When an Analytical Interference Is a Useful Diagnostic Tool: Finding Monoclonal Gammopathies in Routine Analysis

Carmen Quiñones-Torrelo,* María Pilar Villanueva-Gil, Ana Rodríguez-Muñoz, Lidia Abellán-Tejada, Manuel Aparici-Ibáñez, and Arturo Carratalá-Calvo
Bioquímica Clínica y Patología Molecular, Hospital Clínico Universitario, Valencia, Spain

Background: The daily productivity of a clinical laboratory depends on the large number of interferences that affect analytical accuracy. Obviously, they have always been considered as a very important aspect to keep accuracy under control. Nevertheless, we wondered if this aspect would be beneficial. In this article, we propose a method for finding monoclonal gammopathies that are based on the fact that the presence of paraprotein in the sample may interfere with routine laboratory assays, specifically, with the quantification of uric acid and conjugated bilirubin. **Methods:** Over a 5-month period, we evaluated 18,278 sera samples of patients from primary care. None of them were suspected of having plasma cell dyscrasias (not observed hypercalcemia, renal failure, anemia, and/or lytic bone lesions). Although biochemical findings suggested paraprotein

interference, we carried out serum capillary electrophoresis (CE) and quantification of immunoglobulins and serum-free light chains (SFLCs). We also confirmed the results obtained by performing the corresponding immunofixation electrophoresis (IFE). Flow cytometry analyses were conducted for immunophenotypic characterization of plasma cells from these patients.

Results: The proposed detection method allowed us to identify eight patients with previously undiagnosed monoclonal gammopathy. **Conclusions:** The results show that it is possible to use analytical interference for diagnostic purposes, and most importantly, almost all cases were identified at an early stage of the disease, when associated clinical manifestations were not yet observed. *J. Clin. Lab. Anal.* 30:140–144, 2016. © 2014 Wiley Periodicals, Inc.

Key words: analytical interference; monoclonal gammopathy; unconjugated bilirubin; uric acid; detection method

Abbreviations

MM	= multiple myeloma
MGUS	= monoclonal gammopathy of undetermined significance
sMM	= smoldering MM
WM	= Waldenström's macroglobulinemia
CE	= capillary electrophoresis
SFLCs	= serum-free light chain
IFE	= immunofixation electrophoresis

INTRODUCTION

In the very appropriate main title of their editorial, Kazmierczak and Catrou (1) stated that analytical interferences are more than just a laboratory problem. We totally agree, but, what if the enemy becomes our ally?

Paraproteinemia is usually due to underlying monoclonal gammopathies. Currently, potent drugs with reasonable toxicity profiles have been developed, which could facilitate the establishment of early intervention strategies for high-risk patients (2–4). However, we have no suitable biomarkers or molecular imaging techniques that are capable of effectively identifying these strategies (5).

A large number of references in the literature describe how paraproteins can affect routine laboratory assays, but we focused our attention on those articles that describe artifactual hyperbilirubinemia (6, 7) and interferences in conjugated bilirubin (8), and uric acid Olympus assays (9), by using analyzers such as AU clinical chemistry systems (Beckman Coulter, Inc., Brea, CA) in our laboratory.

The aim of our work is to find monoclonal gammopathies in a population that has not yet developed the disease-related symptoms and may benefit from early treatment. Since the presence of paraprotein in the sample

leads to artificially increased results of conjugated bilirubin but not of total bilirubin, a concentration of conjugated bilirubin higher than total bilirubin may suggest the presence of a monoclonal immunoglobulin; so, we propose the calculation of unconjugated bilirubin as a detection method. Obviously, the patient samples must not show signs of hemolysis or jaundice. We also evaluated samples from which uric acid results could not be obtained or were abnormally low because, as stated in the literature, it can be due to the interference of Immunoglobulin M (IgM).

MATERIALS AND METHODS

Serum specimens were collected from primary care patients samples received daily in our laboratory for a period of 5 months. A total of 18,278 samples were evaluated. Target population are the adults (over 18 years) who were not suspected of having plasma cell dyscrasias. Therefore, patients with renal failure, anemia, and/or hypercalcemia were excluded from the study.

Serum levels of total and conjugated bilirubin and uric acid were independently measured using Olympus AU5400 (Olympus Diagnostics, Melville, NY) analyzer that employs colorimetric methods to estimate levels of these three blood components (10–14). In those cases where it was suspected that there may be paraprotein interference, we confirmed the results by performing a new analysis in order to rule out technical problems.

We also carried out CE of these samples. After evaluating the electrophoretic protein pattern, serum-free light chains (SFLCs) and immunoglobulins (IgG, IgA, and IgM) were measured. The presence of a paraprotein was confirmed by performing the corresponding high-resolution immunofixation electrophoresis (IFE).

