rion 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 39-0532-23689, 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, overcame 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 indexes 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
renin assays with a commercially available immunoradiometric method. The program included 48 clinical laboratories studied over a 12-month period with results provided periodically to the participants.

Forty-eight Italian laboratories participated in the first phase of the program, which included the determination of nine reference samples of unknown renin content (one every 3 weeks) during a 6-month period. Once a month a report with the extrapolated “consensus mean” and bias (expressed as SD) of the laboratory was sent to every participant, and at the end of each program a final report carried “precision” and “accuracy” results for the nine sample determinations. No results of this multicenter survey were rejected as outliers.

For the first phase of the program (June 1998 through January 1999), reference samples covering a wide concentration range were prepared from three pools of plasma samples collected for routine assays of renin activity. The three pools contained the following theoretic concentrations: pool 1, 15 ng/L; pool 2, 50 ng/L; pool 3, 150 ng/L. Each vial contained 0.7 mL of lyophilized plasma. The expected renin concentration of each vial was not revealed to the participating laboratories.

Lyophilization (Edwards High Vacuum MFD 0.1) carried out under controlled conditions (pressure, $1 \times 10^{-9}$ Pa; temperature, $-20 \, ^\circ$C; duration, 24 h) did not significantly influence the renin content of the samples.

Forty-nine laboratories participated in the second phase of the program (June 1999 through January 2000). The reference samples for this phase were prepared from normal plasma with or without the addition of the renin International Reference Preparation (IRP) 68/356, which was established as the IRP for renin by the WHO Expert Committee on Biological Standardization (WHO ECBS–WHO TRS 565) (17). The pools were as follows: pool 1 contained normal plasma with 50 ng/L renin IRP [1 ng of IRP 68/356 is equivalent to $1.6 \times 10^{-3}$ Goldblatt Units (GU), with 1 GU being identical to the Medical Research Council International Unit]; pool 2 contained normal plasma; pool 3 contained normal plasma with 110 ng/L renin IRP. The theoretic renin concentrations were 50, 1.7, and 110 ng/L for pools 1, 2, and 3, respectively.

As in the first phase of the quality-control program, each pool was divided into three identical series of vials for a total of nine vials per set (each vial contained 0.75 mL of lyophilized plasma). The lyophilization process was the same as for the first phase.

This quality-control program was open to laboratories performing renin assays with the commercial immunoradiometric assay provided by Eria Diagnostics (Sanofi Pasteur). The first monoclonal antibody recognizes both the active and inactive forms of renin, whereas the second monoclonal antibody, labeled with $^{125}$I, recognizes specifically the active form of renin. The tubes were counted in a gamma counter (Cobra AutoGamma; Canberra Packard), and the renin concentration of unknown samples was calculated from a calibration curve generated with Medical Research Council human renin (1 ng = $1.6 \times 10^{-3}$ GU; WHO 68/356). The detection limit of the assay was $<1$ ng/L for active renin at the half-life of the tracer. The interassay variation given by the manufacturer for renin concentrations of 8–269 ng/L was 14.5–27.2%. Each laboratory also periodically received its accuracy indices with respect to a working consensus mean.

Interlaboratory CVs during the first phase were 14–32% (Table 1); on the other hand, mean intralaboratory CVs for the same run, calculated for every laboratory on the three concentrations assayed three times, were 14–19% (Table 1). During the second phase, interlaboratory CVs were 16–46% (Table 1), and intralaboratory CVs for the second phase, calculated in the same way as for the first phase, were 16–30% (Table 1). The renin concentration range for the second phase included lower concentrations to allow evaluation of precision performance in this portion of the analytical interval.

The survey presented here involved a large group of participating laboratories ($n = 48$) applying the same methodology. The long period of time over which the program was applied (12 months over 2 years) increased our confidence in the reliability of the results achieved.

