Paraproteins Are a Common Cause of Interferences With Automated Chemistry Methods

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Context.—Previous studies have shown that paraproteins caused spurious results on individual analytes including total bilirubin (TBIL), direct bilirubin (DBIL), or HDL-cholesterol (HDL-C). Studies demonstrating paraprotein interferences with multiple analytes measured by different analyzers have not been reported.

Objective.—To systematically investigate interferences of paraproteins on TBIL, DBIL, and HDL-C measured by the Roche MODULAR and the Olympus AU2700.

Design.—Eighty-eight serum specimens with monoclonal gammopathies were analyzed using the Roche MODULAR and the Olympus AU2700. Paraprotein interferences with the MODULAR and AU2700 were identified by abnormal absorbance curves and confirmed by results from the Ortho Vitros 950 or inconsistent laboratory information.

Results.—Spurious results occurred in 89 of 528 measurements; 29 specimens did not demonstrate any interferences whereas 26 specimens gave spurious results in 2 to 4 of the 6 assays. Paraprotein interferences caused spuriously high levels of TBIL in 4 sera measured by the MODULAR. In contrast, paraprotein interferences on DBIL were observed by at least 1 method in 44% (39/88) of sera assayed, occurring almost exclusively with the AU2700. Paraprotein interferences with HDL-C results were present in 35% of specimens assayed with the MODULAR and 16% of specimens assayed with the AU2700. In specimens with interferences, spuriously low AU2700 DBIL, MODULAR HDL-C, and AU2700 HDL-C results occurred with 28%, 90%, and 91% of specimens, respectively.

Conclusions.—We demonstrated that paraprotein interferences with TBIL, DBIL, and HDL-C are relatively common and provided explanations why these interferences occurred. Although it is difficult to predict which specimens cause interferences, spurious results appeared method and concentration dependent.

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Monoclonal immunoglobulins, also known as paraproteins, have been reported to interfere with a number of routine chemistry methods including analyses for creatinine, C-reactive protein, glucose, iron, inorganic phosphate, and uric acid. Recent studies have shown that paraproteins caused false-positive results for total bilirubin (TBIL) and direct bilirubin (DBIL) and false-negative results for HDL-cholesterol (HDL-C). The frequency of paraprotein interferences with these analytes on automated chemistry analyzers was reported to be very rare, and comparative studies of paraprotein interferences with TBIL, DBIL, and HDL-C have not been reported with major automated chemistry analyzers. These interferences may cause incorrect diagnoses, inappropriate treatments, longer hospital stays, and increased morbidity in patients who already may be gravely ill. We systematically investigated the potential interference of paraproteins on measurements of TBIL, DBIL, and HDL-C using 88 serum specimens collected from patients with monoclonal gammopathies and measured using 2 commonly used automated chemistry analyzers.

MATERIALS AND METHODS

This study was conducted as part of a laboratory performance improvement program for patient result reporting. Serum specimens were collected from patients with monoclonal gammopathies determined by serum protein electrophoretic and immunofixation studies during a 1-year period. The sera were free of hemolysis, icterus, and lipemia by routine index checks on automated analyzers. None of the specimens were duplicates or triplicates; if they were from the same patients, they were obtained at different times.

Serum protein electrophoretic and immunofixation studies were performed using the SPIFE 3000 (Helena Laboratories, Beaumont, Tex), and serum total protein concentrations were determined with the Roche P800 MODULAR (Roche Diagnostics, Indianapolis, Ind). Paraprotein concentrations were quantitated by the Quickscan 2000 (Helena Laboratories).

Serum levels of TBIL, DBIL, and HDL-C were measured independently using the Roche P800 MODULAR, Olympus AU2700 (Olympus Diagnostics, Melville, NY), and Vitros 950 (Ortho-Clinical Diagnostics, Rochester, NY) automated chemistry analyzers.

