Immunossays for Serum and Urine Myoglobin: Myoglobin Clearance Assessed as a Risk Factor for Acute Renal Failure

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We compared four immunossays for serum and urine myoglobin. Within-run CVs were 5–13%, with biases seen between assays. Myoglobin was stable for 1 month in serum and 12 days in urine when the pH was adjusted to between 8.0 and 9.5. Hemoglobin caused no interference. We assayed 91 pairs of serum and timed urine specimens from 41 patients admitted for acute trauma or rhabdomyolysis. Most were treated with mannitol and alkalinization. Upon initial presentations, 21 patients with either low serum myoglobin concentrations (<400 μg/L) or high myoglobin clearances (≥4 mL/min) had normal creatinine clearances and no clinical evidence of renal disease. The remaining 20 had low myoglobin clearances. Seven were in rhabdomyolysis-induced acute renal failure, or subsequently developed this complication. We suggest that low myoglobin clearance may indicate a high risk for developing renal failure or may be an early marker for kidney dysfunction. Low myoglobin clearance may prove useful in indicating failure of prophylactic treatment to clear myoglobin.

Indexing Terms: muscle injury/kidney disease/creatinine clearance/rhabdomyolysis/intermethod comparison

Myoglobin, a low-molecular-mass protein found in striated muscles, is released from muscle cells after injury to the skeletal muscles or myocardium and appears in the circulation within a few hours. Numerous case reports implicate high myoglobin concentrations as a cause of acute renal failure (ARF) (1–4). Although the induction of renal disease by myoglobin in patients with crush injuries has been suspected since World War II (5, 6), the underlying pathogenesis is still largely unknown (7). One theory is that under acidic conditions, myoglobin precipitates directly within renal tubules leading to obstruction, an increase in intratubular pressure, and inhibition of the glomerular filtration rate (8). Other studies suggest that inorganic iron causes direct kidney damage through lipid peroxidation and cytotoxicity of proximal tubules (9, 10). Prospective studies have indicated that prophylactic treatment of patients with extensive skeletal muscle injury with mannitol and sodium bicarbonate reduces the prevalence of renal damage, presumably by minimizing precipitation of myoglobin (11). This has become the standard care for such patients (12). Maintenance of optimal acid–base and electrolyte balance, and the assessment of renal and cardiac function, however, is complicated when such treatments are used. In addition, there is a risk of congestive heart failure when critically ill or injured patients are treated with mannitol (8).

Urine myoglobin is qualitatively measured in many laboratories with a urinalysis dipstick. Hemoglobin is also reactive to these dipstick pads, necessitating its removal by addition of 80% saturated ammonium sulfate and centrifugation (13), or by passage through a microconcentrator membrane (14). The salt precipitation method is insensitive, and can produce false-positive results with incomplete removal of hemoglobin and false-negative results if myoglobin is also removed by this process. This has prompted some investigators to believe that the dipstick assay should be abandoned (7).

Quantitative RIAs for myoglobin have been available since the late 1970s and are considerable improvements to the qualitative dipstick assay (15). However, they are laborious, time consuming, and not practical for emergency analysis. Nonisotopic immunossays are currently available for the quantitative measurement of myoglobin in serum (16–18), but, at the time we began this study, none was approved for use with urine. We report here the use of these serum assays for urine, and describe the clinical application of serum and urine myoglobin measurements in patients with skeletal muscle injury.

Materials and Methods
Assays and Controls

We evaluated myoglobin assays on the Opus Plus, Nephelometer, and Turbitime (Behring Diagnostics, Westwood, MA), and Stratus II (Baxter, Miami, FL). All are nonisotopic immunossays. The myoglobin assay on the Opus is a fluorogenic enzyme sandwich immunosassay. The monoclonal capture antibody is linked to glass fibers. A second, polyclonal, antibody is conjugated to alkaline phosphatase. The substrate is 4-methylumbelliferyl phosphate. The rate of production of fluorescent label is proportional to the myoglobin concentration. The other immunossays used here have been described previously (17–19). We followed the manufacturers’ recommendations for the serum assays with no modifications for urine specimens.

