Persistent increase of carbohydrate antigen 19-9 with an unknown reason: A seven-year follow-up case

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Abstract
Background: We described a patient who exhibited a gradual increase in carbohydrate antigen 19-9 (CA19-9) concentrations for 4 years at three hospitals, with no associated clinical manifestations; however, we were unable to define the cause of this increase, forcing us to consider whether it was a false-positive result.

Methods: Given the potential for interference, this study used multiple system detection, gradient dilution, Polyethylene glycol (PEG) precipitation and heterophilic antibody blocking assay to evaluate the reliability of CA19-9 concentration increase.

Results: Analysis of the patient sample using multiple systems indicated that CA19-9 concentrations showed an obvious increase (154.0, and 889.2 IU/ml, respectively) using the Cobas E602 and Advia Centaur XP systems, and were within the reference ranges (<10 IU/ml) on other modules. PEG precipitation on the Cobas E602 and Advia Centaur XP systems reduced the CA19-9 concentration, as did heterophilic blocking tube (HBT-6, HBT-1) blockade.

Conclusion: CA19-9 was incorrectly identified to increase due to the presence of heterophilic antibodies. We recommend that heterophilic antibodies should be evaluated in cases with elevated CA19-9 level but no associated clinical manifestations to prevent false positives.

Keywords
CA19-9, false positive, heterophilic antibody, interferent, pancreatic cancer

1 | INTRODUCTION

Carbohydrate antigen 19-9 (CA19-9) is a well-known glycoprotein distributed in the pancreas, gallbladder, liver, and intestines of the fetus during embryonic development. Normal individuals tend to exhibit very low serum concentrations of this biomarker, making it a very sensitive tumor marker in pancreatic cancer.1 Serum CA19-9 is also substantially increased in patients with cholangiocarcinoma, colorectal cancer, and in some patients with cholestasis.2 Chemiluminescence is an analytical method of high sensitivity and wide linear range. Nowadays it has been widely used in detecting tumor marker. However, the assay still cannot completely avoid the interference and test results can be skewed in response to a range of interferents. Common interferents include cross-reacting...
substances, heterophilic antibodies, human anti-animal antibodies and autoantibodies, etc. Furthermore, preanalytical aspects and certain disease states can also increase the potential for interference in immunoassays. Among these, the presence of endogenous antibodies remains the most under-evaluated issue in these immunoassays. Up to 40% of humans produce endogenous antibodies, mostly as a result of animal contact, vaccination, infection, blood transfusion, or exposure to animal antigens. Several experiments have shown that approximately 0.5% of the data has led to clinical misdiagnosis. The presence of endogenous heterophilic antibodies can play a hidden and unpredictable role in the analysis and is often confounded by the antibodies used in the immunoassay, resulting in false positives or negatives.

Here, we described a patient who presented with a progressive increase in serum CA19-9 concentration, with a peak value of 7000 IU/ml, but no consistent clinical signs or symptoms of disease exacerbation. A series of experiments was conducted and it was finally confirmed that this CA19-9 increase was an experimental artifact produced by the presence of heterophilic antibodies.

2 | CASE DESCRIPTION

The 42-year-old female patient described here was found to have a modestly increased CA19-9 concentration following a medical examination at hospital A in May 2015. This value continued to increase unusually over time. In May 2018, blood testing revealed a CA19-9 concentration >700 IU/ml at hospital B, and the retested result was >7000 IU/ml (Figure 1), strongly suggesting the presence of a malignant tumor, especially pancreatic cancer or cholangiocarcinoma. Therefore, an abdominal computerized tomography (CT) and laboratory tests showed no obvious anomalies. The other examinations performed; results showed only mild dilation of the common bile duct. No additional anomalies were found based on endoscopic retrograde cholangiopancreatography (ERCP). The other examinations were detected using the Cobas E602 (Roche Diagnostics) and the AutoLumo A2000plus (Autobio), and DXI800 (Beckman Coulter). CA19-9 values from these systems were within the reference ranges, substantially different from the results obtained using the CL6000i (Mindray), AutoLumo A2000plus (Autobio), and DXI800 (Beckman Coulter).

