assay. The incubation time of 18 min is also sevenfold that of the second step in the ACS assay (2.5-min incubation with microparticle-bound antibody).

There is strong evidence that BB-PRL is a PRL-IgG complex, and our results confirmed that most of BB-PRL is retained on protein G-Sepharose. Recently, a PRL-binding protein was found in human serum and milk, and on average, 36% of the PRL in the circulation was found to consist of PRL bound to PRL-binding protein (15). In agreement with this, we found by gel filtration that 15–40% of PRL consisted of B-PRL.

Removal of macroprolactin by PEG precipitation is used as a screening method for macroprolactinemia (13, 16, 17), and our results confirmed that PRL measured after PEG precipitation consists mainly of L-PRL. The precipitation step is a manual procedure, which only some laboratories routinely perform. An alternative approach is to retest samples with increased PRL concentrations with a shorter incubation time. A much lower result with the short incubation time indicates the presence of BB-PRL. If our results can be reproduced with other assays, it should be possible to adapt this approach to certain immunoanalyzers to automatically recognize samples with increased PRL attributable to macroprolactinemia.

There are several possible explanations for the effect of incubation time on the PRL results. In addition to slower reaction kinetics of the big forms, BB-PRL and B-PRL may gradually dissociate from their complexes, exposing epitopes hidden in the complexes. In both cases, lower results may be expected with shorter incubation times.

In conclusion, the concentration of PRL measured by immunoassay is dependent on the incubation time if the sample contains macroprolactin. Measurement of PRL with two different incubation times is a potentially useful method for identification of samples containing macroprolactin.

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References

Cystatin C versus Creatinine in Renovascular Disease, Oliviero Olivieri,1* Antonella Bassi,2 Francesca Pizollo,1 Elisa Tinazzi,3 and Roberto Corrocher1 1Department of Clinical and Experimental Medicine, Unit of Internal Medicine, and 2Institute of Clinical Chemistry, University of Verona, 37134 Verona, Italy; † address correspondence to this author at: Department of Clinical and Experimental Medicine, Unit of Internal Medicine, Policlinico G.B. Rossi, P.le A. Scuro 10, 37134 Verona, Italy; fax 39-45-580111, e-mail oliviero.olivieri@univr.it)

Renovascular disease (RVD) is emerging as a common and frequently unsuspected cause of chronic renal failure and end-stage renal disease (1); moreover, it is frequently associated with renovascular hypertension, which generally carries a worse cardiovascular prognosis than other hypertensive conditions (2, 3). RVD and its associated hypertension are often not readily distinguishable from essential hypertension with renal damage, and the currently accepted diagnostic workup strategy is largely based on early clinical suspicion of the disease (4).

Among the main elements contributing to an increased clinical suspicion of RVD is a decreased glomerular filtration rate (GFR), often detected by an increase in serum creatinine concentration. The creatinine concentration is indeed convenient and inexpensive to determine, but it is not ideal because of its sensitivity to changes in muscle mass, dietary protein intake, tubular secretion, and extrarenal metabolism. In addition, it is a relatively poor index of the function of individual kidneys and renal mass because unilateral renal artery stenosis >70% is often
recent evidence suggests that serum cystatin C is a reliable marker of GFR (5–8). Moreover, cystatin C may more readily detect subtle decrements of GFR than does serum creatinine (5, 9, 10). We therefore investigated whether cystatin C can serve as a more sensitive indicator than creatinine in a rule-out strategy for the diagnostic workup for RVD.

We studied 128 free-living individuals (64 males/64 females) who were normotensive and free of any drugs. No renal or other significant diseases were recorded in the history. The participant selection strategy was also aimed at having five age classes (<30, 31–40, 41–50, 50–60, and >61 years; age range, 22–78 years) for each gender composed of 11–13 participants each.

We then studied 150 consecutive hypertensive atherosclerotic patients without overt renal failure who had an intermediate to high index of clinical suspicion of RVD, as defined according to the Mann–Pickering criteria (11). All patients underwent clinical examination, routine biochemical analysis (including serum cystatin C and creatinine), and renal angiographic evaluation [for details, see Ref. (12)]. According to the angiographic results, patients were classified as RVD or RVD-free (at least 70% lumen reduction). We also recruited 12 RVD patients undergoing percutaneous renal angioplasty and stenting and measured their serum cystatin C and creatinine within 48 h (short-term) and 90 days (long-term) after the procedure to evaluate whether angioplasty-induced renal blood flow changes were associated with parallel variations in the GFR markers.