Serum and urine creatinine, and serum creatine kinase (CK), were measured on a Hitachi 717 (Boehringer Mannheim Diagnostics, Indianapolis, IN), an Ektachem 700 (Eastman Kodak, Rochester, NY), and a Cobas Fara (Roche Diagnostics, Nutley, NJ). The reference ranges

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for serum creatinine were 7–17 mg/L (males) and 4–15 mg/L (females), and for creatinine clearance, 97–137 mL/min (males) and 35–124 mL/min (females). For total CK, the upper reference limit for males and females was 230 and 150 U/L, respectively. Urine pH was measured with pH paper or with a glass electrode and pH meter.

Experimental Procedures

**Precision.** We used commercial low, medium, and high controls (Behring) for evaluation of the serum assay, and patients' pooled urine samples for the urine assay. The pH of the urine pools was not adjusted before analysis. Within-run CVs were calculated from 20 determinations. Between-run CVs were calculated from duplicates assayed over 10 days.

**Stability.** We aliquoted fresh pooled serum and urine samples and stored them under various pH values and temperatures. Myoglobin concentrations were measured daily for 12 days.

**Linearity.** Serum myoglobin assays, as stated in the package inserts, were linear up to 500, 400, 650, and 1000 µg/L for the Opus Plus, Nephelometer, Turbitime, and Stratus II, respectively. We did not verify these limits. For linearity in urine, we diluted a specimen with a high myoglobin concentration from 2- to 100-fold with the serum diluent supplied by the manufacturer. The composition of the diluents was not disclosed in the package inserts. However, we measured the pH and total protein (Ektachem) and obtained values of 7.48 and 63 g/L, respectively, for the Opus Plus, 7.13 and <10 g/L for the Nephelometer, and 6.43 and 69 g/L for the Stratus II. The Turbitime was not available when these linearity studies were performed.

**Recovery studies.** The recovery of myoglobin from urine was assessed by supplementation studies. A urine sample containing 464 µg/L myoglobin was diluted with a second urine sample containing a trace amount. The dilution factors were varied from 0.0007 to 0.525. The percent recovery was determined by: [measured]/[expected] × 100.

**Hemoglobin interference studies.** We pooled urine samples containing myoglobin within the linear range of the Opus Plus assay, and added to the pooled urine increasing concentrations of hemoglobin from a hemolyzate to a final urine myoglobin concentration of 332 µg/L and a hemoglobin concentration of 0.1–10 g/L. Samples were assayed for myoglobin on the Opus Plus to determine interferences due to hemoglobin.

**Analytical correlation between methods.** We correlated commercial immunoassays for serum and urine myoglobin with a subset of samples from healthy individuals and patients' samples. None of these assays was considered the "reference" procedure; therefore, we used the Deming regression for calculation of analytical correlation. Because we noticed biases in results for patients' specimens with these different methods, we assayed each manufacturer's calibrators on each instrument to determine if biases were due to assay calibrations.

Clinical Procedures

**Reference ranges.** For the determination of the reference range, we obtained 50 serum and 52 urine specimens from laboratory personnel and from apparently healthy individuals seen at an outpatient clinic. Urine creatinine was measured on 32 of these 52 normal samples.

**Clinical study on trauma patients.** This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. We obtained serum and timed urine specimens (usually 2- or 8-h) from 41 patients who were admitted to the surgical intensive care units at Hermann (Houston, TX) and Hartford Hospitals (Hartford, CT) for rhabdomyolysis or trauma from motor vehicle accidents. However, not all of these patients had extensive skeletal muscle damage as a result of these injuries. The diagnoses included drug abuse, fractures of the femur, pelvis, face, and lumbar/thoracic spine, general skeletal muscle ischemia, and closed head injury. In seven of these patients, a diagnosis of ARF was made on the basis of clinical history and laboratory results including urine volume, urinalysis, osmolality, blood urea nitrogen and serum creatinine concentrations, and creatinine clearances. Myoglobin results from the Opus Plus analyzer were used in the clinical study. Serum samples were collected into evacuated red-top tubes (Becton Dickinson, Rutherford, NJ) and centrifuged before analysis. Urine specimens were collected without preservatives and stored at 2–8°C if analyzed within 8 h, or stored at −20°C or lower if the analysis was 8–72 h after collection. All of the data for the clinical study were from serum assayed within 72 h after collection. Stored serum and urine specimens were used to determine the analytical correlation between instruments. For a given group of samples, all assays were performed on the same day. Urine samples were assayed immediately, or adjusted to a pH between 8.0 and 9.5 with 0.1 mol/L NaOH before analysis. Myoglobin clearances (mL/min) were calculated by multiplying the volume of urine flow (mL/min) times the ratio of urine to serum myoglobin concentrations. Unlike creatinine clearance, which is an estimate of glomerular filtration rate, myoglobin clearance is a function of both filtration and tubular catabolism.

