CLINICAL VIGNETTE

False-positive Serum Protein Electrophoresis due to Natalizumab Therapy

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Case Report

A 53-year-old female with a history of multiple sclerosis (MS) and hysterectomy presented to hematology to evaluate chronic normocytic anemia. She developed a mild, progressive normocytic anemia for two years. She complained of chronic fatigue due to her underlying multiple sclerosis that has been progressively worsening. She reported intermittent bright red blood per rectum due to her hemorrhoids. She had been on natalizumab infusion therapy for the past five years for her MS. She was not on any iron supplementation and her presenting hemoglobin was 9.8 g/dL, and hematocrit was 32.1%. WBC differential and platelet was normal. Upon review of prior CBC's, her hemoglobin had been slowly decreasing from 11.6. Nucleated RBCs were reported on the CBCs. Additional lab included iron level of 60 mcg/dL, iron saturation 20%, TIBC 306 mcg/dL, ferritin 66 ng/mL and soluble transferrin receptor was midly elevated at 5.7 mg/L. ESR was 49 mm/hr. Serum folate was 18.7 ng/mL, B12 was 585 pg/mL, haptoglobin was 105 mg/dL, and serum protein electrophoresis (SPEP) revealed 0.4 g/dL of monoclonal protein. Serum immunofixation revealed monoclonal IgG Kappa protein. Kappa/Lambda light chain ratio was normal at 1.47, and IgG was normal at 992 mg/dL. Random urine protein electrophoresis and urine immunofixation were both negative for M-protein. Her creatinine was normal at 0.95 mg/dL. The total protein was normal at 7 g/dL. Calcium was normal at 10.2 mg/dL. Peripheral blood smear review revealed normochromic, normocytic anemia with minimal polychromasia and rare nucleated RBCs. Her labs were suggestive of mixed anemia of chronic disease and iron deficiency anemia. Iron deficiency anemia evaluation included a negative fecal occult blood immunoassay. She also had EGD and colonoscopy, which showed a non-bleeding AVM at the duodenal bulb and internal hemorrhoids. She was started on a trial of oral ferrous sulfate, and her hemoglobin improved to 11 g/dL. However, given ongoing fatigue, presence of nucleated RBC in her peripheral blood, and the abnormal SPEP and IFE findings, she had a bone marrow biopsy to rule out coexisting plasma cell dyscrasia and other bone marrow disorders. Her bone marrow was hypocellular with mild erythroid predominance and decreased granulopoiesis. Only 3% polytypic plasma cells with both kappa and lambda light chain expression were found, and there was no evidence of plasma cell dyscrasia. Of note, iron stores were not readily detected per iron stain in the marrow, confirming the iron deficiency. She was asked to stay on ferrous sulfate. The abnormal SPEP/IFE and presence of nucleated RBC findings were believed to be secondary to natalizumab infusion, a humanized monoclonal antibody against the cell adhesion molecular α4-integrin.

Discussion

Monoclonal protein (M-protein) is a monoclonal immunoglobulin made by an abnormal clone of plasma cells that can be detected in the blood, urine, soft tissue, or body fluids. It is usually a whole immunoglobulin with a heavy polypeptide chain (IgG, IgA, IgM, IgD) and light chain (either kappa or lambda). Occasionally the M-protein is just the immunoglobulin free light chain (FLC) by itself. One particular type of M-protein is the Bence-Jones protein, a small monoclonal protein with a molecular weight of 22 kDa found in the urine and may be too low in concentration to be detected by routine serum screening tests.[1,2] The presence of M-protein is associated with a variety of pre-malignant (i.e., monoclonal gammopathy of unknown significance (MGUS)) to malignant conditions (i.e., multiple myeloma (MM) and lymphoplasmacytic lymphoma). Serum protein electrophoresis (SPEP) is the most used screening test for M-protein when there is clinical suspicion of multiple myeloma. Other tests commonly used to analyze M-protein include urine protein electrophoresis, serum immunofixation, urine immunofixation, and serum free light chains assay.[2]

SPEP is an easy and relatively inexpensive blood test to screen for monoclonal protein. Electrophoresis is a method to separate proteins based on their properties. The patient's serum is placed on a medium with a charge applied. The charge, size, and shape of the protein differentiate one protein from the others. It is usually done using the agarose gel method. Albumin, the largest protein of human serum, is usually the largest peak, lies closest to the positive electrode. The next five globulins are alpha-1, alpha-2, beta-1, beta-2, and gamma as they lie toward the negative electrode. When M-protein is present, it usually appears as a single discrete band (M-spike) on the agarose gel. The amount can be quantitated using a densitometer tracing of the gel, and the M-protein usually shows up as a single narrow peak on the tracing.[3] However, SPEP is not sensitive when M-protein is small, and an apparent M-protein may represent a polyclonal increase in protein. Serum immunofixation (IFE), a test that overlay different monospecific antibodies (anti-sera) against different heavy and light chains and looks for precipitation of proteins (antibody-antigen complex), can confirm the presence of the M-protein and provide further characterization

and subtype of the M-protein. Urine protein electrophoresis (UPEP) and urine protein immunofixation are tests that quantify and identify urine M-protein. Serum free light chain (FLC) assay is a sensitive antibody-based system that can detect monoclonal kappa or lambda in the serum. FLC assay is useful in detecting some M-proteins that are free light chains by itself without the heavy chain. It is more sensitive to detect monoclonal free light chains than urine immunofixation, and may be used instead of the UPEP to screen for Bence-Jones protein.[4]

It is important to understand how SPEP works and what may cause false-positive findings. False-positive SPEP can lead to patient anxiety and unnecessary bone marrow biopsy. There are several known causes of false-positive SPEP. Any protein elements causing a dense, localized band on the agarose gel or a peak on the densitometer of the SPEP can lead to a finding consistent with an M-spike. Examples include, nephrotic syndrome. which can lead to increased alpha-2 and beta bands, leading to false reporting of an M-protein. Patients with increased acute phase reactants may also have increased alpha-1 band. High transferrin concentration may cause a spike in the beta band.[5] Because of the possibility of false-positive SPEP, it is recommended to have follow-up immunofixation to confirm and characterize the type of M-protein. Subsequent negative serum immunofixation suggests that the M-spike band on the SPEP may be a false positive finding.