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**Table 1. Results of the first and second phases of the renin quality-control program.**

<table>
<thead>
<tr>
<th>Vial</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of laboratories</td>
<td>43</td>
<td>43</td>
<td>34</td>
<td>35</td>
<td>39</td>
<td>36</td>
<td>37</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>Consensus mean, ng/L</td>
<td>61.9</td>
<td>1.71</td>
<td>128</td>
<td>1.62</td>
<td>52.4</td>
<td>117</td>
<td>109</td>
<td>35.4</td>
<td>1.65</td>
</tr>
<tr>
<td>Interlaboratory CV, %</td>
<td>23</td>
<td>46</td>
<td>16</td>
<td>16</td>
<td>27</td>
<td>28</td>
<td>31</td>
<td>31</td>
<td>45</td>
</tr>
</tbody>
</table>

*Intralaboratory precision (CV) for three groups of vials: for vials 1, 5, and 9, intralaboratory CV = 19%; for vials 2, 4, and 8, intralaboratory CV = 16%; for vials 3, 6, and 7, intralaboratory CV = 14%.

* Intralaboratory precision for three groups of vials: for vials 1, 5, and 8, intralaboratory CV = 28%; for vials 2, 4, and 9, intralaboratory CV = 30%; for vials 3, 6, and 7, intralaboratory CV = 16%.
Although the present overall results confirm the previous good reproducibility data obtained with the same immunometric method (11), the data reveal marked imprecision for lower renin concentrations. Blood concentrations of renin are particularly valuable and useful at low concentrations (18) and when the renin-angiotensin system is activated (15). Because various immunometric assays for active renin are used for clinical research (15, 16, 19), it would be advisable to validate each technique with a thorough quality-control program.

We are indebted to our colleagues at 48 Italian centers who participated in the present study.

References

Age and Sex Dependency of Carnitine Concentration in Human Serum and Skeletal Muscle, Jens Rüdiger Opalka, Frank-Norbert Gellerich, and Stephan Zierz (Muskellabor, Universitätsklinik und Poliklinik für Neurologie, Martin-Luther-Universität Halle-Wittenberg, Ernst-Grube-Strasse 40, D-06097 Halle (Saale), Germany; * author for correspondence: fax 49-345-557-3505, e-mail jens.opalka@medizin.uni-halle.de)

Carnitine plays an essential role in fatty acid metabolism. It can be synthesized in the liver, but additional intestinal resorption is necessary (1). Carnitine mediates the transport of activated acyl residues via the carnitine palmitoyl transferase system into mitochondria for β-oxidation (2). Whereas primary carnitine deficiency is attributable to mutations in OCTN2 [a carnitine transporter of plasma membranes (3)], several other conditions can cause secondary deficiency (4). The leading symptom of either primary or secondary carnitine deficiency is weakness of skeletal muscles. In addition to these pathologic conditions in some animal models, a physiologic decline of carnitine concentration with aging has been reported (5, 6). In addition to increasing muscular carnitine concentrations (6), oral treatment with l-carnitine or its acetyl ester has also been shown to restore many of physiologic impairments that accompany aging (7–10). These data indicate the important role of carnitine in the aging process, at least in mice and rats. For human skeletal muscle, confusing results exist with respect to the age dependency of carnitine content: Whereas Costell et al. (5) found a “drastic” age-dependent decrease of carnitine in human skeletal muscle and Gonzalez-Crespo et al. (11) detected reduced free carnitine concentrations in elderly patients undergoing hip surgery, an age-dependent decrease in carnitine concentration could not be confirmed by Starling et al. (12). The answer to the question of whether carnitine in human muscle is also age dependent is hampered by the different populations investigated and the different methods used. No reliable data exist concerning the sex dependency of a possible age-dependent variation.

The present study seeks to clarify whether carnitine concentrations in human skeletal muscle and serum depend on age and sex. The physiologic relevance is discussed. Analysis of carnitine in serum was performed in samples from healthy blood donors (n = 80; 18–57 years) from the local blood-donor service of the University Hospital Halle (Saale). For determination of carnitine in skeletal muscle, routine diagnostic muscle biopsies from patients (n = 52; 18–74 years) were analyzed. Healthy controls were selected among patients who had no muscle disease, as determined by combined clinical, electrophysiologic, histologic, electron microscopic, biochemical, and genetic criteria. Only specimens of proximal skeletal muscles (vastus lateralis or biceps brachii) obtained by open biopsy at standardized locations were included in this study. Biopsy was performed without anesthesia of muscle tissue. The sample was immediately frozen in liquid