Cystatin C was assayed by the Jaffé method on a DAX 96 multichannel analyzer, according to the manufacturer’s procedures (Bayer Diagnostic); the intrassay and interassay CVs were <3% and <5%, respectively. Cystatin C was measured by commercially available particle-enhanced nephelometric immunoassay with antisera, calibrator, and a BNII nephelometer from Dade Behring (13). The intrassay CV was calculated on 10 control replicates and interassay CV on duplicates over 10 days. Imprecision was within the manufacturer’s specifications, i.e., the intrassay CV was <2.1% and the interassay CV was <2.5%. Details concerning the statistical analysis are reported as data supplements accompanying the online version of this Technical Brief at http://www.clinchem.org/content/vol48/issue12/.

The mean (SD) cystatin C and creatinine concentrations in normotensive controls were 0.78 (0.09) mg/L (range, 0.57–1 mg/L) and 74.5 (13.5) μmol/L (range, 44.2–114.9 μmol/L), respectively. The percentile distributions of serum cystatin C and creatinine in healthy normotensive population are reported in Table A in the online data supplements.

A total of 65 patients had angiographically significant RVD, whereas the remaining 85 patients with no lesions and no significant stenosis (<70%) were considered the RVD-free group. In the RVD group, 12 patients also had a contralateral stenosis <50%, and 10 patients had bilaterally significant lesions. The two groups were well matched for most features, but RVD patients were older and had higher serum cystatin C and creatinine (Table B in the online data supplements).

The ROC curves for cystatin C and creatinine were nearly superimposable [areas under the curves, 0.73 (95% confidence interval, 0.64–0.80) and 0.74 (95% confidence interval, 0.66–0.82) for cystatin C and creatinine, respectively]. However, in agreement with the fact that the value corresponding to the 90th (or 95th) percentile of the distribution of the normotensive population was equivalent to the 25th (or 30th) percentile for creatinine and to the 45th (or 55th) percentile for cystatin C and to the 45th (or 55th) percentile for creatinine of the distribution for the hypertensive population, marked differences in the diagnostic performance were observed when either the 90th or the 95th percentile of the normotensive population was used as the decision threshold. The performance of both analytes according to different decision thresholds is reported in Table 1.

The likelihood ratio of a positive test (LR+) and the

Table 1. Cystatin C and creatinine sensitivity, specificity, and negative and positive predictive values for RVD/RVD-free diagnosis according to different decision thresholds.

<table>
<thead>
<tr>
<th>Best performance point by ROC curve</th>
<th>Cystatin C (0.98 mg/L)</th>
<th>Creatinine (92.3 μmol/L)</th>
<th>90th percentile of normotensive population</th>
<th>Cystatin C (0.93 mg/L)</th>
<th>Creatinine (97.2 μmol/L)</th>
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<td>PPV, %</td>
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<td>45–63</td>
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* Threshold selected for optimal separation of groups without weighting for costs of false-positive and false-negative results or prevalence of disease.

* Decision thresholds are in parentheses.

* CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
likelihood ratio of a negative test (LR−) were also calculated. As expected, they were virtually identical at the ROC-selected cutoffs, but for cystatin C, LR+ was 1.55 and 1.57 and LR− was 0.19 and 0.28 at the 90th and 95th percentiles, respectively; for creatinine, LR+ was 1.90 and 2.70 and LR− was 0.45 and 0.39 at the 90th and 95th percentiles, respectively. The value of LR− obtained for cystatin C (0.19 for the 90th percentile) was consistent with an odds ratio of 4.26 (i.e., probability against the occurrence of true RVD diagnosis if cystatin C was <0.90 mg/L), suggesting a possible clinical usefulness of the test.

The changes in cystatin C and creatinine concentrations after renal angioplasty were evaluated over both the short and long term (Fig. 1). Angioplasty decreased cystatin C (Fig. 1A), but did not appear to affect creatinine values over either the short or long term (Fig. 1B).

We also compared the sensitivities of creatinine and cystatin C in reflecting differences in arterial luminal narrowing and the coexistence of significant bilateral stenosis. Artery luminal stenosis of 65–70% was generally associated with a cystatin C concentration >0.90 mg/L, the decision cutoff corresponding to the 90th percentile of the normotensive individuals (Fig. 2 in the online data supplements). In the case of creatinine, however, 80% (or more) lumen reduction seemed necessary to cause a steady increase in concentrations to >92.9 µmol/L, the 90th percentile of the normotensive individuals (Fig. 3 in the online data supplements).

Patients with bilateral lesions had statistically higher cystatin C (1.42 ± 0.43 vs 1.17 ± 0.33 mg/L; P <0.05) but not significantly higher creatinine (114 ± 28 vs 111 ± 28.6 µmol/L) than patients with unilateral stenosis (Fig. 4 in the online data supplements).

