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Case Report

Falsely Elevated Plasma Creatinine Due to an Immunoglobulin M Paraprotein

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The most common method for measuring plasma creatinine is based on its reaction with picric acid. However, enzymatic methods are becoming more popular due to improved specificity. We present a case of falsely elevated plasma creatinine values obtained by an enzymatic method that turned out to be due to a monoclonal immunoglobulin M (IgM) paraprotein. A 63-year-old woman evaluated for lung transplantation had falsely increased plasma creatinine levels (1.54-1.71 mg/dL; corresponding to estimated glomerular filtration rates of 32-36 mL/min/1.73 m^2) as measured by the Roche Creatinine plus enzymatic assay when compared with the picric acid–based procedure and several other enzymatic methods, which gave plasma creatinine values of 0.7 to 0.8 mg/dL. Serum protein electrophoresis revealed an IgM κ light chain paraprotein. Removal of high-molecular-weight (>30 kDa) proteins by ultrafiltration reduced the patient's plasma creatinine level by the Roche enzymatic method to 0.7 mg/dL. Addition of the patient's immunoglobulin fraction to plasma from other patients with normal plasma creatinine levels resulted in values that were increased by 0.58 to 0.62 mg/dL. Furthermore, removal of non-IgM immunoglobulins with protein G–coupled beads did not eliminate the interference from the patient's plasma. Taken together, these studies demonstrate that falsely elevated plasma creatinine values by the Roche enzymatic method can be due to an IgM paraprotein.
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INDEX WORDS: Plasma creatinine; estimated glomerular filtration rate (eGFR); artifact; protein interference; measurement error; laboratory assay; renal function; immunoglobulin M paraprotein; IgM; Jaffe method; enzymatic method; Roche Creatinine plus.

Plasma creatinine is important for assessing kidney function because an increase indicates decreased glomerular filtration rate (GFR). The most widely used method for plasma creatinine measurement is the Jaffé method,[1,2] which is based on the reaction of creatinine with picric acid, which results in an orange-red chromophore. In the United States, 70% of laboratories use the Jaffé method.[2] However, a large number of substances interfere with this method, including bilirubin, acetoacetate, and various drugs.[3,4] Newer enzymatic methods are gaining popularity because they are more specific, but they also are subject to various sources of interference.[3,5] We present a case of a plasma creatinine value obtained by the Roche Creatinine plus enzymatic method that was falsely elevated due to a unique monoclonal immunoglobulin M (IgM). Although similar interference in enzymatic methods has been suggested in previous cases,[6,7] there was insufficient investigation to definitively establish involvement of an IgM paraprotein.

CASE REPORT

A 63-year-old white woman with end-stage chronic obstructive pulmonary disease was evaluated for lung transplantation. During the initial assessment, her plasma creatinine was measured at our center using the Roche Diagnostics Creatinine plus enzymatic method, giving an elevated reading of 1.54 (reference range, 0.6-1.1) mg/dL, corresponding to an estimated GFR (eGFR) of 36 mL/min/1.73 m^2 (as calculated by the MDRD [Modification of Diet in Renal Disease] Study equation).[8] The increase was attributed to treatment with lisinopril and celecoxib, and treatment with both drugs was discontinued.

When plasma creatinine was measured at our center 3 months later, there was no improvement; however, the patient's plasma creatinine was within the reference range when measured at an outside laboratory. Plasma creatinine was subsequently measured on the same day at both our laboratory and the outside laboratory: it was 1.51 mg/dL at our institution by the Roche Creatinine plus method and 0.84 mg/dL at the outside laboratory, which used a Jaffé method on the Beckman-Coulter 5800 platform. Corresponding eGFRs were 37 and 74 mL/min/1.73 m^2.

Direct measurement of the patient's GFR using iothalamate gave a result of 74 mL/min/1.73 m^2, which was comparable to the GFR estimated using Jaffé method–measured creatinine. Cystatin C measured at Mayo Medical Laboratories using the Gentian Cystatin C immunoassay on the Roche Cobas c501 was 0.82 (reference range, 0.66-1.26) mg/L and the resulting cystatin C–based eGFR[9] was 94 mL/min/1.73 m^2. When creatinine was measured in whole blood using an enzymatic method, but with

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electrochemical detection (Abbott i-STAT), the result was 0.8 mg/dL (eGFR, 77 mL/min/1.73 m^2), which matched the value obtained by the Roche Diagnostics Jaffé method using a plasma sample. In addition, the patient had no proteinuria or hematuria, and her urea nitrogen level was unremarkable (16; reference range, 8-25 mg/dL). Taken together, these data strongly suggested an interferant in this patient's sample that affected the Roche Creatinine plus enzymatic assay.

