served were caused by cross-reactivity of venlafaxine and ODV with the RapidTest PCP assay.

Venlafaxine, designated (R/S)-1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl] cyclohexanol, is a phenethyamine derivative that is chemically unrelated to tricylic, tetracyclic, and other antidepressants. It is the first antidepressant in a new drug class referred to as the serotonin noradrenergic reuptake inhibitors (SNaRIs) (4). Aside from possessing phenyl and cyclohexyl groups, venlafaxine bears little structural similarity to phencyclidine [1-(1-phenylcyclohexyl)piperidine; see Fig. 1]. Given this structural dissimilarity, it is somewhat surprising that venlafaxine or any of its desmethyl metabolites would cross-react with the anti-PCP antibody used in the RapidTest device. However, other examples of unexpected interferences with immunoassays for drugs of abuse have been well documented in the literature, e.g., oxaprozin with the Emit assay for benzodiazepines (5) and efavirenz with the CEDIA test for cannabinoids (6, 7).

In conclusion, we believe our data strongly implicate venlafaxine and ODV as the agents responsible for the false-positive PCP results we observed with the RapidTest device. We have reported our findings to the manufacturer and recommend that all laboratories using these devices be made aware of this cause of false-positive results. Until this interference is eliminated, we have implemented a procedure where all positive PCP results obtained with the Rapid Test must be verified with the Emit II Plus PCP assay before being reported to our Emergency Department.

We gratefully acknowledge the generous contribution of pure samples of venlafaxine and ODV from Wyeth-Ayerst Research, Princeton, NJ.

References

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False-Positive Troponin I Attributed to a Macromplex

To the Editor:
Cardiac troponins (I and T) represent a major improvement in the biochemical approach to the detection and evaluation of myocardial damage (1, 2). Here, we describe a case in which the measurement of cardiac troponin I (cTnI) gave erroneous clinical information because of interference caused by the presence of a macromplex.

The patient, a 78-year-old Caucasian woman with a pacemaker, had a long-standing history of cardiac disease, including a myocardial infarction 1 year before (July 1999), transient ischemic episodes with chest pain, and mitral valve failure. On July 22, 2000, the patient was admitted to the cardiac intensive care unit for an episode of chest pain and dyspnea. On admission, no significant electrocardiographic alterations were found. Biochemical markers were measured on several occasions (e.g., Table 1). Reference values were according to the manufacturers and confirmed in our population. At admission, cTnI and myoglobin (RxL Dimension; Dade-Behring) were increased (1.0 and 96 μg/L, respectively; reference values <0.05 and <70 μg/L), but creatine kinase MB (CK-MB) was not (2 μg/L; reference value <5 μg/L). The patient was moved from the intensive care unit to a medical department on the 2nd day after admission. cTnI ranged from 0.9 to 1.1 μg/L, myoglobin gradually decreased to within the reference interval, and CK-MB remained within normal limits, ranging from 1.5 to 2.3 μg/L. After July 29 (7th day of admission), a progressive increase was observed in cTnI, with the maximum of 10.2 μg/L observed on August 17 (the 26th day after admission).

The patient was discharged as the increased cTnI values were not associated with a worsening clinical status. At follow-up, cTnI values were persistently increased (17.4, 19.0, and 18.8 μg/L on the 34th, 39th, and 47th day after initial presentation, respectively), whereas CK-MB and myoglobin were within their reference intervals. On the 74th day after initial presentation (October 4, 2000), cTnI was still increased (4.0 μg/L) but had decreased from the September 7 value. The variations observed were not ascribable to the imprecision of the assay because the CV was <3% at those concentrations. We found no alterations in renal function or in electrocardiographic or clinical findings in relation to the increased cTnI.

The constant increases in cTnI were also confirmed with the Triage System (Biosite Diagnostic; cTnI values, 23.0, 22.8, and 15.6 μg/L; refer-
ence value <0.19 μg/L) and the Status CS (Dade-Behring; cTnI values, 32.7, 32.5, and 3.8 μg/L; reference value <0.05 μg/L) in several specimens retested on August 30, September 7, and October 4 (Table 1). Cardiac troponin T (cTnT; Elecsys 2010, third-generation assay; Roche Diagnostics) was positive (0.55 μg/L; reference value <0.01 μg/L) in the first sample collected at admission and negative in the following four samples (Table 1). The simultaneous increase of both cTnI and cTnT at admission seems to indicate myocardial damage. The discrepancies between biochemical and clinical data were initially ascribed to the presence of heterophilic antibodies, but no differences were found in cTnT concentrations before and after treatment with heterophilic blocking tubes (17.7 vs 17.1 μg/L; Scantibodies Laboratories) (3). Rheumatoid factor, another interference in immunoassays (4), was undetectable (<10.6 kIU/L; BN II; Dade-Behring).