We performed CE using the Sebia Capillarys™ 2 system (Sebia, Inc. Norcross, GA) and measured κ and λ SFLC concentrations by nephelometry on BN-II analyzer (Siemens, Liederbach, Germany). According to the manufacturer's protocols, immunoglobulins were quantified by an immunoturbidimetric assay on Olympus AU5400 system (Olympus Diagnostics). IFE of serum samples was performed using Sebia Hydragel kits on the Sebia Hydrasys electrophoresis system (Sebia, Inc.).

In order to characterize the detected monoclonal gammopathies (demonstration of an abnormal phenotype and/or clonality), the patients were remitted to the hematology service of our hospital for flow cytometric testing. According to the procedure established by the Spanish Myeloma Group, immunophenotypic studies were performed using FACSCalibur flow cytometer (BD Biosciences [BDB], San Jose, CA) and the CellQUEST software (BDB; (15)).

RESULTS

Eight cases of monoclonal gammopathy, which were previously undiagnosed (see Table 1), were found. Three of these were women (37.5%) and five were men (62.5%). There was no evidence of lytic bone lesions in the medical history of such patients. Only for Patient 1, degenerative changes in lumbar spine were previously described. Two cases of monoclonal component were first identified as IgG-lambda (25.0%), one as IgM-lambda (12.5%), and five cases as IgM-kappa (62.5%). No Bence-Jones protein was detected in any urine sample from these patients, except for Patient 5.

These results were confirmed by performing the corresponding serum immunofixation (see Fig. 1), except for Patient 1 who died before the study was concluded, and Patient 2 because the remaining sample size was not enough to ensure good results. Nevertheless, the biochemistry results and protein CE image for this serum were highly suggestive of monoclonal gammopathy.

Flow cytometry studies revealed one case of MM with CD56-positive plasma cells (Patient 3), and another of WM with predominantly negative CD5 B lymphocytes and lambda clonality with strong fluorescence intensity (Patient 8). Hematological studies were not conclusive for Patient 4, for whom two plasma cell populations were found—one with normal biomarker expression (CD45+, CD56-) and another considered to be pathological, with CD19- and CD20-negative cells, but expressing CD117 antigen (weakly positive). Plasma cell infiltration was less than 10% in these three cases. Serum free light chain assays (SFLCA) ratio was abnormal for Patients 4 and 8. These data were not available for Patient 3.

Patient 5 was diagnosed with cryoglobulinemia. This was the only case with a kappa-type Bence-Jones protein. No bone marrow plasma cell infiltration was observed, but the patient had anemia, and radiological studies evidenced vertebral body collapse; moreover, the SFLCA ratio was 0.1 (normal reference 0.26–1.65). These clinical findings mean that the patient had a relatively high likelihood of developing a hematological disorder.

Patient 2 was previously treated for lung cancer. The finding of a monoclonal component was justified as an MGUS, often associated with this type of tumor; nevertheless, a latent lymphoproliferative disease or sMM could not be excluded.

Patients 6 and 7 were not remitted to the hematology service by their respective primary care physicians. In the first case, from our point of view, significant anemia justified more exhaustive studies. Regarding Patient 7, the serum IgM concentration of almost 3 g/dl and abnormal SFLCA ratio were more likely due to a malignant hematological disorder than due to an MGUS. In this case, we also think that it would have been justified to carry out closer monitoring of the patient.

TABLE 1. Clinical, Biochemical, and Hematological Data of Primary Care Patients With No Clinical Suspicion of Monoclonal Gammopathy at the Time of Analysis