**Classification of patients.** For the purpose of proposed interpretations, we classified specimens from trauma patients without renal failure according to the serum and urine myoglobin concentrations measured on the Opus Plus.

Many patients were prophylactically treated with mannitol, volume expansion, urine alkalization, and hemodynamic support throughout the sampling period. Samples from patients not in renal failure and containing low serum myoglobin concentrations (<400 µg/L) were designated group A. Samples with high serum myoglobin concentrations and a high myoglobin clearance (≥4 mL/min) were designated group B. Samples with high serum myoglobin concentrations and a low myoglobin clearance (<4 mL/min) were designated
group C. Samples from six of seven patients with rhabdomyolysis-induced ARF constituted group D. Urine was unavailable after development of renal failure in the seventh patient. In four of these seven patients, serum and urine samples were collected before onset of ARF (including patient 7). We used the Student's t-test for statistical comparison of data between groups.

**Results**

**Analytical Study**

**Precision.** The within-run CVs for serum on the Opus Plus were 6.7, 5.7, and 7.0% for the low, medium, and high controls (50, 107, and 268 μg/L), respectively. The day-to-day CVs for these controls were 6.7, 5.1, and 7.8%. For urine, the within-run CVs were 7.5, 11.9, and 13.2% for urine myoglobin concentrations of 4, 45, and 1324 μg/L, respectively. The day-to-day CVs were 20.0, 14.6, and 14.5%, respectively. The day-to-day CV for the urine pool was adversely affected by the instability of myoglobin at low pHs, and was improved to values seen for serum when the pH of the urine pool was adjusted to between 8.0 and 9.5 before analysis. The CVs for the other analyzers for serum and urine myoglobin also ranged from 5% to 15% (data not shown).

**Stability.** We determined that myoglobin is stable in serum for at least 1 month when stored at 2–8°C. Myoglobin recovery in serum was within 15% for the low pool (mean 55 μg/L) and 10% for the medium and high pools (mean 115 and 300 μg/L) after 28 days. For urine, the stability of myoglobin is dependent on the pH. As shown in Fig. 1, urine samples at pH 5.5 degrade within a few days, even when stored at −70°C. In contrast, samples adjusted to between pH 8.0 and 9.0 exhibited good stability after refrigeration and freezing. We recommend that samples be analyzed immediately, or alkalized before storage at 2–8°C. Our observations of these samples in vitro are similar to clinical observations in vivo: Myoglobin stability and clearance is aided when the pH of urine is high.

**Linearity.** Because very high myoglobin concentrations are often seen in the urine of trauma patients, it was important to determine if the protein-based serum diluent supplied with each kit could be used as a diluent for urine. We diluted high myoglobin samples and plotted observed (y) vs expected (x) myoglobin concentrations. The linear regression curves of these plots produced results that had slopes close to 1.00 with a high degree of linear correlation: Stratus II, y = 0.98x + 6.6 (r = 0.998); Nephelometer, y = 0.98x + 11.8 (r = 0.998); and Opus Plus, y = 1.01x - 14.9 μg/L (r = 0.997). These results indicate that the linearity of the assay in urine is valid to the manufacturer's specification for serum (500, 650, and 1000 μg/L for the Opus Plus, Nephelometer, and Stratus II, respectively), and is maintained when samples are diluted up to 100-fold. Higher dilutions are also likely to produce accurate results. Dilutions of high urine myoglobin concentrations were not as linear when saline or water was used as the diluent (results not shown).

**Recovery.** Table 1 shows that the recovery of myoglobin in urine was 100–117%, mean 108%. These results are within the precision for the urine assay.

**Hemoglobin interference studies.** We detected no significant difference in apparent myoglobin concentrations on either the Opus Plus or Stratus II when urine was supplemented with up to 10 g/L hemoglobin.

