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Case Report

Interference from heterophilic antibodies in Ca 19-9 testing: A case report

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Abstract

Immunoassays are widely used analytical techniques in clinical laboratories, which can be affected by interference, leading to erroneous test results. We present the case of a 38-year-old female patient with a thyroid nodule, who had been followed up with since 2012, was not taking medication, and had an implausible elevation of Ca 19-9, a commonly used biomarker for gastrointestinal malignancies. Interference arising from heterophilic antibodies in the differential diagnosis was considered. Measurements were repeated with the same patient sample on three different immunoassay analyzers. Ca 19-9 levels measured with the Abbott Architect i2000 device were high, while the results from the latter two instruments (COBAS e601 and ADVIA Centaur CP) were within normal limits. Serial dilutions of serum samples revealed non-linearity, suggesting assay interference. The heterophilic blocking tube procedure and polyethylene glycol precipitation resulted in a normal range of Ca 19-9 levels, confirming that a heterophile antibody interaction was the most likely cause of the abnormal results. In this paper, we present a narrative review of the current literature pertaining to false-positive Ca 19-9 caused by interference. We propose close collaboration between clinicians and the laboratory before exposing patients to the cost of additional testing when clinical non-compliance is suspected.

Keywords: Carbohydrate antigen 19-9, heterophilic antibodies, immunoassay, interference

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Immunoassays are analytical techniques widely used in clinical laboratories for the quantitation of analytes in clinical samples and involve various types of labels for signal detection [1]. Despite significant improvements in the reliability of immunoassays, there are numerous reports in the scientific literature of patients receiving inappropriate medical treatment based on false test results, leading to misdiagnosis [1].

Interference is defined as the effect of a substance in the sample, which changes the actual value of a test result [1]. Interference cannot be identified by standard quality management systems, such as quality control practices, device maintenance, and accreditation. Examples of interfering substances include heterophile or human anti-animal antibodies (HAAs), therapeutic antibodies, autoantibodies, rheumatoid factor (RF), cross-reactivity, or other unidentified interfering substances [1]. Depending on the type of immunoassay used and the mechanism of interference, the values of the results may incorrectly increase or decrease [1].

Ca 19-9 is a cell surface glycoprotein complex involved in cell-to-cell recognition processes. Ca 19-9 is the most commonly used serological biomarker for diagnosis and management of gastrointestinal cancer, after the carcinoembryonic antigen [2]. Approximately 5–10% of the population is unable to synthesize Ca 19-9, and the results are incorrectly considered false-negative in these individuals [2]. Because both false-negative and false-positive results are unacceptably high, Ca 19-9 performance is not sufficient for an accurate diagnosis of pancreatic cancer [2].

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The purpose of this case report is to remind laboratory specialists and clinicians of the possibility of false-positive Ca 19-9 levels due to laboratory analytical interference caused by heterophilic antibodies.

Case Report

This retrospective study protocol was approved by the Ethics Committee of the Institution (2011-KAEK-25 2022/11-07). In the routine examination of a 38-year-old patient who had been followed up with for a thyroid nodule since 2012 and was not on medication, Ca 19-9 analysis was performed with the Architect Ca 19-9 kit with the Abbott Architect i2000 device, and its value was found to be >1200 U/mL (reference range = 0–37 U/mL).

As the patient's tumor markers were studied for the early malignancy screening, analysis of Ca 19-9 was requested a 2nd time, and the resulting value was found to be 1040.1 U/mL. The internal (Technopath Clinical Diagnostics Multichem IA plus) and the external quality control results (KBUDEK, Istanbul, Turkey) in our laboratory were at an acceptable level.

The Ca 15-3 level, which is measured simultaneously with the patient's Ca 19-9 level, was 6.1 U/mL (IR: 0–31.3 U/mL). The level of Ca 125 was found to be normal (28.9 U/mL) and that of antithyroglobulin was above the reference values (17.25 IU/mL [IR: 0–4.11 IU/mL]).