Case resolution

3 | CASE RESOLUTION

An evaluation of the full case history indicated a few anomalies. First, tumor markers including carcinoembryonic antigen, carbohydrate antigen 125 (CA125), CA153, and CA72-4 were all within reference ranges, except for CA19-9. Second, CA19-9 values fluctuated widely when tested three times at the same hospital without any treatment, from 4944 to >7000 IU/ml. Third, the results for CA19-9 were considerably different between the two hospitals within 1 week, declining rapidly from 4944 to 558 IU/ml. Theoretically, a drastic change in CA19-9 concentrations should not be observed in vivo without treatment. Therefore, we hypothesized that there might be assay interference. A series of measures and experiments was conducted to investigate the cause of the inconsistency and possible interference.

3.1 | Analysis using different testing systems

Detection of CA19-9 was confirmed using the Advia Centaur XP (Siemens Diagnostics) at hospital B. At hospitals A and C, CA19-9 was detected using the Cobas E602 (Roche Diagnostics) and the same CA19-9 reagents were used. To exclude the influence of the detection system, we subsequently evaluated the patient's serum using other testing systems such as the CL6000i (Mindray), AutoLumo A2000plus (Autobio), and DXI800 (Beckman Coulter). CA19-9 values from these systems were within the reference ranges, substantially different from the results obtained using the Cobas E602 and Advia Centaur XP (Table 1). The results indicated the presence of an interferent in the patient's serum that might interfere with CA19-9 detection using the Cobas E602 and Advia Centaur XP systems.

3.2 | Gradient dilution

When the patient's serum was diluted two-, four-, and eight-fold in Roche universal diluent, CA19-9 concentrations using the Cobas E602 were 73.23, 39.15, and 19.86 IU/ml, respectively (Figure 2). The values for the control serum were 119.7, 66.07, 35.01, and 20.1 IU/ml at one-, two-, four-, and eight-fold dilutions, respectively (Figure 2). The dilution results showed an almost linear trend, which was not typical for dilutions of endogenous interfering antibodies. Further validation was required.
3.3 | Polyethylene glycol (PEG) precipitation

The patient’s serum was mixed with a two-fold volume of 20% PEG, incubated for 1 h at 37°C, followed by centrifugation at 15,000 g for 10 min. CA19-9 levels were detected using the Cobas E602 and Advia Centaur XP systems. PEG precipitation resulted in a drastic reduction in patient’s CA19-9 concentrations from 154.0 to 15.5 IU/ml when evaluated using the Cobas E602 and from 889.2 to 10.6 IU/ml when evaluated using the Advia Centaur XP, with no considerable change observed in the control serum and quality controls (Figure 3A). This indicated that the endogenous interferent in the patient’s serum might have been derived from immunoglobulins, with a greater interference observed with the Centaur XP.

3.4 | Heterophilic antibody blocking assay

An immunoassay interference analysis was performed using heterophilic blocking tubes (HBT) coated with different heterophilic blocking agents targeting mouse antibody systems (Scantibodies Laboratory Inc.). Briefly, 250 µl serum was added to HBT-1, HBT-3, and HBT-6, respectively, and incubated for 1 h at 18–28°C. HBT-6 successfully blocked heterophilic antibodies in the patient’s serum using the Cobas E602 and the Advia Centaur XP to detect CA19-9 (Figure 3B). However, no considerable changes were observed in the control serum and quality controls after HBT-6 treatment (Figure 3B). HBT-1 had a similar effect in removing heterophilic antibodies whereas HBT-3 had no effect (Table 2). These implied that the presence of heterophilic antibodies in the patient’s serum falsely increased CA19-9 concentrations.