Paraprotein interferences with the Roche MODULAR and Olympus AU2700 were identified by an abnormal reaction absorbance curve obtained directly from the reaction monitor integrated into the automated analyzers and confirmed by at least 1 additional finding. Absorbance curve data from the Olympus AU2700 printouts were plotted manually, whereas similar data...
Figure 1. Scatter plot of total bilirubin (TBIL) concentrations measured by 2 automated chemistry analyzers. ○, specimens without interferences by paraproteins; △, specimens displaying interferences by paraproteins measured by the Roche MODULAR. * To convert to SI units in micromoles per liter, multiply values in milligrams per deciliter by 17.104.

from the Roche MODULAR were used directly. Because it had been shown that the Vitros 950 did not provide spurious results for DBIL and TBIL, it was used for confirmation of interferences.12,14,16 The Ektachem slide method used in the Vitros 950 was interference free because the specimens had to penetrate through several layers before reaching the reaction layer, and the interfering compounds were probably filtered out.20 Therefore, when potential interferences were identified and an adequate specimen remained, confirmation of the spurious results was made by additional measurements using the Vitros 950, except for HDL-C, as our instrument did not have this method included in its test menu. Paraprotein interferences also were confirmed by specimens with negative DBIL or negative HDL-C results, specimens with abnormally high TBIL levels from nonicteric patients without evidence of liver diseases and hemolysis, or specimens with DBIL levels higher than TBIL levels.

The Roche MODULAR TBIL assay is a chromogenic 2-point end point assay modified from the diazo method21 that is performed in 1 cuvette. First the patient’s serum is incubated with reagent 1 (R1), which contains surfactant to accelerate the reaction and to solubilize protein. Then, reagent 2 (R2) containing a reagent 1 (R1), which contains surfactant to accelerate the reaction, is added in the Vitros 950 was interference free because the specimens had to penetrate through several layers before reaching the reaction layer. Before R1 is added, the serum specimen should not be turbid. In contrast, the Olympus AU2700 TBIL assay is a 2-cuvette, 1-point end point assay that uses similar reagents. Both the reaction cuvette and the blank cuvette contain surfactant, whereas the chromogen is not added to the blank cuvette.

The Roche MODULAR and Olympus AU2700 DBIL assays resemble their manufacturers’ TBIL assays, other than that a solubilizing agent such as surfactant is not added. When paraprotein interferences with DBIL initially were suspected in the specimens with either negative DBIL or negative HDL-C results, specimens with abnormally high TBIL levels from nonicteric patients without evidence of liver diseases and hemolysis, or specimens with DBIL levels higher than TBIL levels were used directly. Because it had been shown that the Vitros 950 did not provide spurious results for DBIL and TBIL, it was used for confirmation of interferences.

RESULTS

The present study included 88 serum specimens from 52 patients with monoclonal gammopathies treated at a major municipal hospital or a university hospital. The paraprotein concentrations of these specimens ranged from 0.5 to 8.9 g/dL with a mean of 3.67 g/dL. The majority (60%) of the paraproteins were immunoglobulin (Ig) (κ, 18% were IgG κ, 11% were IgA λ, and 7% were IgM κ). Specimens with paraprotein concentrations less than 3.0 g/dL had spurious results in 7.2% of assays; when paraprotein concentrations were 3.0 to 5.0 g/dL, spurious results were seen in 22.4% of assays, and when paraprotein concentrations were greater than 5.0 g/dL, spurious results were seen in 31.3% of assays.

Spurious results occurred in 89 of 528 measurements; 29 specimens did not demonstrate any interferences, whereas 26 specimens gave spurious results in 2 to 4 of the 6 assays. Of 88 serum specimens, 4 specimens (4.3%) from 3 patients with monoclonal gammopathies displayed spuriously high Roche MODULAR TBIL concentrations. The interferences in 3 specimens initially were suggested by nonicteric sera with abnormally high TBIL values of greater than 25 mg/dL (428 μmol/L), (Figure 1) and subsequently were identified and confirmed along with a fourth specimen by abnormal reaction absorbance curves (Figure 2, C and D) compared with those obtained from normal control serum (Figure 2, A), measurements by the Vitros 950, and icteric serum without measurable paraproteins (Figure 2, B). In our study, the interferences were found exclusively in specimens from patients with IgG κ or IgG λ monoclonal gammopathies, and in these 4 specimens, the paraprotein concentrations (mean, 5.45 g/dL) were higher than the overall mean of the 88 monoclonal proteins studied.

When we manually performed the Roche MODULAR TBIL assay in test tubes according to manufacturer’s suggested protocol using a specimen with the suspected in-
terference, the specimen was slightly turbid visually before adding R1 but became clear after the addition of R1. However, the addition of R2 caused the reaction mixture to become clear instead of the red translucent appearance observed with the control patient's specimens. When the TBIL concentrations of these sera were measured independently by the Olympus AU2700 and Vitros 950 automated chemistry analyzers, no interferences were identified with these 2 instruments.