While reviewing the patient's chart, it was noticed that a paraprotein monoclonal gammopathy of unknown significance had been detected. Serum protein electrophoresis showed a restricted peak in the gamma region (0.5 g/dL), and immunofixation revealed a monoclonal IgM κ light chain paraprotein. Interference in some, but not all, enzymatic creatinine assays by monoclonal IgM paraproteins has previously been suggested in several patients.[6,7] Ultrafiltration to remove high-molecular-weight proteins reduced the interference in one of the patients.[6] To determine whether this patient's elevated plasma creatinine level was an artifact caused by her paraprotein, we measured creatinine before and after removal of high-molecular-weight (>30 kDa) proteins by ultrafiltration (Amicon; EMD-Millipore). This reduced the patient's Roche Creatinine plus enzymatic assay plasma creatinine value by ~50%, but did not affect results from the Roche Jaffé method (Table 1). To verify that ultrafiltration removed IgM, the patient's serum was examined by immunofixation before and after. As expected, no IgM paraprotein was detected in the filtrate (Fig 1). Importantly, removal of proteins did not alter the creatinine values measured by the Roche Creatinine plus enzymatic assay in another patient sample with elevated plasma creatinine values due to decreased kidney function (1.88 vs 1.90 mg/dL before and after filtration [eGFR, 29 mL/min/1.73 m^2 for both]).

To test whether Roche Creatinine plus enzymatic assay interference occurs in all patients with a monoclonal IgM κ light chain, plasma creatinine was measured in samples from 4 other patients with IgM κ light chain paraproteins (concentrations, 0.6-1.9 g/dL) before and after filtration to remove proteins. The filtration did not affect enzymatic plasma creatinine values in these patients (Table 1). To further test the effect of the patient's IgM on the Roche Creatinine plus enzymatic assay, we isolated immunoglobulins from her serum by precipitation with saturated aqueous ammonium sulfate (40% vol/vol), followed by washing and reconstitution in 0.9% saline solution. The reconstituted immunoglobulins were added to plasma samples from 3 patients with normal plasma creatinine levels to a final concentration of ~0.5 g/dL, which was similar to the index patient's paraprotein concentration. As a control, another aliquot from each patient was diluted with an equal volume of 0.9% saline solution. In all cases, addition of immunoglobulin from the index patient increased the measured creatinine value (Table 1). The mean increase was 0.59 ± 0.01 (standard error) mg/dL. To confirm that the interference in our sample was caused by IgM and not another immunoglobulin, we incubated the patient's plasma sample to protein G Sepharose beads (Sigma), which bind IgG but not IgM. Protein G adsorption did not significantly affect the measured plasma creatinine values (Table 1).

Table 1. Plasma Creatinine Values

Method	Plasma Creatinine, mg/dL: Before	After
Creatinine Before and After Ultrafiltration[a]		
Sample with interference		
Enzymatic	1.53	0.79
Jaffé	0.7	0.6
Patient with kidney failure		
Enzymatic	1.88	1.90
Jaffé	1.8	1.7
Other IgM κ light chain paraprotein samples		
Patient 1		
Enzymatic	0.89	0.90
Jaffé	0.9	0.7
Patient 2		
Enzymatic	1.14	1.13
Jaffé	1.1	0.9
Patient 3		
Enzymatic	0.92	0.90
Jaffé	0.8	0.7
Patient 4		
Enzymatic	0.99	0.97
Jaffé	0.9	0.7
Creatinine Before and After Addition of Immunoglobulin to Control Plasma[b]		
Sample 1		
Enzymatic	0.57	1.19
Jaffé	0.6	0.6
Sample 2		
Enzymatic	0.42	1.00
Jaffé	0.4	0.5
Sample 3		
Enzymatic	0.50	1.08
Jaffé	0.5	0.5
Creatinine Before and After Removal of Non-IgM Immunoglobulin[c]		
Enzymatic	1.66	1.44
Jaffé	0.7	0.6

Note: "Enzymatic" refers to the Roche Creatinine plus enzymatic assay method on the Roche P-modular analyzer. "Jaffé" refers to the Roche Jaffé method on the Roche P-modular analyzer. Conversion factor for serum creatinine in mg/dL to μmol/L, ×88.4.

Abbreviation: IgM, immunoglobulin M.

[a]Creatinine was measured before and after ultrafiltration to remove proteins > 30 kDa in the patient with interference and in 2 controls also with monoclonal IgM κ light chain.

[b]Creatinine was measured in control samples before and after addition of immunoglobulins from the original patient's serum.

[c]Creatinine was measured in the original patient's sample before and after removal of non-IgM immunoglobulin by protein G absorption.