Parameter	Patient							
	1	2	3	4	5	6	7	8
Uric acid	Reference interval	10.1	3.7	0.7	Unreadable	-1.96	0.6	3.7
Total bilirubin	3.5–7.2 mg/dl (men) 2.6–6 mg/dl (women)	-4.39						
Conjugated bilirubin	0.1–1 mg/dl	0.56	0.48	0.84	0.53	0.45	0.39	0.44
Unconjugated bilirubin	0.1–0.2 mg/dl	0.6	1.6	0.1	0.2	0.2	0.7	3
Total proteins	0.0–0.8 mg/dl	-0.04	-1.12	0.74	0.33	0.25	-0.31	-2.56
Albumin	6.3–8.5 g/l	8.2	9.1	6.9	6.6	5.5	9.5	9.8
Calcium	3.5–5.2 g/l	2.8	4.4	3.9	3.5	2	4	3.9
Urea	8.1–10.5 mg/dl	-	9.7	9.2	8.8	8	-	11
Creatinine	20–50 mg/dl	23	25	27	25	31	32	54
IgA	0.67–1.17 mg/dl	1.21	0.83	0.95	0.57	0.27	0.68	0.83
IgG	70–400 mg/dl	196	39	15	91	433	181	30
IgM	700–1,200 mg/dl	1,326	2,992	1,231	682	942	971	628
Kappa light chains	40–230 mg/dl	2,403	50	606	1,406	749	2,884	4,255
Lambda light chains	170–370 mg/dl	709	61	353	192	360	632	685
Free kappa light chains	90–210 mg/dl	205	926	154	198	214	141	112
Free lambda light chains	3.3–19.4 mg/l	Not available ^a	Not available ^b	53.1	18.7	34.5	20.7	77
Free lambda light chains	5.7–26.3 mg/l	Not available ^a	Not available ^b	17.2	195	34.8	12.3	8.18
SFLCA kappa/lambda ratio	0.26–1.65	Not available ^a	Not available ^b	3.09	0.1	0.99	1.68	9.41
Red blood cells	3.8–5.8 × 10 ¹² /l (men) 3.5–5 × 10 ¹² /l (women)	3.78	4.25	5.1	3.22	3	4.29	4.6
Hemoglobin	12–16 g/dl (men) 11.2–15.5 g/dl (women)	13.1	12.8	15.3	9.3	8.6	12.8	14.9
Hematocrit	33–55% (men) 30–45% (women)	39	38	46	28	26	39	41
MCH	27–31 pg/cell	35	30	30	29	29	30	32.89
MCV	80–99 fl	103	89	90	88	86	90	89
Age		87	50	83	82	85	84	70
Sex		Man	Woman	Man	Woman	Woman	Man	Man
Bone lesions	Degenerative changes in lumbar spine	No	No	No	Evidence of vertebral body collapse	No	No	Diffuse bone demineralization
Initial diagnostic suspicion	Essential hypertension	Checkup	Preoperative study for intervertebral disc pathology surgery	Uninformed	Anemia	Anemia and hypercholesterolemia	Uninformed	Uninformed

SFLCA, serum free light chain assays; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume.

^aExitus.^bDue to personal reasons, the monitoring of this patient is being carried out at another hospital.

Fig. 1. Serum protein capillary electrophoresis and immunofixation images corresponding to samples of patients from primary care with no clinical suspicion of monoclonal gammopathy at the time of analysis.

No other source of analytical interference was established in such samples. We did not detect any false-positive result. According to the obtained results, the positive predictive value of the proposed method would be 100%.

Due to bureaucratic restrictions, it was not possible to carry out the corresponding serum proteins CE and immunofixations in those samples in which indirect bilirubin and uric acid values were in the range of normality. Therefore, it is not possible to provide information about false-negative results.

In this study, among the other cases that were detected using the proposed method, it is worthy to note the case of a 33-year-old female patient who consulted the doctor for a corneal ulcer (total bilirubin = 0.81 mg/dl, conjugated bilirubin = 1.2 mg/dl, unconjugated bilirubin = -0.39 mg/dl). A monoclonal IgG-lambda protein was identified. Hematological studies revealed the presence of a CD56-positive and CD138-negative plasma cell population with lambda clonality. Bone marrow plasma cell infiltration was less than 5%. During the study, the other biochemical and hematological parameters were within normal values. No Bence-Jones protein was isolated from the urine sample. The patient had no bone lesions.

DISCUSSION

Although a comprehensive study of serum proteins is recommended only to be conducted when the patient may

be suspected of having MM or other plasma cell proliferative malignant disorders, at least in three of the cases detected in this work, to wait for the clinical evidence to justify the study would very likely have led to serious consequences.

According to the diagnostic criteria provided by the International Myeloma Working Group (IMWG) in 2010 and disease definitions for the monoclonal gammopathies reported by Rajkumar et al. (16–18), if we check the results obtained by the patients' evaluation, we could classify Patients 3 and 8 as sMM and sWM, respectively. Patients 5 and 6 could not be cataloged since they had serum IgM < 3 g/dl, but anemia was evident in both cases. The remaining patients would have to be classified as MGUS. Taking into account the 2010 IMWG risk stratification model, in this work, it should be considered that all the patients with MGUS (except Patient 6) had adverse risk factors for disease progression.

In almost all diagnosed cases, the immunoglobulin concentration required to produce analytic interference is at least between two or three times the upper limit of the normal range. Probably, there will be cases of monoclonal gammopathy, which have not been detected since the heavy chain concentration is less than the mentioned values. These cases would correspond to false-negative results that have not been identified.

The fact that most of detected paraproteins are IgM type is because it is the monoclonal component that most

often appears to cause the considered analytical interferences (especially, in the determination of uric acid). Nevertheless, IgG or IgA paraproteins may also cause unreliable results.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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