**Analytical correlation between methods.** Table 2 summarizes the Deming correlations between the serum and urine assays. Although acceptable correlation coefficients were observed, there were significant proportional biases in these data sets. The Stratus II assay

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**Table 1. Recovery of myoglobin from urine.**

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>Myoglobin, μg/L</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>Expected</td>
</tr>
<tr>
<td>0.0007</td>
<td>16.8</td>
<td>16.2</td>
</tr>
<tr>
<td>0.017</td>
<td>25.7</td>
<td>23.9</td>
</tr>
<tr>
<td>0.070</td>
<td>55.8</td>
<td>47.5</td>
</tr>
<tr>
<td>0.175</td>
<td>108</td>
<td>94.6</td>
</tr>
<tr>
<td>0.350</td>
<td>182</td>
<td>173</td>
</tr>
<tr>
<td>0.525</td>
<td>251</td>
<td>252</td>
</tr>
</tbody>
</table>

* Urine sample containing 484 μg/L myoglobin to which was added another containing 16.1 μg/L.

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**Table 2. Analytical correlation of commercial assays for serum and urine myoglobin.**

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Urine*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opus Plus</td>
<td>Nephelometer</td>
<td></td>
</tr>
<tr>
<td>Stratus II</td>
<td>Nephelometer</td>
<td></td>
</tr>
<tr>
<td>Stratus II</td>
<td>Opus Plus</td>
<td></td>
</tr>
<tr>
<td>Opus Plus</td>
<td>Turbitime</td>
<td></td>
</tr>
<tr>
<td>Stratus II</td>
<td>Opus Plus</td>
<td></td>
</tr>
<tr>
<td>Opus Plus</td>
<td>Turbitime</td>
<td></td>
</tr>
</tbody>
</table>

* Myoglobin concentrations in 13 other patients' samples were below the limit of detection for one or more of these assays (Nephelometer 6, Opus Plus 1, Stratus II 1.2, and Turbitime 50 μg/L). Urine samples were adjusted to a pH between 8.0 and 9.5 before analysis.
normally produced the highest results, followed by the Opus Plus and the Nephelometer for serum. To determine if these biases were due to differences in the assignment of myoglobin calibrators, we assayed these calibrators on each analyzer. Slopes from the calibrator regressions did not always match sample regressions: The Nephelometer calibrators produced a slope of 0.50 when assayed on the Opus Plus (expected slope 1.33, from Table 2); the Opus Plus calibrators produced a slope of 1.64 when assayed on the Stratus II (expected 1.30); and the Nephelometer calibrators produced a slope of 1.41 when assayed on the Stratus II (expected 1.71). Biases observed in patients’ samples are probably due to a combination of factors, including differences in antibody specificities, matrix effects, and lack of an accepted reference standard for myoglobin. The American Association for Clinical Chemistry CK-MB Mass Standardization Committee obtained similar results when it attempted to correlate immunoassays for CK-MB (20).

The Opus Plus and Turbitime produced results that were nearly identical. The correlation of the urine assays produced the same degree of biases between instrument methods (See Table 2). Higher y-intercepts were reported, however, because the range of myoglobin concentrations used in the correlation study was higher.

**Clinical Studies**

Reference ranges. The reference range for myoglobin in serum was determined from a population of healthy individuals. The mean (SD, range) for the Opus Plus was 23.7 μg/L (18.1, 6.6–120), 36.9 μg/L (17.5, 10.5–124) for the Nephelometer, and 46.8 μg/L (25.1, 1.6–178) for the Stratus II. The distribution of values is shown in Fig. 2A. Using the central 95% of the reference population distribution, we determined the reference range for these assays to be 8–66, 13–71, and 19–98 μg/L, respectively (although values below the reference range have no clinical significance). In normal urine, myoglobin concentrations are present at much lower concentrations. For the Opus Plus, all of the 52 urines were above the detection limit of the assay (1.0 μg/L) with a mean, SD, and range of 4.2, 1.3, and 2.2–10.1 μg/L, respectively (Fig. 2B). For the Nephelometer and Stratus II, all but nine and two of the urine specimens, respectively, were below the detection limit of 1.2 μg/L. Based on these values, we used a reference range of 0–7.0 μg/L for the Opus Plus assay and 0–4.0 μg/L for the Nephelometer and Stratus II. For the Opus Plus, using the central 95% of the reference population, we determined the reference range for urine to be 1.3–17 μg myoglobin/g creatinine. We also studied the distribution of normal urine myoglobin results with respect to age and exercise status. In contrast to normal ranges reported for total CK (21), no significant difference in mean results was observed in any of these groups (P < 0.005).

