

Unusual Serum Electrophoresis Pattern in a Woman with Pancreatic Carcinoma

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CASE DESCRIPTION

A 51-year-old woman presented with progressive severe icterus associated with advanced inoperable pancreatic carcinoma. Biopsy of the liver revealed metastasis of a moderately differentiated adenocarcinoma, possibly of pancreatic origin. Imaging studies did not show any primary tumor in the pancreas but did reveal a deep vein thrombosis of the left lower extremity and a pulmonary embolism. Bone scans revealed metastases. The patient began treatment with chemotherapy (gemcitabine), radiotherapy to alleviate pain, and placement of a percutaneous transhepatic stent in the right biliary system because of progressive icterus.

Routine biochemical investigation of the patient's serum revealed increased total bilirubin (275 $\mu\text{mol/L}$, reference value $<22 \mu\text{mol/L}$), which consisted mainly of direct bilirubin (215 $\mu\text{mol/L}$, reference value $<4 \mu\text{mol/L}$). The cytosolic liver enzymes alanine aminotransferase (115 U/L) and aspartate aminotransferase (143 U/L) were moderately increased. The serum protein concentration was 68 g/L (reference interval 60–85 g/L), but capillary electrophoresis of serum proteins (CAPILLARYS 2, Sebia) demonstrated a low albumin fraction together with a marked additional peak observed between the β and the γ region (Fig. 1). Results of immunofixation with antibodies against G, M, and A heavy chains and κ and λ light chains were negative, indicating that this additional electrophoretic fraction did not indicate the presence of a paraprotein. Agarose gel electrophoresis confirmed the abnormal pattern, ruling out analytical interference caused by atypical ultraviolet absorbance.

DISCUSSION

Paraproteins are the most common cause of abnormal patterns in electrophoretic analysis results for plasma proteins. However, abnormal serum protein electrophoresis patterns may also indicate the presence of

transitory plasma proteins originating from tissues as well as nonprotein interfering substances, most commonly iodinated contrast agents and antibiotics showing ultraviolet absorption at 200 nm, the wavelength used to quantify proteins in capillary electrophoresis (1). The presence of paraproteins can be excluded by use of immunofixation or immunoelectrophoresis. The presence of drug interferences can be excluded by reanalysis of the sample by use of dye-stained gel electrophoresis.

FURTHER ANALYSIS

Electrochemiluminescence immunoassay (Modular E, Roche) of the patient's serum revealed an exceptionally high concentration of the mucin tumor marker CA 19–9 (2.2×10^6 kU/L, reference value <37 kU/L). The serum concentration of carcinoembryonic antigen was also increased (423 $\mu\text{g/L}$, reference value $<3 \mu\text{g/L}$).

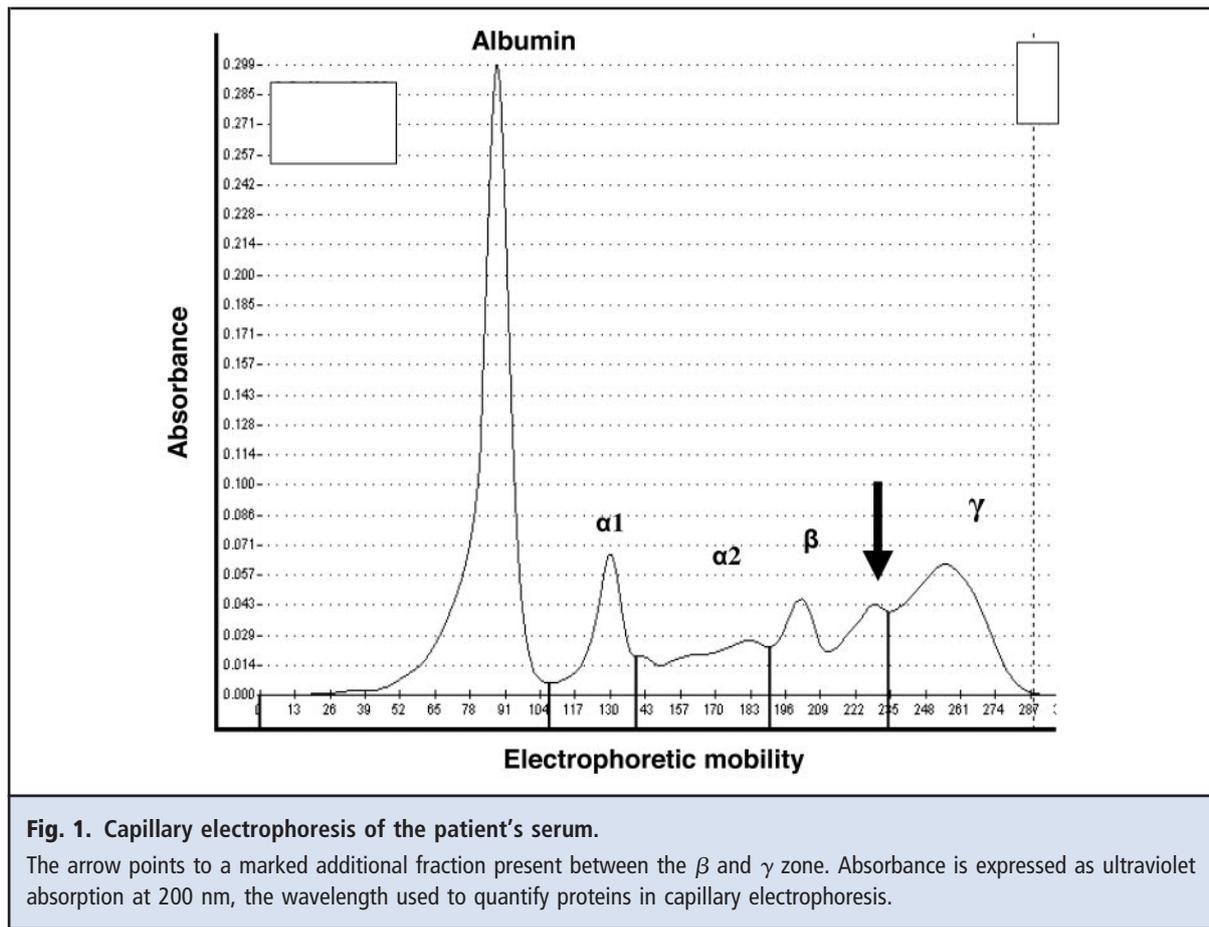
To further analyze the mucins we used high-pressure gel permeation chromatography performed by use of a Waters 650E advanced protein-purification system. We used the Wisp 712 automatic sampler to inject 25 μL of serum, and we performed chromatographic separations on an 80×300 mm Protein PAK glass 300 SW column (Waters Nihon Millipore). The obtained fractions were analyzed for CA 19–9, and this analysis showed that the elution of the CA 19–9 fraction mirrored the void volume of the column, a result corresponding to a molecular mass of >1000 kDa. Electrophoresis of the isolated CA 19–9 fraction confirmed that the mobility of the glycoprotein was in the β - γ region.