Monoclonal antibody (mAbs) are produced by B-cells and are designed to target specific antigens. There is increasing use of therapeutic mAbs (tmAbs) to treat various medical conditions ranging from autoimmune diseases to malignancies. As of December 2019, 79 tmAbs have been approved by the USA FDA, and the list is growing rapidly.[6] Among these tmAbs, humanized mAbs are becoming more common as they may be less likely to elicit an immune reaction against the tmAB itself. Because of this, it is crucial to know whether a tmAb could be misinterpreted as an endogenous clonal M-protein on SPEP and/or immunofixation. Several do not commonly lead to false-positive SPEP/IFE. Infliximab, adalimumab, eculizumab, vedolizumab, and rituximab generally do not form a quantifiable M-protein on SPEP. However, at the expected peak concentrations of vedolizumab and rituximab, a small IgG Kappa M-protein on immunofixation can be detected.[7] On the other hand, daratumumab and elotuzumab, both human IgG Kappa monoclonal antibodies to treat multiple myeloma, can be seen on SPEP as an M-spike. It is important to recognize this issue as multiple myeloma treatment decisions are frequently based on the SPEP result.

In our case, the patient had been on natalizumab therapy for five years before her initial presentation to hematology. Natalizumab is a recombinant humanized monoclonal IgG4 kappa antibody which binds to $\alpha_4\beta_1$-integrin. $\alpha_4\beta_1$-integrin is found on the surface of inflammatory lymphocytes and monocytes responsible for the inflammatory lesions in patients with MS. These inflammatory leukocytes gain access to the brain parenchyma by binding $\alpha_4\beta_1$-integrin to the vascular cell adhesion molecule-1 (VAM-1) found on the vascular endothelial cells. Natalizumab works by blocking $\alpha_4\beta_1$-integrin's interaction with VCAM-1 and inhibits the migration of these inflammatory leukocytes into the brain tissues.[8] Natalizumab is currently approved as disease-modifying therapy for relapsing-remitting multiple sclerosis (RRMS). In the key Phase 3 trial, three years of natalizumab reduced the relapse rate at one year by 68%.[9] It is also effective for moderate-to-severe Crohn's disease and currently has an FDA approval in patients refractory to anti-tumor necrosis factor (TNF) biologic therapy.[10,11]

During her anemia evaluation, her SPEP had a restricted band, and her serum IFE revealed a monoclonal IgG Kappa protein, the same type of protein as natalizumab. These were likely false-positive findings from natalizumab use because her bone marrow biopsy was negative for monoclonal plasma cells. This finding was unexpected from prior clinical experience with other tmABs and is crucial as it can potentially prevent future unnecessary testing, such as bone marrow biopsies.

Mass spectrometry (MS) is currently under investigation as an improved way to identify and classify M-protein in the serum. MS can determine the molecular mass of the M-protein heavy and light chain with high accuracy. The process also involves purification of immunoglobulins and thus removes potentially interfering serum proteins from the sample.[12] Because each endogenous M-protein is made of a unique sequence of amino acids that are specific to each clone, it will reveal a distinct peak on high-resolution mass spectrometers. By comparing tmAb's mass to the patient's endogenous M-protein, one can accurately discriminate between the two. When MS becomes more readily available in clinical medicine, it will provide a way to eliminate the possibility of misleading false-positive SPEP/IFE results during treatment.[13]

Interestingly, natalizumab therapy is also associated with several notable changes in CBC values. An increasing number of lymphocytes, monocytes, eosinophils, and basophils have all been reported. It is believed that these changes are due to the expression of $\alpha_4\beta_1$-integrin on these white cell subgroups and the inhibition of transmigration of these cells into the CNS.[9,14] Erythroblastemia, the presence of nucleated red cells in circulating blood, can also be seen in as high as 92% of natalizumab-treated patients. It is believed that nucleated RBC usually stays in the bone marrow due to its surface $\alpha_4\beta_1$-integrin's interaction with bone marrow's fibronectin and natalizumab reduce this particular interaction.[14,15]

Clinical Case Follow-up

Our patient underwent evaluation for worsening fatigue and anemia and was noted to have an M-spike on SPEP and abnormal monoclonal IgG Kappa protein on serum immunofixation. She also had abnormal nucleated RBCs in her peripheral blood. As a result, a bone marrow biopsy did not show any monotypic plasma cell in her bone marrow, thus ruling out plasma cell dyscrasia. Her abnormal SPEP/IFE and

nucleated RBC findings were thought to be due to natalizumab infusions. She was given reassurance, and her anemia was concluded to be secondary to anemia of chronic disease and iron deficiency. The exact cause of her iron deficiency was unclear at the time, but it may have been due to inadequate oral intakes and intermittent hemorrhoidal bleeding. She is currently doing well, and her MS is under control with natalizumab.

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