immunoreactivity in the void volume but preserved the later peak (Fig. 1B).

The proCNP concentrations in venous plasma from the controls and from the heart failure patients are shown in Fig. 1C. There was no difference between the 2 groups [median (range), 8.3 (7.0–12.0) pmol/L vs 8.0 (4.9–11.0) pmol/L; \(P = 0.47\)]. The proCNP concentrations in plasma from different cardiovascular regions in the heart failure patients are shown in Fig. 1D. In plasma from the inferior caval vein, the proCNP concentration was marginally higher than in plasma from the coronary sinus [median (range), 7.5 (6.2–11.0) pmol/L vs 8.3 (7.0–12.0) pmol/L; \(P = 0.013\)].

The present study demonstrates that measurement of precursor peptide is a feasible way to achieve gene product specificity. Extraction by Sep-Pak cartridges, which has been applied in other studies (1, 2, 9), offers the advantage that samples can be concentrated. On the other hand, peptide recovery and, consequently, measurements can be highly variable. To our knowledge, no previous studies have used ultrafiltration for plasma extraction. We established that the recovery is reproducible and sufficient to allow accurate measurement of proCNP.

The proCNP concentration in ultrafiltered venous plasma was not increased in heart failure patients compared with controls. The use of plasma proCNP as a marker in stable heart failure patients is thus not supported by our findings. However, patients with destabilized, severe exacerbations were not included in our study. Whether proCNP could be a marker in such patients remains to be investigated.

The proCNP concentration was marginally higher in peripheral venous plasma than in plasma sampled from the coronary sinus. Interestingly, cardiac secretion of CNP has been reported recently (10). Although our results do not exclude a minor cardiac secretion, they suggest that circulating proCNP is not secreted from the failing heart. Referring to CNP as a “cardiac” natriuretic peptide is thus misleading (11).

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References

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False-Positive Results in the Detection of Methadone in Urines of Patients Treated with Psychotropic Substances, Frédérique Lancelin, Linda Kroul, Nadia Flatischler, Sophie Brovedani-Roussel, and Marie-Liesse Piketty (Laboratoire Central de Biologie, Centre Hospitalier Sainte Anne, 1 rue Cabanis, F 75014, Paris, France; * author for correspondence: fax 33-1-45657446, e-mail m.piketty@ch-sainte-anne.fr)

In the context of methadone maintenance programs, immunochemical detection of methadone in urine has evolved, with rapid and sensitive techniques gaining impact in clinical laboratories because of their cost-effectiveness and suitability for automation (1). However, immunochemical methods may lack the specificity necessary for accurate measurement. We report spurious methadone results encountered in a psychiatric hospital.

We have been detecting urinary methadone with the Kinetics Interaction of Microparticles in Solution (KIMS) methodology on the Roche Integra 800 since April 2003 (Roche Diagnostics). The test is used in the qualitative mode, with a methadone concentration exceeding 0.300 mg/L being reported as positive. This technology is known for its steep dose–response curve around the cutoff of the assay (1). In its first formulation, the reagent included a polyclonal antibody that remained free in solution and captured either methadone or a methadone multivalent conjugate coupled on microparticles. In September 2004, Roche Diagnostics switched from the polyclonal antibody to a monoclonal antibody and modified the original KIMS technology by coupling the antibody to the microparticles and keeping the multivalent drug conjugate in solution. This second-generation inhibition assay was expected to improve the sensitivity and specificity of the test (2).

Soon after this change, some clinicians contacted the laboratory about a mismatch between the clinical information and the methadone detection result. Urines from some patients tested positive for methadone without a...
history of methadone ingestion (n = 45). We therefore performed additional tests on these samples, using another immunoassay for methadone (Emit II Plus Methadone; Dade Behring), a gas chromatography assay, and an assay for the methadone metabolite ethylene dimethyl diphenyl pyrrolidine (EDDP; Microgenics). The limits of detection of these assays are 0.02, 0.01, and 0.006 mg/L, respectively, and all of them gave negative results. This problem of false positives concerned 8.4% of samples. In the patients whose urine tested erroneously positive, medical records showed the administration of cyamemazine (n = 40; dose per day ranging from 100 to 600 mg) or of levomepromazine (n = 2; dose per day of 125 and 150 mg), both compounds administered either alone or with alimemazine (n = 8; dose ranging from 30 to 40 mg per day). These psychotropic molecules with a phenothiazine structure are widely prescribed in our hospital and are excreted in urine. In 3 cases, no therapy except olanzapine was recorded.

To address the question of cross-reactivity of psychotropic drugs in the reformulated KIMS methadone assay, drug-free urine was supplemented with different antipsychotic or antidepressant substances dissolved in methanol (final concentrations, 5–100 mg/L). To evaluate the matrix effects of methanol, we added different mixtures of methanol to a negative urine in ratios ranging from 1/20 to 1/4 and recorded constant negative signals. We obtained positive results from 8 mg/L cyamemazine and 57 mg/L alimemazine, giving ~3.8% and 0.5% cross-reactivity, respectively. As Roche Diagnostics describes in the reagent package insert (3), we also obtained positive results from 5 mg/L levomepromazine, 20 mg/L chlorpromazine, 100 mg/L clomipramine, and 100 mg/L thioridazine (giving ~6%, 1.5%, 0.3%, and 0.3% cross-reactivity, respectively). The following psychotropic compounds not mentioned in the package insert did not cross-react at concentrations up to 100 mg/L (cross-reactivity <0.3%): the antipsychotic substances propirizacine, pipothiazine, trifluoperazine, fluphenazine, flupenthixol, zuclopenthixol, olanzapine, clozapine, loxapine, amisulpride, and risperidone and the antidepressant substances tianeptine, dosulepine, viloxazine, paroxetine, citalopram, venlafaxine, and sertraline.

In our experience, only samples from patients treated with cyamemazine or levomepromazine give false-positive results. Nevertheless, in 3 olanzapine-treated cases, no therapy could explain the false-positive result because olanzapine is unlikely to interfere. In these 3 cases, we cannot exclude that a cross-reacting substance may have been administered before the hospitalization. Some metabolites of the psychotropic drugs tested could also induce a cross-reactivity-related artifact in these patients.

Some false-positive results for methadone have already been reported with other techniques and were attributable to metabolites of verapamil (4), diphenhydramine (5), or doxylamine (6). In our patients, these compounds are not an issue of concern. Moreover, the manufacturer’s package insert states that interference from these compounds is potentially negligible (3).

The cross-reactivity related to psychotropic drugs (or their metabolites) represents a drawback of the new Roche assay: psychotropic therapy may be used in a patient on methadone maintenance therapy because relationships frequently exist between mental illness and substance abuse disorders (7). Methadone and EDDP may well be absent in the urine initially, but will appear when the dosage has been increased to a sufficient amount. Thus, methadone or EDDP in urine can serve as a guide to appropriate dosage (8), and interference such as described above will hinder correct dosage adjustment. Adulteration is also a major problem in methadone maintenance, where patients have strong incentives to cheat (9). Therefore, either another methadone assay or detection of the methadone metabolite EDDP should be preferred in a methadone maintenance program. Roche Diagnostics has now included a notice concerning the cyamemazine cross-reactivity in the reagent information. Although the new assay format provides significant analytical improvement for detection of other drugs (2), as far as methadone testing is concerned, the monoclonal antibody used is a source of error, particularly for cases in which drug abuse is combined with a clinical psychiatric condition.

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References

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