A paraprotein interference and its management in clinical laboratory

Özlem Çakır Madenci*, Nihal Yücel, Lale Köroğlu Dağdelen, Yusuf Temel, Aycan Bölük, Asuman Orçun Kaptanağası

Abstract: In the present study we describe a patient who has interference due to paraproteinemia in her laboratory results. In a patient with a total protein concentration of 10.8 g/dL, a direct bilirubin result higher than total was detected. She also had discordant results in her whole blood count parameters. Further investigation was performed on this patient. Presence of any cold agglutinin and cryoglobulin was tested and excluded first. After 2-mercaptoethanol (2-ME) treatment, patient was identified as Ig-M Kappa monoclonal gammapathy on immunofixation electrophoresis (IFE). Direct bilirubin interference disappeared after removal of the paraprotein by polyethylene glycol (PEG) precipitation. Laboratory specialist should know paraprotein interference and be able to manage it.

Keywords: Monoclonal Gammapathy, paraprotein interference, direct bilirubin

Introduction

Monoclonal gammopathy is characterized by the presence of a monoclonal immunoglobulin or paraprotein in the serum or urine and occurs in clinical situations as multiple myeloma, Waldenstrom's macroglobulinemia (WM), plasmacytoma, amyloidosis and monoclonal gammopathy of undetermined significance. Monoclonal gammopathies may or may not be together with cryoglobulins. In cryoglobulinemias, there is mono, oligo- or polyclonal immunoglobulins (cryoglobulins) precipitating at low temperatures and re-solubilize with warming. Type I cryoglobulinemia is associated with IgM gammopathy of Waldenstrom's disease [1]. The cold agglutination disease is another situation in
which patients have anti-erythrocyte antibodies, usually IgM, that bind to red blood cell antigens at temperatures less than 37°C. Cold agglutinins account for 30% of immunohemolytic anemias. Hemolysis is complement-mediated. Cold agglutinins are not generally cryoglobulins. Cryoglobulins should be assessed in all patients with M-component and cold sensitive complications, especially in those with monoclonal immunoglobulin M [2]. In some of these patients, the disease gradually evolves into typical WM. In both cases, soluble immune complex aggregates that may or may not be cryoglobulins may appear as a paraprotein band on protein electrophoresis. In IFE, a band is seen in all lanes or in the IgG, IgM, kappa, and lambda lanes. Both paraproteins and cryoglobulins, have been shown to interfere with several assays and can produce falsely increased or decreased test results [3,4].

It is important for clinical laboratories be aware of the possibility and recognize artifactual alterations in laboratory parameters.

### Case History

A 79-year-old woman was sent to our laboratory by the hematology department of our hospital with a suspected diagnosis of anemia/hematologic disorder. Routine biochemistry was held on AU 5800 analyzer. The whole blood count was determined on the LH 750 Hematology Analyzers (Beckman Coulter Inc, US).

### Methods and Results

Among clinical chemistry tests; creatinine, calcium, urea nitrogen, total, HDL or LDL cholesterol levels were found in the normal range, but her total bilirubin was 0.45 mg/dL while direct bilirubin was 4.53 mg/ dL (to convert to micromoles per liter, multiply by 17.1). Other pathologic results were total protein 10.8 g/ dL and albumin 2.7 mg/ dL. In her whole blood count discordant results were; hemoglobin; 11.4 mg/ dL and hematocrit; 23.5%. Patients’ results are shown in Table 1. Visual analysis of serum in the tube didn’t show any evidence of icterus, lipemia or hemolysis. We obtained an informed consent from the patient.

We performed following procedures.

1. **Serum protein electrophoresis and IFE:** Protein electrophoresis revealed a monoclonal gammopathy in gamma fraction (Fig. 1). In IFE; first we determined bands in all globulin fractions (Fig. 2). We thought of any protein aggregate as an interfering agent.

2. **Cold agglutinin evaluation:** We repeated whole blood count inside the laboratory just near the operation site. After incubating the sample at 37°C for 2 hours another whole blood count was carried. No difference was observed in the results. This was not a cold agglutinin we concluded.

3. **Cryoglobulinemia evaluation:** Fresh specimen of blood was taken directly into a pre-warmed container of 37°C. We allowed the blood to clot 1 hour at 37°C and centrifuged warm for 10 minutes at 2500 RPM. Aliquots of serum was kept at + 4°C and 37°C for 7 days [4]. No cryoprecipitate formation was observed.

4. **Treatment of serum with 2-ME to dissolve paraprotein precipitation:** As a reducing agent, 2-ME destroys disulfide bands in protein precipitates [3] After this procedure a monoclonal band of IgM-lambda became obvious. Thus patient was identified as IgM-Lambda gammopathy (Fig. 3).

