Pseudohypoproteinemia and multiple myeloma

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■ Paraproteinemia is an important diagnostic feature of multiple myeloma. The M-protein level reflects tumor burden and helps to determine the response to chemotherapy. A case is described that illustrates the phenomenon of reversible in vitro gelification of an M-protein. Paraprotein IgG_1-kappa formed a concentration- and temperature-dependent gel, which was reversed by agitation. Measurement of paraprotein without previous vortexing of the specimen can erroneously lower the apparent M-protein level as well as reduce serum viscosity levels. This phenomenon can downstage the disease at diagnosis, produce inappropriate assessment of treatment response, or lead to premature withdrawal of chemotherapy. In addition, misdiagnosis of hyperviscosity syndrome can occur with serious clinical consequences.

□ INDEX TERMS: MULTIPLE MYELOMA □ CLEVE CLIN J MED 1990; 57:298–300

A 69-year-old white woman presented to a community hospital with fever, sore throat, and unproductive cough of 3 days' duration. She was treated with cefazolin and gentamicin for a right-sided pneumonia diagnosed by chest radiograph. Initial laboratory workup revealed anemia (hemoglobin, 7.0 g/dL) and thrombocytopenia (platelet count, 20,000/mm^3). She received packed red blood cells and platelet transfusions.

She was referred to the Cleveland Clinic. On hospital admission, she denied any history of chronic fever, weight loss, or generalized bleeding. She complained of chronic lumbar back pain and infrequent, self-limiting episodes of epistaxis for the previous 6 months. She was taking hydralazine and diuretics for hypertension.

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Physical examination showed no lymphadenopathy, petechiae, or ecchymosis. She was afebrile. There was no palpable organomegaly. The results of the neurologic examination were normal.

LABORATORY DATA

On admission, hematologic values were: white blood cells, 6,000/mm^3; hemoglobin, 9.7 g/dL; hematocrit, 29%; and platelets, 159,000/mm^3. Peripheral smear showed significant rouleaux formation with mild leukoerythroblastic reaction. Erythrocyte sedimentation rate was 157 mm/h. The results of coagulation studies, liver function tests, blood urea nitrogen, creatinine, and electrolyte levels were all within normal limits. Skeletal survey showed multiple osteolytic lesions in the skull and pelvis. Bone marrow examination revealed significant marrow infiltration by sheets of immature plasma cells, confirming the diagnosis of multiple myeloma (MM).

Visual inspection of a refrigerated serum specimen with transillumination revealed a compact, dense, translucent, gel-like bottom separated from a yellowish super-

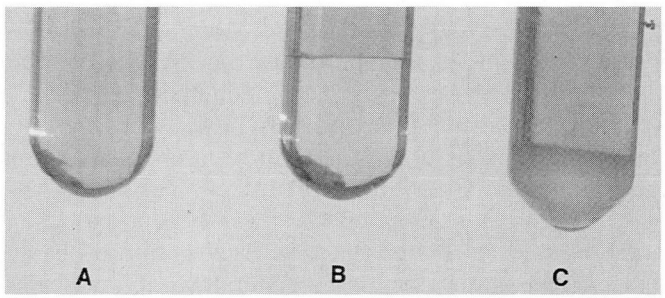

FIGURE 1. Comparison of the physical appearance of the cryogelified M-protein (B) with a typical M-cryoglobulin precipitate (C) and normal human serum (A). The tubes were transilluminated to enhance the color difference between the two layers of the sample.

natant fluid by a sharp interface (*Figure 1*). This gelification process was temperature- and concentration-dependent: Gel formation did not occur at room temperature but, at 4°C, gelification of undiluted serum occurred in 15 to 20 minutes; at dilutions of 1:2 and 1:4, gelification took 40 to 45 minutes and 50 to 60 minutes, respectively. The process was reversed by vortexing. This physical appearance contrasted with that of a refrigerated normal serum sample and with a serum sample containing a typical whitish monoclonal cryoprecipitate (*Figure 1*).

Total serum protein was 6.3 g/dL. Serum protein electrophoresis (SPEP) showed a homogeneous spike of 0.81 g/dL in the gamma fraction (*Figure 2*). Serum viscosity was 1.6 (normal 0.8 to 1.8). However, after vortexing the specimen, the total serum protein was 8.2 g/dL. SPEP showed a homogeneous spike of 3.85 g/dL in the same location (*Figure 3*), with serum viscosity increased to 2.8. Serum immunoelectrophoresis (IEP) revealed a monoclonal IgG-kappa gammopathy. Free monoclonal kappa light chains were present in the urine by IEP. Quantitative serum immunoglobulin measurement after vortexing of the specimen revealed IgG of 6,160 mg/dL, IgA of 21 mg/dL, and IgM of less than 40 mg/dL. IgG subclass measurement revealed IgG_1 of 4,796 mg/dL, IgG_2 of 46 mg/dL, IgG_3 of 14.3 mg/dL, and IgG_4 of 63.7 mg/dL. Cryoglobulin studies using standard laboratory procedures showed a cryoglobulin level of 67 µg/mL (normal less than 50 µg/mL), consistent with a low level of immune complexes.

