False Elevation of Serum CA-125 Level Caused by Human Anti-Mouse Antibodies

Roger L. Bertholf,1 Laura Johannsen,2 and Guy Benrubi 3
1 Department of Pathology, 2 Clinical Laboratories, and 3 Department of Obstetrics and Gynecology, University of Florida Health Science Center/Jacksonville and Shands Hospital, Jacksonville, Florida

Abstract. Discordant results were observed for serum CA-125 (carbohydrate antigen-125) assays in a patient who was monitored for recurrence of ovarian cancer. Serum CA-125 levels in this patient were normal when measured in one laboratory, but >5-times the upper limit of normal (35 U/mL) when measured in another laboratory. Both laboratories used dual antibody heterogeneous immunoassays, but from different manufacturers. Cross-linking heterophilic antibodies were suspected as a cause of the discrepancy, but the interference was not alleviated after 10-fold dilution. Assay of the patient’s serum for human anti-mouse antibodies was positive, but only slightly above the reference range. Addition of blocking antibodies eliminated the interference, showing that human anti-mouse antibodies were the cause of the discrepant CA-125 results. These findings indicate that relatively low concentrations of human anti-mouse antibodies can cause significant interference in two-site immunoassays. (received 8 May 2002; accepted 8 June 2002)

Keywords: ovarian cancer, carbohydrate antigen-125, immunoassay interference

Introduction

Carbohydrate antigen 125 (CA-125) is a large glycoprotein that is expressed by tissues that are derived from the Mullerian ducts. Assay of serum CA-125 level is primarily useful for detecting and monitoring ovarian tumors, although its serum concentration is elevated in other tumors, including endometrial, pancreatic, breast, colorectal, lung, and gastrointestinal carcinomas [1-3]. CA-125 derives its numeric designation from monoclonal antibody OC-125, isolated in 1981 by Bast et al [2] using cells from a serous papillary cystadenocarcinoma of the ovary. An immunoradiometric assay for CA-125 was developed in 1983 [4], and several immunochemically-based methods are now available for measurements of this widely used tumor marker.

The clinical problem that is described in this report arose when a gynecologist contacted our clinical laboratory concerning serum CA-125 results in a patient who had received surgical and chemotherapeutic treatment for ovarian cancer several years earlier, but had been disease-free in the interim. Blood specimens from the patient had been sent to a reference laboratory, and the serum CA-125 levels showed progressively abnormal elevations. When blood specimens were submitted to our clinical laboratory, the results of serum CA-125 assays were within the normal limits. Our laboratory was asked to explain this discrepancy.

Materials and Methods

In the clinical laboratory of Shands Jacksonville Hospital (SJH), serum CA-125 is assayed with an Elecsys 2010 analyzer using an electrochemiluminescent method (Roche Diagnostics, Indianapolis, IN). Serum specimens from the patient were sent to two reference laboratories operated by Laboratory Corporation of America (Tampa, FL,
and Burlington, NC), and one operated by Quest Diagnostics, Inc., (Nichols Institute, San Juan Capistrano, CA). All three reference laboratories measure serum CA-125 with an AxSym analyzer using a microparticle enzyme immunoassay (MEIA, Abbott Laboratories, Abbott Park, IL). Dilution studies were performed at SJH and Laboratory Corporation of America (Tampa, FL). Specimens were diluted 2-fold, 5-fold, and 10-fold using the manufacturer-supplied diluents. The Laboratory Corporation of America's facility in Burlington, NC, measured human anti-mouse antibodies (HAMA) in the patient's serum, using an enzyme-linked immunosorbent assay. Blocking studies were performed at Laboratory Corporation of America (Burlington, NC) by adding 15 µl of heterophilic blocking reagent (murine IgG; catalog #3KC534, Scantibodies, Inc., Santee, CA) per 100 µl of serum specimen prior to CA-125 analysis by MEIA and the AxSym analyzer.

The Institutional Review Board of the University of Florida Health Science Center/Jacksonville approved the publication of laboratory and clinical data from this unidentified patient.

Results

Pertinent Case History. The patient, a 55-yr-old woman, underwent surgical treatment for ovarian cancer in 1995. Since the surgery, she had been free of any detectable recurrence or metastatic spread of the carcinoma. Periodic measurements of serum CA-125 were within normal limits until October 1998, when a reference laboratory found an elevated level of CA-125 in serum of this patient. Subsequent CA-125 levels increased steadily to nearly 160 U/ml.

In July 2001, a blood specimen for serum CA-125 analysis was sent to the SJH clinical laboratory. The result was 11 U/ml. The assay was repeated in August 2001 on another specimen; the SJH laboratory reported the serum CA-125 level to be 14 U/ml. The patient's gynecologist contacted the laboratory director and inquired about the discrepancy. The serum specimen that had been assayed in August was retrieved, and the CA-125 measurement was repeated at SJH; the results were consistent with the initial report. Aliquots of the serum were sent to reference laboratories (Laboratory Corporation of America; Quest Diagnostics, Inc.), and the reported results were 156 and 187 U/ml, respectively. Serum CA-125 results for the patient during 90 mo post-surgery are shown in Fig. 1.

