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The pH of chemistry assays plays an important role in monoclonal immunoglobulin interferences

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ABSTRACT

Objectives: Immunoglobulin paraproteins can interfere with multiple chemistry assays. We want to investigate the mechanisms of immunoglobulin interference.
Design and methods: Serum samples containing paraproteins from the index patient and eight additional patients were used to investigate the interference with the creatinine and total protein assays on the Beckman Coulter AU5400/2700 analyzer, and to determine the effects of pH and ionic strength on the precipitation of different immunoglobulins in these patient samples.
Results: The paraprotein interference with the creatinine and total protein assays was caused by the precipitation of IgM paraprotein in the index patient's samples under alkaline assay conditions. At extremely high pH (12–13) and extremely low pH (1–2) and low ionic strength, paraprotein formed large aggregates in samples from the index patient but not from other patients.
Conclusions: The pH and ionic strength are the key factors that contribute to protein aggregation and precipitation which interfere with the creatinine and total protein measurements on AU5400/2700. The different amino acid sequence of each monoclonal paraprotein will determine the pH and ionic strength at which the paraprotein will precipitate.

1. Introduction

Cases of monoclonal immunoglobulin (or 'paraprotein') interference with a wide variety of chemistry and immunochemistry tests on various automated chemistry or nephelometry (including turbidimetry) analyzers have been reported. Besides a few systematic studies—such as paraprotein interference with the measurement of total and direct bilirubin and high density lipoprotein cholesterol (HDL-C) on two automated chemistry analyzers using 88 samples with paraproteins [1], and the study of interference with a direct bilirubin assay using 117 samples containing paraproteins

Abbreviations: ; AMR, analytical measurement range; AU2700, Beckman Coulter AU2700 analyzer; AU5400, Beckman Coulter AU5400 analyzer; CV, coefficient of variation; HCl, hydrochloric acid; HDL-C, high density lipoprotein cholesterol; IFE, immunofixation electrophoresis; IRB, Institutional Review Board; LPL, lymphoplasmacytic lymphoma; NaCl, sodium chloride; NaOH, sodium hydroxide; Cobas 8000, Roche Cobas 8000; SPEP, serum protein electrophoresis; WM, Waldenström's macroglobulinemia

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[2]—the majority of these reports described specific cases of either IgM, IgG, or IgA paraprotein interference with various laboratory tests. There have been several reports of IgM paraprotein interference with albumin [3], glucose [4,5], C-reactive protein [6], uric acid [7], HDL-C [8–10], total bilirubin [8,11], phosphate [5], creatinine [12–15], calcium [16], and total protein [17–19]. Interestingly, each of these cases described individual IgM paraprotein interferences with a particular assay on a particular platform. When other samples containing IgM paraproteins were tested with the same assay, the interferences were typically not observed. When the original samples were tested on different platforms, the interference may or may not be observed [8,11,13–15]. In some reports, the interference was believed to be caused by the precipitation of paraproteins under assay conditions [1,2,5,11]. We have encountered a case of a unique IgMκ paraprotein that exhibited interferences with the creatinine, total protein, and direct bilirubin assays on the Beckman Coulter AU5400 (AU5400) and AU2700 (AU2700) analyzers (Beckman Coulter, Brea, CA).