4 | DISCUSSION

CA19-9 immunoassays have high sensitivity and good specificity, but interference cannot be completely avoided. Common conditions (hemolysis, jaundice, and lipemia) have less influence on immunological detection currently, and interference by endogenous antibodies is receiving increasing attention. Endogenous antibodies include heterophilic antibodies, human anti-animal antibodies, and autoantibodies, among others. Reportedly, rheumatoid factor can interfere with CA19-9 detection. Other studies have shown that heterophilic antibodies and a low-molecular-weight interference result in the false positives of CA19-9 concentration. Further, human anti-mouse antibody generation after immunotherapy (panitumumab and cetuximab) results in an increase of CA19-9 concentrations in a patient with rectal cancer after surgical resection. In the current case report, the patient’s rheumatoid factor was within the reference range. The patient did not have any autoimmune disease, did not receive any immunotherapy, and tested negative for autoantibodies. Therefore, we hypothesized that the discrepancy in CA19-9 detection might have resulted from the presence of heterophilic antibodies.

In general, the binding capacity of heterophilic antibodies and target antibodies is weak, and the result will usually change in a nonlinear fashion following dilution. However, our patient’s results showed a clear linear change in CA19-9 concentrations after two-, four-, and eight-fold dilution. That the binding capacity of these endogenous antibodies is strong and that the affinity does not change with dilution cannot be ruled out, but this should be evaluated with caution.
Capture and labeled antibodies for CA19-9 from different manufacturers are derived from different species and bind to different antigen epitopes. Therefore, heterophilic antibodies have variable effects on different testing systems. The same 1116-NS-19-9 mouse monoclonal antibody is used to detect CA19-9 in both the Roche and Siemens systems, which can explain why the Cobas E602 and Advia Centaur XP assay results are inconsistent with those of the other three systems.

Polyethylene glycol precipitates proteins via steric hindrance. Reportedly, PEG 6000 precipitates immunoglobulin M (IgM) and IgG completely and up to 80% of IgA. Previous studies have shown that PEG pretreatment can reduce the interference of heterophilic antibodies. Here, PEG precipitation confirmed that the interferent resulting in false-positive results for CA19-9 on the Cobas E602 and Advia Centaur XP systems might have been derived from immunoglobulins. The effect of this interferent was more pronounced with the Advia Centaur XP. Several studies have reported that the Advia Centaur is more sensitive to rheumatoid factors and other interferents, which is consistent with our results.

HBT-1, HBT-3, and HBT-6 were then used to perform heterophilic antibody blocking assays. The use of HBTs cannot always identify the presence of heterophilic antibodies; positive results indicate the presence of an interferent, but negative results are not evidence of no interferent. Hence, if one type of HBT provides a negative result, another tube should be tested. In this study, HBT-3 could not decrease the CA19-9 concentrations, whereas the CA19-9 concentrations decreased when treated with HBT-6 and HBT-1.

How long heterophilic antibodies remain in the body is still unknown. In this study, the patient’s work brought her into contact with mice between 2011 and 2017. The patient was no longer exposed to mice as of November 2017. CA19-9 concentrations continued to increase after 2015 and peaked in 2018, appearing more like the progression of a disease than an interferent. Second, the systems (Advia Centaur XP and Cobas E602) used at the three hospitals were coincidentally affected by heterophilic antibodies in the patient’s serum. The results obtained from the analyses at the three hospitals were abnormal, which prevented clinicians from investigating the discrepancies between clinical assessments and the CA19-9 results. Owing to the unknown cause of elevated CA19-9, the patient underwent a general examination every 6 months. Therefore, although common malignant tumors were excluded, the results still placed great psychological and economic burdens on her. Notably, the patient suffered from operational pancreatitis while undergoing ERCP and developed gallstones 1 year later, which could have been avoided if the situation was identified earlier. This makes timely communication between clinicians and laboratorians imperative.

5 | CONCLUSION

In summary, this study described an abnormal increase in CA19-9 concentration resulting from the presence of heterophilic antibodies in the patient’s serum. It was observed that heterophilic antibodies could interfere simultaneously with the detection of CA19-9 using multiple detection systems and exist in the body for several years. When the test results are inconsistent with the clinical signs, interference of heterophilic antibodies should be considered as early as possible to avoid unnecessary examination and treatment of patient.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this work.

DATA AVAILABILITY STATEMENT

All data generated during this study are included in this published article.
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