5. **Quantification of Individual immunoglobulins for comparison:** IgG level was 535 mg/dL (reference interval; 700–1600), IgA 44 mg/dL (70–400), and IgM 3458 mg/dL (40–230).

6. **Elimination of paraprotein interference on bilirubin assays:** We tried two procedures [5].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rbc</td>
<td>2.66</td>
<td>3.9–5.4 (10⁹/μL)</td>
</tr>
<tr>
<td>Hb</td>
<td>11.4</td>
<td>12–16 (g/dl)</td>
</tr>
<tr>
<td>Hct</td>
<td>23.5</td>
<td>37–47 (%)</td>
</tr>
<tr>
<td>MCV</td>
<td>88.4</td>
<td>81–99 (fl)</td>
</tr>
<tr>
<td>MCH</td>
<td>42.8</td>
<td>27–31 (pg)</td>
</tr>
<tr>
<td>MCHC</td>
<td>48.4</td>
<td>32–36 (g/dl)</td>
</tr>
</tbody>
</table>

Table 1: Whole blood count result of the patient.

![Figure 1: Protein electrophoresis of the patient.](image)
a. Dilution of the sample: Diluting sample in the ratio of 1/2 and 1/4 didn’t make any difference in results.
b. Pretreatment with polyethylene glycol: Results were 0.30 mg/dL for total and 0.08 mg/dl for direct bilirubin (with a CV of 4.1% and 4.5% respectively).

Discussion

Paraproteins can interfere with the methods utilized in automatic blood analyzers, leading to erroneous readings. In a two-phase medium like whole blood, formation of microaggregates have been described causing erroneous values of blood cells on particle counters (Beckman Coulter, Inc, Fullerton, CA). False elevation of Hb measurement by automated methods was observed in several patients with paraproteinemias. This anomaly is related to high levels of Ig that interact with reagents of the lysis solution. Red blood cell count and MCV are unaffected in this situation but as Hb is overestimated, MCHC usually exceeds 36 g/dl [6]. It is also noted that leukocyte and platelet counts remained unchanged [7]. It is strongly advised that before identifying any patient as M-paraproteinemia, cold agglutinin disease in which cryoglobulins and/or cryofibrinogen precipitate and cause the same interference, must be identified. In this study, we tried to rule out cold agglutinins by heating and cryoglobulins by freezing methods.

The most widespread mechanism reported for assay interference is through increased sample turbidity or precipitation interfering with absorbance readings [8]. In the literature total and direct bilirubin assays have been reported to show such interference on a few analysers. One report is about paraprotein interference on Beckman Coulter AU2700 direct bilirubin assay. It concludes that this interference causes falsely increased values, thus exceeding the total bilirubin concentration [9]. The total bilirubin (catalog number 6761) and direct bilirubin (catalog number: 6642) assays in Beckman Coulter are both end-point assays. Both bilirubin assays use a sample blank cuvette and calculate the difference of absorbance readings. Both couples conjugated bilirubin (direct bilirubin) with the diazonium salt of 3,5-dichloroaniline, at low pH to form azobilirubin.

Total bilirubin assay uses caffeine and surfactant to stabilize unconjugated bilirubin and to accelerate the reaction. In direct bilirubin assay caffeine and surfactant is lacking and the medium is strongly acidic (pH 1) to eliminate conjugated isomers of bilirubin. At this low pH, proteins typically precipitate. To avoid this precipitation, this reagent contains a “protein stabilizing agent.” It seems that this solubilization capacity is not sufficient when protein concentrations are too high. So in direct bilirubin assay, paraproteins precipitate. On the other hand, this protein precipitation and turbidity formation in both cuvettes is not reproducible and varies in continuous absorbance readings of each individual cuvette and so the absorbance differences in a sample-blanked method may be variable [8]. In our study direct bilirubin results of the same serum changed between -0.59 to 4.82 mg/dl in repeating analysis; that means this poor reproducibility causes a random result; either false positive or a false negative result if analysis is repeated. Pre-dilution of an interfering gammopathy in some cases reduces the interference or avoids it completely, leading to more correct result. But especially in cases in which the M-protein interacts directly with the test system, it will not show any significant effect [10]. In our study dilution of the
sample didn't change the result. Removal of paraprotein by PEG, ammonium sulphate or ultrafiltration are other suggested strategies [5]. In our patient, we had reproducible and plausible results after PEG precipitation.

**Conclusion**

In a patient with paraproteinemia, when an artifactual test result is suspected, a laboratory medicine specialist should identify the interferences related to the method and overcome the interference if possible.

**Conflict of Interest:** The authors have no conflict of interest.

**References**