The results of our study suggest that cystatin C may be a possible “discovery test” for RVD and a more sensitive marker of ischemic GFR impairment than creatinine. The difference in diagnostic sensitivity between cystatin C and creatinine was not directly observed by comparing operationally equivalent points on the ROC curve, but hypertensive atherosclerotic patients with cystatin C values within the reference interval had a much higher probabil-

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**A**

Cystatin C mg/L

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**B**

Creatinine µmol/L

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*Fig. 1. Angioplasty-induced changes in cystatin C (A) and creatinine (B) concentrations 48 h (short-term) and 90 days (long-term) after the procedure. Individual patients (Pt) are indicated with the same Arabic numbers in both panels. *, P <0.05 compared with baseline values (by t-test for paired data). NS, not statistically significant.*
ity of being free of disease than did patients with creatinine values within the reference interval. The probability against the occurrence of true RVD diagnosis in an atherosclerotic hypertensive patient with cystatin C <0.90 mg/L was 4.26:1, which appears a reasonable odds ratio for screening purpose. Conversely, the same probability in a patient with creatinine values in the reference interval was 1.22:1.

Compensatory hyperfunction of the unaffected kidney generally minimizes the consequences of unilateral ischemic damage, and this is indeed what currently happens in the so-called creatinine blinded area, in which creatinine concentrations exceed the upper limit of the reference interval only when GFR is reduced to <75 mL/min (5). In our study, such an increase in cystatin C reflected an earlier stage of renal damage because it increased progressively above the 90th percentile of the normotensive range as the lumen of renal artery was reduced by 65–70%, whereas a corresponding increase in creatinine was recognizable when at least 80% lumen stenosis was present. These data agree with the notion that serum cystatin C increases when GFR is <88 mL/min (5). Additional evidence was also provided by our analysis of RVD patients treated with angioplasty and stenting of the stenosed vessels. Improved renal blood flow induced by successful angioplasty was followed by changes in cystatin C but not creatinine (Fig. 1), suggesting that relatively subtle GFR variations were adequately reflected by cystatin C but not by creatinine concentrations. This finding highlights the possibility that improvement of renal function may be underestimated or not recognized by creatinine measurements in some ischemic patients treated with angioplasty. It is possible that this reduced sensitivity is partially attributable to the aspecific chromogenic interferences inherent in the Jaffe method and that our conclusions cannot be applied to the enzymatic method, which does not imply such interferences. Although the Jaffe method is less expensive and more widely used, a comparison between enzymatically measured creatinine and cystatin could be an interesting issue for future research in the field. Taking into account that the value of the present results must be restricted to the specific clinical setting of atherosclerotic hypertensive patients, cystatin C assay may be of clinical utility in a rule-out diagnostic strategy for RVD.

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References
4. Vasbinder GBC, Nelemans PJ, Kessels AGH, Kroon AK, de Leeuw PW, van


Determination of Gabapentin in Plasma by Liquid Chromatography with Fluorescence Detection after Solid-Phase Extraction with a C18 Column, Donna Gauthier* and Ram Gupta (Department of Laboratory Medicine, St. Joseph’s Hospital, 50 Charlton Ave. East, Hamilton, Ontario, L8N 4A6 Canada; *author for correspondence: fax 905-521-6185, e-mail dgauthie@stjosham.on.ca)

Gabapentin (Neurontin®), one of the relatively new anti-epileptic drugs, has a novel mechanism of action that is not fully understood. Gabapentin has been shown to increase γ-aminobutyric acid concentrations in the brain of epilepsy patients (1, 2). There is an approximate linear relationship between dose and plasma concentrations. A therapeutic range of 2–20 mg/L has been commonly accepted (2), although a higher minimum concentration of 6 mg/L has also been suggested (1).

Various analytical methods have been used for the determination of gabapentin in plasma, with liquid chromatography being the most commonly used approach in clinical laboratories. Gabapentin does not have a natural chromophore; therefore, it must be derivatized to be detected spectrophotometrically or fluorometrically unless detected by mass spectrometry (3). Gabapentin has been derivatized with 2,4,6-trinitrobenzenesulfonic acid for photometric detection (4, 5). Several approaches have been described for the preparation of fluorescent derivatives. Garcia et al. (6) derivatized gabapentin with fluorescamine for determination by capillary electrophoresis. However, the preparation of o-phthalaldehyde (OPA) derivatives of gabapentin has been the most popular and practical approach for liquid chromatographic determinations (7–12).

In all of these procedures, plasma proteins are precipitated by different reagents and the resulting supernatant