Patients. The results of samples collected from trauma and rhabdomyolysis patients are summarized in Table 3. The urine pH range was similar for all groups and offered no discriminatory ability for predicting renal problems. Total CK was slightly lower in sera of patients with minimal myoglobin release (group A), indicating the minor extent of skeletal muscle injury. Significantly higher total CK was seen in patients in group B than in group A (P <0.05), indicating a higher degree of muscle injury (Table 3). However, no significant difference was observed when comparing CK values between groups B, C, and ARF patients.

Better correlation was seen with myoglobin clearance values and clinical outcome for ARF. Samples from patients in group A all had normal or high creatinine clearances (mean 172 mL/min, Table 3), and none had any clinical or laboratory evidence of renal dysfunction. Only one of these patients had a urine myoglobin concentration >1000 μg/L. Mean creatinine clearance values in this group were higher than the upper reference limit of 137 mL/min; this may have been caused by the increased release of creatinine from skeletal muscles from trauma (8). Inaccuracies in urine volume may also have artificially increased clearances, since bladder rinses were not used to empty the contents before the collection of urine. Fig. 3A illustrates data from one patient from group A with minor skeletal muscle injury. The creatinine clearance was normal in all of the urines produced by the Opus Plus and Nephelometer.

![Fig. 2](image-url) Distribution of normal myoglobin values for serum (A) and urine (B) with the Opus Plus.
Table 3. Summary of mean (range) laboratory results in trauma and rhabdomyolysis patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Samples/patients</th>
<th>pH, range</th>
<th>CK, U/L</th>
<th>Myoglobin, μg/L</th>
<th>Urine myoglobin, μg/g creatinine</th>
<th>Myoglobin Clearance, mL/min</th>
<th>Serum creatinine, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normals</td>
<td>25/17</td>
<td>5.3-8.0</td>
<td>0-230 (males)</td>
<td>0-150 (females)</td>
<td>8-66</td>
<td>0-7</td>
<td>NA</td>
</tr>
<tr>
<td>A</td>
<td>25/17</td>
<td>5.3-8.0</td>
<td>0-230 (males)</td>
<td>0-150 (females)</td>
<td>8-66</td>
<td>0-7</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>21/13</td>
<td>5.2-7.5</td>
<td>28-5000</td>
<td>13-380</td>
<td>218-162000</td>
<td>247-49000 (1230-7500)</td>
<td>500-94000</td>
</tr>
<tr>
<td>C</td>
<td>25/20</td>
<td>4.9-8.0</td>
<td>13-360</td>
<td>218-162000</td>
<td>247-49000 (1230-7500)</td>
<td>500-94000</td>
<td>5-67.4</td>
</tr>
<tr>
<td>ARF</td>
<td>20/6</td>
<td>5.0-8.2</td>
<td>18-800</td>
<td>218-162000</td>
<td>247-49000 (1230-7500)</td>
<td>500-94000</td>
<td>5-67.4</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time between onset of injury and sample collection 2 h-7 days. NA, not applicable.

Fig. 3. Examples of representative cases.
Serial serum (A) and urine (B) myoglobin concentrations (lines, left axis) and creatinine clearance (bars, right axis) in study patients. (A) Patient in group A with minimal skeletal muscle injury. Corresponding myoglobin clearances were 2, 8, and 0.4 mL/min, respectively. (B) Patient in group B with ongoing injury. Myoglobin clearance: 0.01, 0.22, and 5.1 mL/min. (C) Patient in group C with high risk for renal failure. Myoglobin clearance: 0.01, 0.22, and 5.1 mL/min. (D) Patient in group D with rhabdomyolysis-induced nonoliguric renal failure on day 4. Myoglobin clearance: 4.0, 2.3, 2.8, and 2.0 mL/min.

from this patient. The myoglobin clearance was low, indicating minor skeletal muscle injury or the ability of renal tubules to sufficiently catabolize the amount of filtered myoglobin.