A progressive discrepancy was found between the observed and expected cTnI concentrations in serial dilutions with either the level 1 (0 μg/L) cTnI RxL calibrator or TnI-free human serum (data not shown). An immunoprecipitation procedure (5) was carried out with anti-IgG, IgA, IgM-specific antisera (Dako Ltd). When the patient’s sample of August 30 was treated with an anti-IgG antiserum, a large decrease in the cTnI concentration was obtained (from 19.0 to 3.0 μg/L; 16% recovery in the sample diluted 1:10 (1 mL in 9 mL) with anti-IgG antiserum), whereas treatment with other antisera did not affect the measured cTnI. These results suggest the presence of an immunocomplex involving cTnI and IgG that produces the persistently high cTnI concentrations, probably because of the prolonged half-life of the IgG. Furthermore, when our patient’s immunoglobulins were separated by precipitation with ammonium sulfate (at 55% saturation) and tested against a sample from another myocardial infarction patient (cTnI = 3.1 μg/L) to verify their behavior toward cTnI of different origins, the cTnI concentration of the patient was totally recovered when treated with IgG.

Our findings suggest the possibility that a modified molecule of cTnI (6) induced the immunocomplex formation, differently from most cases of macroenzymes described in the literature (7) in which a modified immunoglobulin molecule (IgG or IgA) was the responsible complexing factor that bound the normal protein. An immunoblotting analysis carried out in 1.5% agarose with the same anti-cTnI antibodies (kindly provided by Dade-Behring) used in the RxL Dimension assay confirmed the presence of a complex characterized by a molecular mass similar to that of apolipoprotein B-100 (~500 kDa).

Our report is not intended to question the role and value of cardiac troponins in the diagnosis of acute coronary syndromes (8), but to underline the need to identify analytical and pathophysiologic situations that could compromise the measurement of cTnI (and, potentially, cTnT).

References


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To the Editor:

Since the first demonstration of the presence of cell-free fetal DNA in maternal plasma of pregnant women (1), many reports have been published, mainly about fetal sex and RhD determinations, published, mainly about fetal sex and RhD determinations, mainly about fetal sex and RhD determinations.

We evaluated a fully automated sample preparation system (8) combined with real-time PCR. We extracted DNA from maternal serum by use of the MagNA Pure LC apparatus (Roche Biochemicals) with the Total Nucleic Acid LV reagent set (Roche) according to the manufacturer’s instructions. The eluted DNA and PCR reagents were automatically dispensed into PCR capillaries by an integrated PCR reaction set-up procedure. The operator only placed the reagents and samples in the apparatus.

In the first part of the study, cross-contamination between samples was evaluated. We introduced 1 mL of serum from a male and a female in an alternating pattern (Fig. 1A). DNA was eluted in 50 μL of elution buffer. We used 10 μL each of the 32 eluates for real-time PCR amplification of the SRY gene (3). Two clearly distinguishable groups of curves were observed (Fig. 1B): as expected, the 16 extracted DNA samples from male serum gave positive results for the SRY gene, whereas all DNA extracts from the female serum gave negative results. Quantitative results for the male serum, expressed as crossing points (cp), defined as the maximum of the second derivative of the fluorescence curves, revealed a CV of 0.4% at a 30.6 cp value (n = 16).

In the second part of the study, 108 sera from pregnant women (mean gestational age, 11.7 weeks) were analyzed by the above fully automated procedure. Results were compared with those obtained using the conventional manual procedure (3). The results of the two methods were completely concordant. All sera from pregnant women carrying a male fetus were negative for the SRY gene (n = 62), whereas all sera from pregnant women carrying a male fetus were positive (n = 46). With the fully automated procedure, the concentration of male fetal DNA in maternal serum during the first trimester of pregnancy was estimated to range from 5.2 to 64.9 copies/mL (mean, 26.3 copies/mL). This result was similar to those obtained by others (9). The CV of the test in this range of concentration was 2% (one sample analyzed 12 times during one analytical run; mean cp, 38.6; SD, 0.8 cp), which reflects the reproducibility of both DNA extraction and PCR assay.

Analysis of fetal DNA in maternal serum offers new possibilities for noninvasive prenatal diagnosis. A lack of contamination is crucial for subsequent PCR applications, particularly in the field of prenatal diagnosis. Exclusion of contamination during extraction has become a major challenge since the introduction of real-time PCR. The results described here indicate that nearly complete automation of the DNA extraction, amplification, and detection steps can be achieved. This automated procedure could have implications for systematic analysis, such as RhD fe-

Fig. 1. Evaluation of cross-contamination during DNA extraction using MagNA Pure LC instrument.

Thirty-two samples were processed during the same run. (A) 1 mL of either a male or a female serum was introduced in the sample cartridge in an alternating pattern. (B) all extracted DNA samples were analyzed for the presence of the SRY gene by real-time PCR.