Investigation. There were three potential scenarios that might explain the discrepant CA-125 results: one assay could be experiencing positive interference, the other assay could be experiencing negative interference, or both assays could be affected to various degrees by interference from an endogenous compound. Since endogenous interferences in

![Fig. 1. Sequential assays of serum CA-125 levels in a patient after surgery for ovarian cancer (solid squares: assays by the Abbott MEIA method; solid triangles: assays by the Roche ECIA method).](image)
immunochemical assays ordinarily display a threshold effect, it is often possible to eliminate the interference by diluting the specimen. The specimen collected in August 2001 was diluted and assayed at SJH and Laboratory Corporation of America (Tampa, FL). As shown in Fig. 2, the dilution studies were inconclusive; results for the diluted specimens were only slightly increased with the Roche assay, and were not decreased with the Abbott assay.

Human anti-mouse antibodies have the potential to interfere with two-site heterogeneous immunoassays using mouse IgG capture and label antibodies, so the serum specimen was sent to the Laboratory Corporation of America's laboratory in Burlington, NC, for HAMA measurement. The result was 196 ng/ml (reference range: 0-188 ng/ml). The specimen was then analyzed using a blocking agent (mouse IgG) against HAMA. In the absence of heterophilic blocking reagent, the CA-125 result was 212 U/ml; in the presence of blocking reagent the result was 11 U/ml. A serum specimen was also sent for CA-125 analysis at Abbott Laboratories, which confirmed a normal level in the presence of heterophilic blocking reagent (personal communication).

Discussion

The list of biochemical markers used for detecting, staging, and monitoring neoplastic diseases has grown steadily over the last two decades, and some of these tests (eg, prostate-specific antigen) have become so commonplace that frequent references to the test appear even in the lay literature. Tumor markers typically demonstrate high sensitivity for disease, but comparatively low specificity. The predictive value of a positive result in an unselected population may be very low, and therefore the use of tumor markers for mass screening is, under most circumstances, discouraged. Perhaps the most clinically useful application of tumor markers is for detecting recurrence of disease, when baseline values following therapy have been established. A significant increase of tumor marker concentration above post-therapy levels may signal a recurrence of tumor, and prompt additional therapeutic interventions.

Serum CA-125 is widely used as a biochemical marker for ovarian cancer, which is the fifth most common cancer of women in the United States. Serum CA-125 concentrations exceed 35 U/mL in 80% of patients with nonmucinous epithelial

**Fig. 2.** Relative CA-125 levels in diluted serum specimens (results are multiplied by the dilution factor); solid squares: assays by the Abbott MEIA method; solid triangles: assays by the Roche ECIA method.
ovarian cancer [4], and the concentrations correlate well with the progression or regression of ovarian cancer [3,5].

The effect of heterophilic antibodies on immunodiffusion assays was described >30 yr ago [6], and their interference in two-site “sandwich” immunoassays became recognized as these methods became available in the mid-1980s. The presence of human anti-animal antibodies most often results in falsely elevated results for two-site immunoassays using mouse monoclonal antibodies by bridging the capture and detection antibodies [7,8], but negative interferences have also been described [9].

Estimates of the incidence of human anti-animal antibodies vary considerably, likely due to the large number of animal antigens to which humans are exposed and the heterogeneity of the antibodies that are produced in response to these exposures [6,7,10,11]. By far the most prevalent human anti-animal antibodies are against mouse immunoglobulins, which are used in imaging reagents and antibody-targeted drugs [7]. Development of human anti-mouse antibodies, and subsequent falsely elevated CA-125 measurements, has been directly linked to the use of OC125-conjugated immunoscintigraphy reagents [12,13]. In the subject of this case report, there was no record of exposure to OC125-containing pharmaceuticals or imaging reagents. The origin of the HAMA in this patient is unknown.

Several methods have been proposed for removing human anti-animal interferences in two-site immunoassays. Boerman et al [14] reported falsely elevated CA-125 results due to human anti-murine antibody interference with an immunofluorimetric assay, and suggested inclusion of murine serum or antibodies in the assay buffer to block the interference. Others have reported elimination of human anti-mouse antibody interferences by addition of mouse IgG to two-site immunoassays or use of M11 monoclonal antibody [11,15-19]. The M11 monoclonal antibody is used in many second-generation CA-125 sandwich immunoassays, and the Roche method cited in this report uses the M11 monoclonal as the capture antibody.

For interferences from anti-idiotypic antibodies, chromatographic removal of immunoglobulins prior to CA-125 assay has been suggested [20]. Morrissey et al [21] used an acid/heat extraction procedure to remove interfering anti-mouse antibodies from human sera prior to measurement of carcinoembryonic antigen. Kricka et al [22] demonstrated positive interference of rabbit and goat anti-mouse antibodies on an CA-125 immunoassay and noted that addition of mouse IgG did not eliminate the interference.

Interference by human anti-mouse antibodies in two-site “sandwich” immunoassays has been known for many years, but is widely regarded as rare and only associated with high antibody concentration. A notable finding in this case is interference in the presence of a relatively low concentration of anti-mouse antibodies, only slightly above the reference interval. Dilution factors up to 10-fold failed to alleviate the interference. The anti-mouse antibody concentration was near the upper limit of the reference range, which suggests that as many as 1 in 40 healthy individuals will have anti-mouse antibodies at a concentration as high as this patient, and dilution studies indicate that the interference is present at one-tenth of that concentration.

In conclusion, the potential exists for significant interference in serum CA-125 measurements from the presence of human anti-mouse antibodies. The magnitude of positive bias resulting even from relatively low amounts of antibody is sufficient to raise clinical suspicion of disease, and possibly prompt therapeutic or surgical interventions. Methods are available to measure serum CA-125 levels accurately in the presence of anti-mouse antibodies, and any suspicious results should be thoroughly investigated before clinical conclusions are drawn.

References