Using the criteria we established for interference, 39 (44%) of 88 specimens were found to display interferences with DBIL on the Olympus AU2700 (Figure 3). However, only 1 specimen was found to have interferences with DBIL when measured by the Roche MODULAR, and this result was confirmed as spurious by the Vitros 950.

We found that these interferences occurred when the reaction absorbance curve of the blank cuvette (DBIL-B) did not parallel the absorbance curve of the reaction cuvette (DBIL-C) (Figure 4). The Olympus instrument was programmed to subtract the absorbance of the blank cuvette from the absorbance of the reaction cuvette at the end point, then tally the difference and convert this difference into a numerical result. If the absorbance of the blank cuvette was lower than the end point absorbance of the reaction cuvette, the instrument reported a positive value (Figure 4, A); in contrast, if the absorbance of the

Figure 2. Reaction absorbance curves of the Roche MODULAR total bilirubin (TBIL) assay monitored. A, Normal control serum. B, An icteric patient serum without paraprotein. C and D, Nonicteric, nonhemolytic serum specimens containing paraproteins. The gray vertical bars indicate the points at which measurements are taken to calculate results. IgG indicates immunoglobulin G.
Figure 3. Scatter plot of direct bilirubin (DBIL) measurements made by 2 automated chemistry analyzers. ● Specimens without evidence of paraprotein interferences; △, specimens displaying interferences by paraproteins measured by the Olympus AU2700; ■, specimens displaying interferences by paraproteins measured by the Olympus AU2700 and the Roche MODULAR. * For SI units in micromoles per liter, multiply results in milligrams per deciliter by 17.104.

Figure 4. Absorbance curves from the Olympus AU2700 direct bilirubin (DBIL) assay. A and B, Abnormal reaction absorbance curves of the Olympus AU2700 DBIL assay from 2 patient specimens containing paraproteins. The DBIL assay is a 2-cuvette, end point assay utilizing a sample blank; the sample blank cuvette (DBIL-B) absorbance is subtracted from the color cuvette (DBIL-C) absorbance to determine the net reaction absorbance. The arrows indicate reaction end point.

Among the 39 specimens with Olympus AU2700 DBIL interferences, 14 specimens had spuriously high values and 25 specimens had spuriously low values. However, the specimens displaying interferences with the Olympus AU2700 DBIL method tended to give irreproducible DBIL values, as demonstrated by a specimen that varied from falsely positive (13.0 mg/dL [222 µmol/L]) to falsely negative (–17.3 mg/dL [296 µmol/L]) values when measured repeatedly (data not shown).

Figure 5 depicts 31 (35%) of 88 specimens that demonstrated HDL-C interferences with the Roche MODULAR, and of these, 90% were spuriously low. In contrast, 14 (16%) of 88 specimens demonstrated HDL-C interferences when measured by the Olympus AU2700. Of these interfering sera, 7 demonstrated interferences with both methods.

Examples of absorbance curves demonstrating interferences with HDL-C as identified by abnormal reaction absorbance curves compared with those from control specimens and confirmed by negative HDL-C values are shown in Figure 6. In Figure 6, B and C, the Roche MODULAR absorbance values of the blank obtained just before the addition of R2 were higher than after the addition of R2, thereby giving spurious results.

There was a much higher baseline absorbance before the addition of R2 in the specimens displaying interferences with the Olympus AU2700 HDL-C method compared
with that in the control specimens; after the addition of R2, absorbance values did not increase as much as expected (Figure 6, D), thereby accounting for the spuriously low values of HDL-C. Visually checking cuvettes containing specimens with the interference also demonstrated white precipitates that were formed after the addition of R1.

The paraprotein interferences appear to be concentration dependent, as dilution of patient sera and treatment of the patient by therapeutic plasmapheresis or chemotherapeutic agents causing decreasing monoclonal proteins minimized paraprotein interferences (data not shown).

COMMENT

Our studies demonstrate that the interferences with TBIL, DBIL, and HDL-C are relatively common in the presence of paraproteins. Comparing results among different analyzers and monitoring absorbance curves led us to conclude that paraprotein interferences are more prevalent than previously reported. Pantanowitz and coworkers11 found that artifactual TBIL occurred in 2 (1.2%) of 115 patient sera, whereas Nauti et al14 reported that paraprotein interferences with the Olympus AU2700 DBIL were observed in only 3 (1.5%) of 200 serum specimens. Spuriously low levels of HDL-C on the Roche 917 analyzer caused by paraprotein were found in 2 (13%) of 15 patient sera, whereas Nauti et al14 reported that paraprotein interferences with the Olympus AU2700 DBIL were observed in only 3 (1.5%) of 200 serum specimens. Spuriously low levels of HDL-C on the Roche 917 analyzer caused by paraprotein were found in 2 (13%) of 15 patient sera, whereas Nauti et al14 reported that paraprotein interferences with the Olympus AU2700 DBIL were observed in only 3 (1.5%) of 200 serum specimens.