The patient's serum showed pronounced increases in the membrane-bound liver enzymes alkaline phosphatase (1002 U/L, reference interval 30–120 U/L) and γ -glutamyltransferase (1153 U/L, reference interval 9–36 U/L). The molecular mass distribution of γ -glutamyltransferase and alkaline phosphatase activity levels (determined by use of high-pressure gel permeation chromatography) also showed a macromolecular character: 75.5% of serum γ -glutamyltransferase and 20.5% of alkaline phosphatase activity were attributable to a macromolecular complex, suggesting that membrane vesicles were present in the serum (2). After the serum sample had been extracted with 1-butanol, the macromolecular fraction of the membrane-bound

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enzymes vanished. In contrast, the molecular mass distribution of the CA 19–9 mucin was unchanged after 1-butanol extraction, a finding that argues against the hypothesis that the high apparent molecular mass of the mucin was attributable to lipid binding.

CA 19–9

The CA 19–9 monoclonal antibody 1116 NS 19–9 (used in the assay) reacts with sialylated Lea-active pentasaccharide (sialylated lacto-N-fucopentaose II), which is enzymatically synthesized by sialylation of type 1 carbohydrate chains. CA 19–9 contains oligosaccharide structures present on heavily glycosylated high molecular mass mucins (3). The structure of the apomucin backbone typically reveals the presence of Ser/Thr/Pro-rich regions containing tandemly repeated stretches of amino acids that constitute potential O-glycosylation sites (4). Mucins are synthesized either as membrane-bound or as secreted glycoproteins. These molecules are widely synthesized and expressed by epithelial cells of the gastrointestinal, respiratory,

and genitourinary tracts (4). In cells the increase in cAMP concentrations increases the synthesis and release of the carbohydrate antigen 19–9. cAMP is involved in the expression of glycoprotein-associated sialyl Lewis(a) antigen in LS174T cells (5).

The increased exposure of peptide epitopes of mucin glycoproteins in biliopancreatic cancer is due to abnormal glycosylation and/or altered transcription levels of mucin genes (3). The dosage of the Lewis gene [fucosyltransferase 3 (galactoside 3(4)-L-fucosyltransferase, Lewis blood group)] increases the amount of CA 19–9, whereas the dosage of secretor genes decreases it (6). CA 19–9 is the standard tumor marker for pancreatic cancer (7). Increased CA 19–9 concentrations are also found in other cancers, in chronic pancreatitis, and in benign gastrointestinal conditions (7). The majority of tumor cells in gastrointestinal carcinomas, including adenocarcinomas of the stomach, intestine, and pancreas, are strongly positive for CA 19–9 (8). In the patient we describe the cancer was accompanied by cholestasis. Controversy exists regarding the

role of cholestasis as a physiopathological mechanism that affects serum CA 19–9 concentrations (7).

Little is known about the molecular heterogeneity of CA 19–9. In colon adenocarcinoma and cell plasma membranes this antigen is expressed on various glycoproteins with molecular masses ranging in size from ± 100 kDa to >200 kDa. In cytosol and culture medium the epitope is carried by a single complex glycoprotein that has a very high molecular weight and resembles mucin (5). High molecular mass forms of CA 19–9 have been reported in serum from patients with pancreatic tumors (9).