DISCUSSION

The difference in our patient's plasma creatinine values between the Roche Creatinine plus enzymatic method used at our institution and those obtained from the Jaffé method on the Olympus/Beckman Coulter 5800 (0.69 mg/dL) far exceeded known bias and variation between methods and platforms.[1,2] One study using fresh-frozen human serum and isotope-dilution mass spectrometry–traceable standards found that bias varies more across manufacturers than across methods, and that the maximum average bias across 50 manufacturer/method groups was 0.36 mg/dL.[1] Our investigation clearly showed that

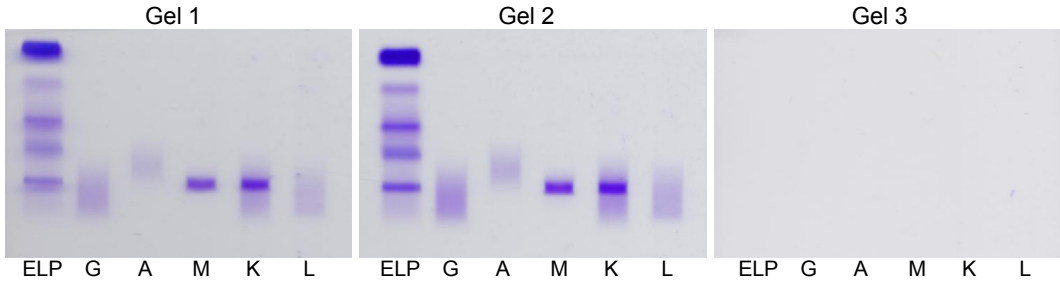

Figure 1. Immunofixation before and after ultrafiltration of serum. Serum from the patient was filtered to remove protein and immunofixation was performed on the samples to test for the presence of immunoglobulin M (IgM) κ light chain. Gel 1, serum before filtration. Gel 2, concentrate. Gel 3, filtrate. Abbreviations: ELP, serum protein electrophoresis; G, IgG; A, IgA; M, IgM; K, κ light chains; L, λ light chains.

the interference in the Roche enzymatic assay observed with this patient's samples was due to her IgM κ light chain paraprotein.

This interference had a direct effect on the patient's care because the elevated plasma creatinine values from the Roche Creatinine plus enzymatic assay delayed her from being on the lung transplant list. Our demonstration that the elevated values were the result of an analytic interference allowed her transplantation evaluation to continue. This case highlights the importance of communication between clinical teams and the laboratory when unexpected and seemingly unexplainable results are reported from the clinical laboratory. Here, the patient's outside plasma creatinine results were known to the clinicians but not to the laboratory. Fortunately, the discrepancy was brought to the attention of the laboratory by the clinical team. Without this communication, the patient's lung transplantation evaluation may have been further delayed.

The biochemical mechanism of this paraprotein interference is unclear. Both the i-STAT and Roche enzymatic methods used in our laboratory measure hydrogen peroxide generated in the sarcosine oxidase–catalyzed conversion of sarcosine to glycine and formaldehyde. However, the methods of hydrogen peroxide detection differ. The i-STAT uses electrochemical detection, whereas the Roche method measures a chromogenic quinone produced by oxidative condensation of 4-aminophenazone and 2,4,6-triiodo-3-hydroxybenzoic acid by hydrogen peroxide. It is possible that the IgM interfered in this reaction, which is not part of the i-STAT chemistry. Interestingly, IgM paraprotein interference has been reported in the Siemens ADVIA enzymatic creatinine method, but not the Abbott Architect method,[7] both of which use an enzymatic reaction similar to that of the Roche Creatinine plus method. It has been suggested that precipitation of IgM may occur under certain reaction conditions.[6] However, the reaction conditions used in all the enzymatic methods are similar. The remaining difference among these 3 enzymatic methods is the chromogen. It is possible that certain chromogens bind to specific IgM paraproteins depending on their variable region. The fact that the IgM paraproteins from 4 other patients did not cause interference suggests that the phenomenon depends on the variable region of the monoclonal antibody. Thus, only a small number of patients would be expected to have erroneous results due to a paraprotein.

In conclusion, we presented a case of a patient who was misdiagnosed with decreased GFR based on a laboratory plasma creatinine measurement using the Roche Creatinine plus enzymatic assay. A thorough evaluation revealed that the patient did not have decreased GFR and that the elevation of plasma creatinine level was due to a monoclonal paraprotein that was interfering with the assay. It is important that clinicians be mindful that substances, including some paraproteins, can interfere with plasma creatinine and not label patients inappropriately with a diagnosis of decreased GFR without contacting their laboratory.

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