High creatinine and myoglobin clearances were observed for patients in group B. The mean creatinine clearance for this group (114 mL/min, Table 3) is significantly different for patients in group A (P < 0.05). All of these samples had urine myoglobin concentrations >1000 μg/L. Fig. 3B is from a patient who had very high serum and urine creatinine concentrations, indicating extensive ongoing skeletal muscle injury. In this case, the high serum myoglobin concentrations were offset by an even higher urine myoglobin concentration, suggesting that the kidneys were able to excrete this myoglobin load. Creatinine clearance values remained within the reference range, and no evidence of renal dysfunction was observed.
Group C patients had decreased myoglobin clearance with significantly lower mean creatinine clearances (87 mL/min) when compared with patients in either groups A or B (P <0.005, Table 3). None of these patients was in renal failure at the time of sample collection. The mean myoglobin clearances in this group were low and were within the range of values observed for patients with ARF (Table 3); 12 of 20 patients had a urine myoglobin concentration that was <1000 µg/L. One of these patients developed ARF. In the remaining eight with urine myoglobin concentrations >1000 µg/L, three developed ARF. Fig. 3C is from a patient who had a low myoglobin clearance at admission and was possibly at high risk for ARF. With therapy, serum myoglobin concentrations decreased with a concomitant increase in myoglobin clearance, indicating that therapy was successful in removing myoglobin. On day 3, we predicted that this patient would be at low risk for acute tubular failure unless additional skeletal muscle injury and release of myoglobin occurred. The data on days 5 and 6 demonstrated no renal impairment on the basis of creatinine clearance, and urine myoglobin concentrations returned to normal. Retrospective review of the medical record revealed that this patient had only a single episode of acute muscle injury that resolved within 2–3 days.

The results of the seven patients with rhabdomyolysis-induced renal failure are given in Table 4. Creatinine and myoglobin clearances were decreased in all samples tested from this group. Fig. 3D shows the results of samples collected on one rhabdomyolysis patient. Before onset of ARF, this patient had high serum and very high urine myoglobin concentrations. Unlike the case in Fig. 3B, however, the myoglobin clearance was low and the patient was classified into the high-risk group C. Acute nonoliguric renal failure developed within a few days after admission, as demonstrated by increasing blood urea nitrogen and creatinine concentrations, and a low creatinine clearance.

Discussion

The development of ARF is highly complex and dependent on many factors such as plasma renal flow, urine flow rate, tubular fluid pH and salt concentrations, and the condition of the kidneys. Severe skeletal muscle disease or injury is also an important cause of ARF. Measurement of total CK in serum is important in assessing the extent of skeletal muscle turnover in these patients. For prediction of renal failure, however, our data show that total CK values do not distinguish patients in group B (no renal failure) from groups C (higher risk) and D (renal failure).

Qualitative dipstick assay for urine myoglobin after precipitation of hemoglobin has been used for years as a basis for initiating prophylactic therapy, although numerous false positives and negatives may result. As a more useful approach, we examined rapid quantitative immunoassays for myoglobin on serially collected timed urine, in conjunction with quantitative myoglobin results on serum collected at the same time.

There are several possible interpretations of our data. Because myoglobinuria has been identified as a cause of renal failure, we tried to determine if myoglobin clearance measurements can be prospectively used to determine risk for ARF. Feinfeld et al. found that four of five patients with myoglobin concentrations >1000 µg/L at admission developed renal failure (2). In our study, only six of 20 patients with urine myoglobin concentrations >1000 µg/L had or developed renal failure. In contrast, all of our patients with rhabdomyolysis-induced renal failure had myoglobin clearances <4 mL/min. We suggest that in the presence of high serum myoglobin concentrations, a low urine myoglobin clearance rate indicates a high risk for subsequent renal disease. We considered this group to be at high risk because four of seven rhabdomyolysis-induced ARF patients initially had serum myoglobin concentrations and clearances that were in this range before the onset of renal failure. In contrast, patients with high serum myoglobin concentrations and clearances (>4 mL/min) had adequate renal function and only one developed kidney failure. For example, the patient shown in Fig. 3C had very high urine myoglobin concentrations, but had no subsequent renal problems. Because the myoglobin clearance was high, the rate of renal excretion exceeded the rate of release from the injured tissue. A low myoglobin clearance alone does not necessarily indicate a risk for renal damage, because patients with minor striated muscle