A recent study showed that paraprotein caused only spuriously high levels with Olympus AU2700 DBIL measurements.14 However, in contrast, our study demonstrated that most DBIL interferences (64%) were spuriously low when measured by the Olympus AU2700 DBIL. Because the Olympus DBIL assay gave exceedingly variable repetitive results ranging from a markedly positive to markedly negative interferences in specimens containing a monoclonal protein, we believe that describing an interference as negative or positive is unsatisfactory.

The paraprotein interferences with TBIL, DBIL, and HDL-C appeared to be method dependent. The interferences with TBIL and HDL-C mainly were observed with the Roche MODULAR, while DBIL interferences occurred most frequently with the Olympus AU2700.

The Roche TBIL assay is a 1-cuvette, 2-point end point assay. Because the addition of R2 to the reaction mixture changed the assay conditions to more acidic (pH 1–2), paraproteins could form insoluble complexes with R2, which cause the interferences. On the other hand, the Olympus TBIL assay is a 2-cuvette end point assay. Both cuvettes, the sample blank cuvette and the reaction cuvette, contain the same reagents, except the chromogen is only in the reaction cuvette. Therefore, the condition in the assay is not changed as there is no addition of R2.

The chromogenic reaction of the DBIL assay occurs at a very low pH (<1). A “protein stabilizing agent” is included to avoid protein precipitation. It appears that the solubilization capacity of the Olympus AU2700 protein stabilizing agent is not sufficient to prevent the proteins in solution from precipitating at such an acidic pH, which may cause more interferences with DBIL on the Olympus AU2700.

As mentioned in the “Results,” spuriously low HDL-C results observed in the patients with monoclonal gammopathies on the Roche MODULAR and the Olympus AU2700 may be due to the high baseline absorbance in the presence of R1 reagent. The R1 reagent contains a buffer system to selectively form a water-soluble complex.
Paraprotein Interferences With Automated Methods

Paraprotein interferences with automated methods can lead to inaccurate results. This can be particularly problematic with cholesterol measurements, as demonstrated by the comparison of high-density lipoprotein cholesterol (HDL-C) measurements made by two automated chemistry analyzers: the Roche MODULAR and the Olympus AU2700. The study shows that paraprotein interferences with DBIL and HDL-C are relatively common, and spurious results of TBIL, DBIL, and HDL-C appear to be methodology and concentration dependent. In a few patients followed for a period of time, treatment that decreased paraprotein concentrations caused interferences to disappear, while disease progression and increasing monoclonal protein concentrations caused interferences to increase. It is important to appreciate the occurrence of these interferences to avoid misinterpretation of the results, especially because low HDL-C (<40 mg/dL [684 μmol/L]) is considered to be an independent risk factor for coronary heart disease. To automatically detect the interference, Smogorzewska et al suggested manually programming the analyzer to trigger a flag when the difference of absorbance between certain points was above a certain threshold.
preset value for the Roche MODULAR TBIL assay or the absorbance immediately before the addition of the R2 agent was above a designated value for the Roche AU2700 HDL-C assay. This may help when the abnormal absorbance curves follow a similar kinetic pattern. However, our studies showed that the abnormal reaction curves of the Roche MODULAR TBIL assay and the Olympus AU2700 DBIL assay have different patterns (Figures 2 and 4), which may lead to missing some interferences if a programmable rule is used. An approach that we favor for bilirubin assays is to program the analyzer to automatically flag results for confirmation when specimens have TBIL results greater than 20 mg/dL (342 µmol/L) and the icteric flag is not markedly positive. To prevent results preceded by a negative sign from being reported, we recommend programming the laboratory information system to reject patient DBIL and HDL-C results that are preceded by a minus sign.

In conclusion, we found that paraprotein interferences with clinical laboratory measurements are far more frequent than previously reported. If an interference occurs with one assay, it is likely that other assays also will be affected. These types of interferences appear in part to be concentration dependent. Specimens with paraproteins cause spurious results by causing precipitation in TBIL, DBIL, and HDL-C assays.

References