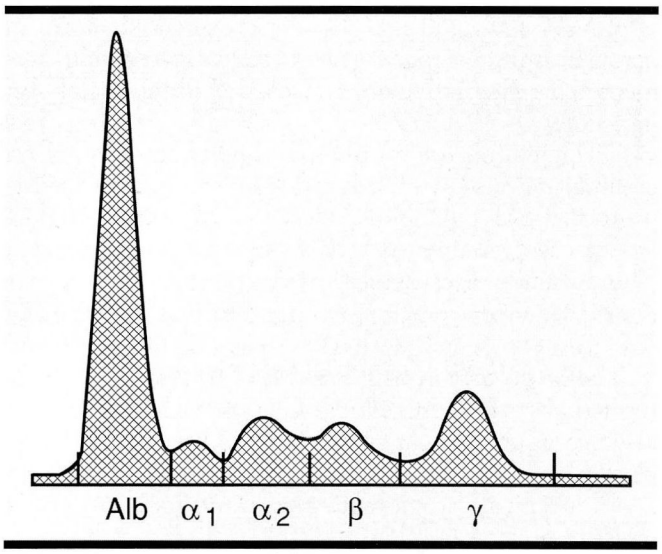

FIGURE 2. SPEP performed without previous vortexing of the sample to illustrate a remarkable erroneous decrease in the M-spike in gamma fraction (concentration, 0.81 g/dL).

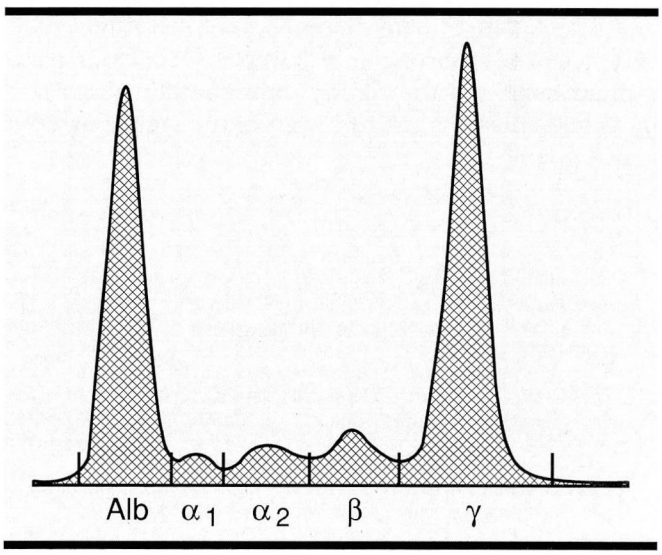

FIGURE 3. SPEP performed after vortexing of the sample with prominent M-spike in gamma fraction (concentration, 3.85 g/dL).

DISCUSSION

Paraproteinemia is an integral, diagnostic feature of MM,[1] which is a clonal (malignant) plasma cell disorder. It is a sensitive tumor marker[2] and a major criterion for staging MM.[3,4] Serum M-protein levels correlate so well with tumor bulk that remission in myelomatosis is defined in terms of percentage reduction of initial paraprotein levels.[2]

IgG myeloma is the most common variant. Among IgG myelomas, 65% are IgG_1.[5] Hyperviscosity is common with Waldenstrom's macroglobulinemia, IgG_3, and

IgG_1 MM. IgG_3 and IgG_1 have a propensity to form Ig complexes linked together by specific aggregating sites located in the Fd fragments of the immunoglobulin molecules.[6]

Cryoglobulins are proteins that precipitate or gel on cooling and dissolve when heated. Five percent of M-proteins are cryoglobulins.[7] However, the exact percentage of cryoglobulins in IgG_1 M-protein is not known. The presence of cryoglobulins causes purpura, cold urticaria, leg ulcers, gangrene of the toes, and symptoms of cold intolerance manifested by Raynaud's phenomenon.

The M-protein in this case was of IgG_1 kappa type. It formed a translucent gel at 4°C (*Figure 1*). The process of gelification was concentration- and temperature-dependent and was reversed by vortexing. The M-protein is not a typical cryoglobulin because it does not form a whitish precipitate that redissolves at 37°C. In fact, specific quantification of cryoglobulins revealed no significant levels even though three-fourths of the monoclonal protein was cryogelified. This was because the abnormal protein was completely redissolved during the washing process and discarded with the washing fluid.

As documented here, the process of reversible gelification can also erroneously lower the apparent paraprotein level and the corresponding serum viscosity if measured only from the top layers of the sample without previous vortexing (*Figures 2* and *3*). Clinically, it can cause misdiagnosis, inappropriate downstaging of the disease, or premature withdrawal of chemotherapy.

The mechanism of M-protein gelification-aggregation is unknown. Due to structural polymorphism, monoclonal immunoglobulins easily interact with one other. The reversible self-association may be explained by weak protein-to-protein binding. Aggregation probably involves a combination of dispersion forces,[8,9] hydrogen binding,[10] and hydrophobic interactions.[10] The dominant factors are unknown.

Similar phenomena have been noted in three other patients with IgM paraproteinemia at this institution. At present we have no clinical evidence that this unusual phenomenon could occur in vivo. The prevalence of the phenomenon is unknown. We feel that it is uncommon but, because of its significant clinical impact in the management of MM, every laboratory should look routinely for this phenomenon. We strongly recommend vortexing of all serum samples just prior to performing the relevant protein studies.

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Commentary

This article describes a laboratory phenomenon that can have significant clinical consequences. The authors report a case of a patient with multiple myeloma who has a gelifying IgG_1-kappa paraprotein. This gelification process was temperature- and concentration-dependent, and was reversed by vortexing.

The phenomenon of M-protein gelification has been observed in three other patients, and its mechanism is not understood. Although this phenomenon is uncommon, it can erroneously lower the measured paraprotein level and affect the serum viscosity. Thus it can affect the diagnosis and treatment of certain patients with multiple myeloma.

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