The patient's Ca 19-9 level was studied in different centers and by different immunoassay devices. The results obtained with the COBAS e 601 (Roche Diagnostic System, Switzerland) and ADVIA Centaur CP immunoassay system (Siemens Healthcare Diagnostics, Germany) were within the normal range (Table 1).

The Architect i2000 analyzer (Abbott) uses a chemiluminescent microparticle immunoassay, the COBAS e 601 uses an electrochemiluminescence immunoassay, and the ADVIA Centaur CP immunoassay system uses direct chemiluminescent technology [2].

Serial dilutions (1/2, 1/4, 1/8) of the serum sample performed with the manufacturer's diluent (Multi Assay Manual Diluent, Abbott Laboratories, USA) revealed non-linearity (Table 2).

Heterophile antibody analysis was performed with commercially available heterophile antibody blocking tubes (HBT; Scantibodies, USA) according to the manufacturer's instructions. The result of this measurement was 14.83 U/mL (RI= 0–37 U/mL).

Our patient's serum RF level was 10.6 U/mL (RI: 0–20 U/mL) with a BN II nephelometer (Siemens HealthCare Diagnostics Products, Germany). The Ca 19-9 test has been shown to be affected by RF interference [3].

A precipitation test was performed with PEG 6000 [1]. The Ca 19-9 result was found to be within the normal reference range of 6.84 U/mL (0–37 U/mL).

Recovery in PEG precipitation was 1.4%. A recovery rate of <40% indicates the effect of high-molecular-weight proteins on test results [4, 5].

Result

Reported assay interference in the measurement of Ca 19-9 is relatively rare. Substances that commonly interfere with Ca 19-9 assays include heterophile antibodies, human anti-mouse/animal antibodies, and RFs [3, 5–10].

In 1995, Biguet et al. [5] described the possible interference of RF with Ca 19-9 for the 1st time in a 67-year-old female patient with chronic hepatitis C and a benign spinal cord tumor. High Ca 19-9 values were detected in eight different samples during a 4-month period with an Abbott IMx Device (Abbott Diagnostics, USA). The results were within the reference range when Ca 19-9 levels were measured with an immunoradiometric assay kit (CIS Bio International, France) and an ELISA kit (Enzymun test, Germany).

Berth et al. [3] published false-positive Ca 19-9 results due to RF interference in the ADVIA Centaur (Siemens Diagnostics, USA) analyzer in 2006 (A 61-year-old male was admitted for evaluation of fatigue and weight loss). The Ca 19-9 test provided normal results in the VIDAS (bioMérieux), AxSYM, and Architect i2000 (Abbott Diagnostics, USA) systems. The patient's RF concentration was too high (900 kIU/L). It has been suggested that the ADVIA Centaur system appears more sensitive to RF interference than other systems [3].

Monaghan et al. [6] reported that the level of Ca 19-9 in an apparently healthy male was high on the ADVIA Centaur device (Siemens Diagnostics, Germany) in 2009. When they analyzed the same patient sample using the Roche Modular Analytics E170 (Roche Diagnostics, Germany) and B·R·A·H·M·S KRYPTOR (Thermo Fisher Scientific, Germany) autoanalyzers, the Ca 19-9 level was within the limits of the reference range. Gel filtration chromatography showed the interference of low-molecular-weight compounds in the sample.

Liang et al. [7] found a Ca 19-9 value of 1047 U/L (through AxSYM) and 12 U/L (through Elecsys) in 2008 in a patient with a history of biliary polyps. Although the patient had a high RF level (122 IU/mL), they experimentally attributed the false-positive to the presence of HAMA.

Nakano et al. [8] reported on a 64-year-old male patient diagnosed with rectal cancer with liver metastases. The patient's Ca 19-9 concentrations were within normal limits when measured before his palliative surgical resection for rectal cancer in June 2010. After resection, the patient was treated with mouse antibodies, panitumumab and cetuximab, and his Ca 19-9 concentration was increased significantly higher with an AIA 1800 analyzer (TOSOH, Japan) at more than 400 kU/L. Based on the conflict with the clinical data, the value for Ca 19-9 was 9.5 kU/L using a UniCel Dxl 800 (Beckman Coulter, USA). An IgM-type HAMA was found to cause interference in this patient.

