clinical Physiology, National Center of Research, 56100 Pisa, Italy; * address correspondence to this author at: Department of Experimental and Clinical Medicine, Section of Nuclear Medicine, Via Luigi Borsari, 46, 44100 Ferrara, Italy; fax 39-0532-236589, e-mail pif@unife.it)

For the last 20 years, the most widely used method for assessment of the renin-angiotensin system has been the plasma renin activity assay (1). Although the assay gives useful information on the enzymatic function of the renin molecule, the intrinsic characteristics of this method limit its analytical accuracy (2). Immunoradiometric assays for renin, developed in 1985, overcome this limitation of the enzymatic assay because the well-defined monoclonal antibodies provided the means for direct quantification of specific active forms of the enzyme molecule (3, 4). Nevertheless, a critical debate has developed over the use of these assays (5–10).

A pilot study (11, 12) in a limited number of laboratories (eight Italian centers) found satisfactory indexes of precision for a direct immunoradiometric renin assay (Eria Diagnostics, Sanofi-Pasteur). In our previous experience, the inter- and intralaboratory reproducibility indices of this commercial immunoradiometric assay appeared to be better than those that had been achieved with an enzymatic assay (REN-CTK; Sorin Biomedica), with the differences probably being attributable to the greater complexity of the procedure for the latter assay (11).

Various reports have been published during the past 30 years describing useful tools to monitor the performance of immunoassays (13, 14). Regarding the active renin assay, it is of paramount importance to assess the methodologic accuracy, especially for “low renin” concentrations, which are clinically relevant (15, 16).

Here we describe the results of a supraregional quality-control program, open to laboratories performing direct