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Table 4. Mean laboratory results for individual patients with renal failure.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>No. samples</th>
<th>Time to ARF onset, days*</th>
<th>Myoglobin, µg/L</th>
<th>Urine myoglobin, µg/g creatinine</th>
<th>Clearance, mL/min</th>
<th>Serum creatinine, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>2</td>
<td>3828</td>
<td>761</td>
<td>0.8</td>
<td>0.007</td>
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<td>2</td>
<td>1</td>
<td>2</td>
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<td>31 000</td>
<td>46 000</td>
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<td>3</td>
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<td>2</td>
<td>36 500</td>
<td>4170</td>
<td>5040</td>
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<tr>
<td>4</td>
<td>3</td>
<td>4</td>
<td>32 800</td>
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<td>5</td>
<td>1</td>
<td>4</td>
<td>2661</td>
<td>1310</td>
<td>12.7</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>2</td>
<td>21 444</td>
<td>10 587</td>
<td>28 951</td>
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<tr>
<td>7b</td>
<td>1</td>
<td>2</td>
<td>NA</td>
<td>16 300</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* Time from admission to onset of ARF.

b Patient expired on day of collection. One sample was collected before onset of ARF, included in group C. Urine was not available. (This sample was not counted as a paired sample under ARF in Table 2.)

NA, not available.
injury will necessarily have low myoglobin clearances, reflecting the normal renal capacity for reabsorbing myoglobin from the tubular filtrate.

An alternative interpretation of these data is that low myoglobin clearances are the result rather than the cause of abnormal renal function. In this regard, myoglobin clearance measurements might only serve as another renal function test, such as serum creatinine or creatinine clearance. However, even if this were the case, myoglobin clearances might still have some clinical utility as an early marker of renal disease, as we are able to identify patients that have abnormally low myoglobin clearances (such as that observed in patients with overt renal failure) with serum creatinine and clearances near the reference ranges (patients in group C had serum creatinine and clearances of 13.3 mg/L and 87 mL/min, respectively, as shown in Table 3). A high urinary myoglobin clearance may also indicate saturation or impairment of tubular reabsorption and catabolic functions, suggesting the early onset of renal damage. Our data do not currently support this view, since only one of 20 patients with a high myoglobin clearance (15 mL/min) progressed to renal failure. In this one patient, the myoglobin clearance dropped to 2.5 mL/min within 12 h, and renal failure occurred later that same day.

We cannot determine which of these interpretations is correct. We cannot withhold treatment and allow rhabdomyolysis patients to develop renal failure; all patients with severe skeletal muscle injury should be treated prophylactically. However, serum and urine myoglobin measurements may be useful for determining a subset of patients with minor skeletal muscle injury (group A). In these patients, a decision to treat prophylactically with mannitol or alkalization might be delayed or canceled with a consequent reduction in unnecessary risks and costs.

Most of our patients were indeed prophylactically treated with urine alkalization, mannitol, and volume expansion, with the objective of clearing myoglobin. Such treatment affects both the creatinine and myoglobin clearance rates. Nevertheless, because serum creatinine is routinely used to monitor renal function after therapy, the myoglobin clearance rate might be useful to determine the success of therapy in removing myoglobin from the blood. Low myoglobin clearances, in advance of overt renal disease, might indicate the need for more aggressive treatment.

Analytical assays are now available for directly measuring serum and urine myoglobin concentrations. With proper attention to handling and dilution of urine samples and pH adjustment, automated nonsitopetic immunoassays are sufficiently rapid to permit stat analysis. If samples are not assayed on a stat basis, accurate analysis requires alkalization of urine to maintain myoglobin immunoreactivity. The Turbitime analyzer is portable, has a 90-s assay time, and is ideal for satellite or near-patient testing, although the sensitivity is only 50 μg/L. The Nephelometer, Opus Plus, and Stratus II are more automated, have slightly longer assay times (10 min), and have lower detection limits.

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References