Çokluk et al. [9] evaluated a 57-year-old female patient who was treated with a malignant neoplasm of the cervical uterus in 2019. The Ca 19-9 result was found to be high in multiple studies with the Abbott Architect device (Abbott Diagnostics, Germany). The patient's result was found within the reference

Table 1. Results of serum CA19-9 from a 38-year old female patient on three immunoassay analyzers

Date	Our Laboratory[a]	Laboratory 1[a]	Laboratory 2[b]	Laboratory 3[c]
October 15, 2021	>1200 U/ml[a]			
October 18, 2021	1040.1 U/ml[a]			
October 19, 2021		1127 U/ml	5.5 U/ml[b]	0.0 U/ml[c]
October 20, 2021	936.83 U/ml[a]	5196 U/ml	1 U/ml[b]	0.0 U/ml[c]

[a]: ABBOTT Arcitech i2000 (Abbott Diagnostics, Abbott Park, IL, USA), [b]: COBAS e 601 (Roche Diagnostic System, Basel, Switzerland), [c]: Advia Centaur Cp Immunoassay System (Siemens Healthcare Diagnostics, Munich, Germany), (Reference interval: 0–37 U/ml). CA19-9: Carbohydrate antigen 19-9.

ranges with Roche Diagnostics (Roche Diagnostic System, Switzerland). Heterophilic antibody interference was considered in the patient, and the Ca 19-9 measurement was repeated with the HBT tube. In this repeated measurement, the level of Ca 19-9 was found within the reference interval with the Abbott Architect device (Abbott Diagnostics, Germany).

Passerini et al. [10] compared Ca 19-9 levels in 2007 using 500 consecutive samples on Abbott Architect i2000 and Roche COBAS 410 analyzers. Consistency between the two tests was found to be 90.6%. The data obtained in this evaluation clearly show that interference is a problem in both systems.

Discussion

In this study, falsely elevated Ca 19-9 in a patient was corrected by intervention with simple methods available in many laboratories.

Immunoassay tests are prone to interference due to the complexity of the antigen–antibody interaction [1]. By adding blocking reagents, test manufacturers have reduced the incidence of heterophile interference, but have not completely eliminated the problem [1]. Different devices use different blocking antibodies, which might lead to different results.

Cancer patients often show tumor-induced activation of their immune system or may suffer from infections that can lead to the production of polyspecific antibodies. Given sufficient incubation time, these are bound by blocking reagents. However, there may not be enough time to achieve complete blocking in modern automated assays [1, 11].

Interference with the analytes should be included in the patient chart, and all immunoassay analyses of the patient should be carefully reviewed and interpreted. Communication and a collaborative approach between clinicians and laboratory staff are essential. Laboratory professionals must look at the results with a critical eye and not blindly trust the instruments.

Laboratories should stop choosing assays with their price in mind rather than quality. A single false result can trigger extremely costly interventions, including direct costs, such as radiological examinations, surgical interventions, and hospitalization. Feedback from the laboratory to manufacturers allows manufacturers to improve the robustness of the immunoassay based on the risk of interference.

Table 2. Results of serial dilutions of serum sample performed with the manufacturers diluent

	Results
Initial	>1200 U/mL
1/2 dilution	1853 U/mL
1/4 dilution	2244 U/mL
1/8 dilution	2726 U/mL

Limitations

Due to technical inadequacy, the type of heterophile antibodies could not be determined. Gel filtration chromatography, the protein G addition test, and the HAMA interference test were not examined.

Conclusion

Test results that conflict with the general clinical picture should be investigated further. When interference is suspected, several approaches should be available or laboratory professionals to detect and verify it, including serial dilutions, PEG precipitation, antibody blocking tubes, and alternative analytical platforms.

Informed Consent: Informed consent was obtained from the patient included in this study.

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