CASE RESOLUTION

After we excluded the possibility that the unusual serum electrophoresis pattern was due to the presence of paraproteins or to analytical effects attributable to ultraviolet absorption, we found that this case illustrates

that excessive release of a mucin marker protein may affect the serum electrophoresis pattern. Because 1 U of CA19.9 antigen corresponds to approximately 0.8 ng of glycoprotein (10), we estimated that the patient's serum contained approximately 1.6 g/L of CA 19–9 mucins, corresponding to 2.5% of the patient's total serum protein, which can be detected by use of standard electrophoresis.

The patient showed a partial radiological and biochemical response to treatment (decrease of serum CA 19–9 from 2.2×10^6 to 5.5×10^4 kU/L). The decrease in CA 19–9 correlated with a decrease in the electrophoretic fraction migrating between the β and γ region. The patient died 13 days after admission.

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POINTS TO REMEMBER

- Drug interferences, paraproteins, and proteins originating from tissues can cause additional serum protein electrophoresis fractions.
- In extreme cases, tumor markers such as CA 19–9 may be so abundant in serum or plasma that they become visible as an additional fraction on serum protein electrophoresis.
- In such extreme cases, the laboratory should be proactive in further investigating unexpected results and should communicate appropriately with the clinical team. First, the presence of paraproteins should be ruled out by immunoelectrophoresis or immunofixation. If capillary electrophoresis has been used, then interfering substances that effect ultraviolet absorbance should be investigated (by carrying out standard gel electrophoresis in parallel). Further immunochemical investigations and biochemical characterizations can also be helpful.

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Commentary

Jerry A. Katzmann

The abnormal serum protein electrophoresis findings in this patient had no impact on her unfortunate rapid

death, and the correct (or incorrect) identification of the abnormal fraction did not impact treatment. The detection of this electrophoretic abnormality, however, reinforces the need for cautious interpretation of serum and urine protein electrophoresis patterns.

A number of “classic” serum electrophoresis patterns require further confirmatory tests for diagnosis. Absence of an α -1 band, for example, suggests A1AT deficiency disease; polyclonal elevation of the γ frac-

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tion suggests autoimmune, infectious, or liver disease; decreased albumin and increased α -2 fractions suggest nephrotic disease; and an additional discrete band suggests monoclonal gammopathy. The diagnosis of monoclonal gammopathy is usually confirmed by immunofixation electrophoresis. The most common serum electrophoretic abnormalities that are detected on agarose gels and are confused with monoclonal gammopathies are fibrinogen (due to incomplete blood clotting) and hemoglobin (due to hemolysis). The ultraviolet detection systems used in capillary electrophoresis have additional interferences that must be recognized (e.g., radioopaque imaging agents as well as some antibiotics). Initial diagnostic observations of abnormal electrophoretic bands must therefore be con-

firmed by immunotyping of heavy and light chain isotype.

Serum protein electrophoretic abnormalities that are monoclonal proteins may represent significant disease and should be pursued to define the specific plasma proliferative disease that is leading to the secretion of the monoclonal immunoglobulin. In addition, abnormalities, such as in this case, in which there is no monoclonal gammopathy should not be ignored and may provide insights into the disease.

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Commentary

Jim D. Faix

The presence of serum monoclonal immunoglobulin indicates abnormal clonal proliferation of either plasma cells or lymphocytes. Although this finding may be a marker of multiple myeloma or lymphoma, it is often seen in the absence of any clinical signs or symptoms, especially in elderly patients. In these cases the presence of serum monoclonal immunoglobulin is usually considered a benign monoclonal gammopathy, but this diagnosis has been renamed "monoclonal gammopathy of undetermined significance" because long-term follow-up in these patients has shown a high risk (approximately 25%) for the development of myeloma or lymphoma.

The traditional way to detect serum monoclonal immunoglobulin has been protein electrophoresis followed by visual inspection (looking for an abnormal band). This procedure has been automated and is now also performed by capillary electrophoresis for high-throughput testing. No matter how the abnormal band is visualized, its identity as a monoclonal immunoglobulin must be confirmed by use of antibodies specific for

immunoglobulin heavy and light chains. This confirmation is necessary because a number of nonimmunoglobulin proteins may masquerade as abnormal bands.

Fibrinogen, probably the most common "pseudo-band," will of course be present if plasma (rather than serum) protein electrophoresis is performed, but some residual fibrinogen is often present in serum specimens. Other causes of confusion include polymorphisms (such as bis-albuminemia) and in vitro alterations (such as complement degradation).

Serum proteins which migrate outside of the traditional bands are usually present at concentrations well below the detection limit of routine protein electrophoresis (approximately 1 g/L), but in some patients they reach much higher concentrations and may appear as unexplained abnormal bands. This situation has been noted most frequently in patients with increased C-reactive protein. The authors of this case study have shown that tumor markers may also produce such an effect. Although it is not clear exactly why serum protein electrophoresis was performed in this case, laboratorians who review these procedures may want to add elevated tumor markers to the list of things that can mimic a monoclonal immunoglobulin.

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