Use of Serum Myoglobin Assays for Urine Myoglobin Measurements Can Cause False-Negative Results

To the Editor:

Several conditions, including rhabdomyolysis, trauma, and surgery, are associated with the release of large amounts of myoglobin into the circulation. Saturation of the salvage system of the kidney will produce myoglobinuria, a condition associated with acute renal failure (ARF). The exact mechanism is not known, but precipitation of myoglobin in the tubules and myoglobin-mediated formation of free radicals have been postulated (1).

Early identification of severe myoglobinuria permits acute treatment, thereby avoiding ARF. Quantitative myoglobin assays have several pitfalls, however, including discrepancies in myoglobin recovery (2). The lack of quantitative information precludes differentiating mild myoglobinuria, which probably does not cause ARF, from severe myoglobinuria. Therefore, myoglobin immunoassays developed for serum applications have been adapted for use with urine samples (3–5). Prior reports have recommended alkalinizing urine specimens before storage.

Because quantification of urine myoglobin might be valuable in the early evaluation of patients who might have rhabdomyolysis, a rapid in-house myoglobin assay is desirable. To this end, we evaluated 4 different commercial serum myoglobin assays for their potential to measure myoglobin in urine. These 4 assays can be run on the analyzers present in our laboratory [Integra 700 (Roche), Elecsys 1010 (Roche), Aeroset (Abbott Diagnostics), and BN ProSpec (Dade Behring)]. All 4 assays are based on immunogenic detection of myoglobin, but by different techniques, namely turbidimetry, nephelometry, and a heterogeneous immunoassay. The assays were evaluated for imprecision with control materials provided by the manufacturers. For all assays, interassay CVs were <5% for all tested concentrations (50–1100 μg/L).

Although spiking of purified myoglobin into urine samples could be used to study myoglobin stability in urine, we preferred to use patient-derived material, thereby circumventing the intrinsic instability of purified myoglobin. We collected urine samples from patients with rhabdomyolysis, who were selected because of their dramatically increased serum activities of creatine kinase. The samples were alkalinized with sodium hydroxide solution to a pH >8. The measured myoglobin concentrations in these patients exceeded the linear ranges of the 4 assays. We therefore diluted the urine samples in myoglobin-free urine, which was adjusted to pH 4.5, pH 7, or pH 8.5. Fig. 1 shows the results for patient A. The original myoglobin concentration in the urine of patient A was approximately 30 000 μg/L as measured with the Elecsys 1010 kit. This sample was diluted to a concentration of approximately 2000 μg/L myoglobin. Acidifying the samples dramatically decreased the measured myoglobin concentrations on all 4 platforms (Fig. 1). The lower measured concentration for the Elecsys platform probably reflects variation in the calibration procedure, which was not investigated further. Similar results were obtained with 2 other patient samples, which showed a decrease in the measured myoglobin concentration of at least 5-fold after the samples were acidified. The decrease in measured myoglobin was also observed with urine samples acidified up to pH 6, although to a less pronounced extent (data not shown). These findings, which were obtained with assays that are currently widely available, are in line with older studies of myoglobin stability that used spiked myoglobin as well as urine from patients with rhabdomyolysis (4, 5). To our knowledge, only the BN ProSpec method has previously been investigated (5). We found that the measured myoglobin concentration decreased up to 50% within 1 week of storage of alkalinized urine at 4 °C, −20 °C, or −70 °C, with different kinetics.
in different patients (data not shown).

Because alkalinizing urine can stabilize myoglobin, we attempted to recover myoglobin immunoreactivity in acidic urine. We did not recover any of the measured myoglobin but did observe that myoglobin was stabilized. Immediate alkalinization of urine at the bedside has been suggested as an appropriate sample-collection procedure for myoglobin, because it prevents a major decrease in the myoglobin concentration (4, 5). Urine resides for varying lengths of time in the bladder, however, where it might be acidic. To investigate the influence of a physiological delay in voiding urine from the bladder on the myoglobin concentration in acidic urine, we mimicked this condition by diluting an alkaline urine sample containing 8000 µg/L myoglobin with myoglobin-free urine to 300 µg/L myoglobin. The urine was then acidified to pH 4.5 and incubated aliquots of the acidified urine at 37 °C for various times (0.5, 1, 2, 3, and 4 h). The measured myoglobin concentration was reduced to <10% in <2 h. Similar results were obtained with urine from another patient (data not shown). Thus, the myoglobin concentration in urine may already have become markedly reduced by a physiologically low pH and a long retention time in the bladder by the time a sample can be collected. Such conditions can produce negative results even while the patient is at high risk for ARF.

This process may also be dependent on the magnitude of the myoglobin concentration, causing even more variation in the potential decline in immunoreactivity (5).

Our results show that none of the 4 myoglobin assays developed for serum applications are suitable for measuring myoglobin in urine, suggesting that probably all immunoassay-based myoglobin assays are unsuitable. This finding should alert laboratories that have adapted serum myoglobin assays for measuring myoglobin in urine to re-evaluate their validation process.

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