The patient was a 72 year-old male (index patient) who was referred to UCLA Medical Center for workup of his renal insufficiency indicated by elevated serum creatinine levels which fluctuated between 1.5 and 3.2 mg/dL over a six month period with an unrevealing renal ultrasound study. The initial laboratory evaluation at UCLA revealed an M-spike protein (paraprotein) with a concentration of 3.4 g/dL by serum protein electrophoresis (SPEP) which was identified as IgMκ by immunofixation electrophoresis (IFE). The index patient was diagnosed with Waldenström's macroglobulinemia (WM) supported by immunohistochemical and flow cytometric studies on a bone marrow biopsy sample. Thereafter, serum total protein, creatinine, quantitative IgM, viscosity, and M-spike by SPEP were tested on a regular basis to follow the patient's disease course. About three months after the presentation to our institution, a discrepancy in the patient's total protein results was recognized by the clinical pathologist who was interpreting the patient's SPEP. A total protein result of 7.1 g/dL was reported on the sample which had an albumin result of 3.0 g/dL and IgM concentration of >6.4 g/dL. This finding triggered a laboratory investigation of the total protein and other chemistry tests performed on this sample which revealed that the incorrect low total protein result of 7.1 g/dL was generated by the on-board dilution protocol. When the sample (Sample 1A) was initially tested with AU5400, a total protein result of 10.2 g/dL was obtained which exceeded the upper limit of the validated AMR of 10.0 g/dL. The instrument initiated the rerun with on-board dilution and obtained a result of 7.1 g/dL. This problem was recapitulated when the same sample was repeated on the AU2700 (the AU5400 and AU2700 use the same chemistry assays) with an initial result of 10.2 g/dL and a rerun result of 7.2 g/dL obtained with the on-board dilution. We also found the index patient's creatinine results puzzling, which in contrast to his previous elevated results (both the method and platform are unknown to the authors), became undetectable (below the lower limit of detection) on three occasions after being referred to our institution without having any treatment for renal infufficiency. The creatinine result on Sample 1A was cancelled due to technical difficulties to obtain a consistent result. Therefore, this study investigates the causes of the interference with the creatinine and total protein assays on the AU5400/2700 and identifies the factors that cause the IgM paraprotein to precipitate.

2. Materials and methods

2.1. Patient specimens

Serum samples from the index patient (Subject 1) and randomly selected thirteen other patients (Subjects 2–14) were used in this study. Nine samples from the index patient (Samples 1A–1I) collected from different times, and eleven samples from eight additional patients who had elevated monoclonal IgM (Samples 2A, 2B, 3A, 3B, 3C, 5A–10A), three samples from a patient with elevated biclonal IgMλ and IgMκ (Samples 4A, 4B, and 4C), a sample with elevated monoclonal IgG (Sample 11A), and a sample with polyclonal hypergammaglobulinemia (Sample 12A) were used in the studies of creatinine interference as well as the causes for protein precipitation. In addition, two samples (Samples 13A, and 14A) without elevated immunoglobulins were used as controls for the studies. Note that both numbers and letters are used to name a sample with the numbers denoting subjects and the letters denoting samples collected at different times. This study was performed using de-identified and discarded samples that were obtained for patient care and not for the purpose of research; therefore, Institutional Review Board (IRB) approval was not required.

2.2. Creatinine and total protein assays

Creatinine and total protein were measured on the AU5400/2700 analyzers and the Roche Cobas 8000 (Cobas 8000) (Roche Diagnostics, Indianapolis, IN) respectively. The creatinine assay on the AU5400/2700 uses a modified kinetic Jaffé method under alkaline conditions (pH around 12.7–12.9) and the result is determined by the rate of change of the absorbance at 520 nm. The creatinine reagent contains 120 mmol/L sodium hydroxide (NaOH), 2.9 mmol/L picric acid and some unspecified preservatives in the final reaction. The order of addition for samples and reagents to the reaction cuvette is as follows: 8 μL of the sample, 48 μL of the R1 reagent (containing NaOH), 36 μL of water, and 48 μL of the R2 reagent. The analytical measurement range (AMR) of the creatinine assay is 0.2–25.0 mg/dL. The coefficient of variation (CV) is 0.8% at the creatinine level of 0.93. The creatinine assay on the Cobas 8000 uses an enzymatic method performed under a less alkaline condition (pH around 8.1) with an AMR of 0.06 to 30.5 mg/dL, and CV of 1.3% at the creatinine level of 1.0 mg/dL. Both the AU5400/2700 and the Cobas 8000 use a biuret method for testing total proteins at alkaline conditions (pH around 12–13).