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CASE REPORT

A Pseudo Elevation of CEA Caused by Epidemic Hemorrhagic Fever

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SUMMARY

Background: **Heterophilic antibodies (HA) are one of the main substances that interfere with immunology, especially chemiluminescence immunoassay. Non-specific binding, labeling antibodies, bridging to capture antibodies, or labeling antigens can interfere with the detection process, leading to serious discrepancies between the measured results and clinical manifestations, and even delaying clinical diagnosis and treatment.**
Methods: **This paper is a case of epidemic hemorrhagic fever causing pseudo CEA elevation caused by heterophagy induced antibodies in the body.**
Results: **The patient's CEA detected on the ABBOTT detection platform was 51.1 ng/mL, and on the ROCHE detection platforms it was 4.66 ng/mL, and treated by PEG precipitation it was 45.2 ng/mL, after diluting the sample the CEA was 50.2 ng/mL, meanwhile the patient's platelets were 96 x 10^9/L and serum creatinine was 188.4 µmol/L, epidemic hemorrhagic fever IgM antibody was positive.**
Conclusions: **When the test results do not match clinical symptoms, further confirmation is required through additional testing. Patients who use mouse monoclonal antibody preparations for diagnosis or treatment may have human anti-mouse antibodies in their serum, and the test results may falsely increase or decrease.**
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KEYWORDS

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INTRODUCTION

Carcinoembryonic antigen (CEA) can be widely present in digestive system cancers originating from the endosperm. CEA is a broad-spectrum tumor marker, which can reflect the existence of a variety of tumors to people. It is a good tumor marker for curative effect judgment, disease development, monitoring, and prognosis estimation of colorectal cancer, breast cancer, and lung cancer. The serum CEA level has a clear relationship with the stage of colorectal cancer. The more advanced the lesions are, the higher the CEA concentration. An abnormal increase in CEA in patients should be taken seriously [1].
Heterophilic antibodies (HA) are a class of multi specific immunoglobulins with sufficient titers and relatively

weak binding to immunoglobulins from multiple species, produced by known or unknown antigenic substances stimulating the human body [2]. This antibody can react with antigens that are unrelated to the original antigen. This substance, which has a different chemical structure but similar activity to the tested substance, can bind to fragments of many animal immunoglobulins, interfere with experiments, and make the test results inconsistent with clinical manifestations, leading to erroneous results [3].

Epidemic hemorrhagic fever is a natural infectious disease mainly transmitted by rodents. Patients usually come into contact with contaminated mouse blood, saliva, feces, and urine, and the incubation period after infection is usually 2 - 3 weeks. The main symptoms of epidemic hemorrhagic fever include fever, bleeding, hypotension shock, and kidney damage [4].

CASE PRESENTATION

The patient was a 67 year old female who was previously healthy without a history of tumor, chronic disease, or blood transfusion. Living in rural areas of Shaoxing City, she had no obvious fever for 10 days, with a maximum of 39.8℃. She came to our hospital and was hospitalized. The ABBOTT detection platform revealed that CEA was 51.1 ng/mL (normal < 5.0 ng/mL). Blood routine examination results were platelets 96 x 10^9/L and serum creatinine 188.4 μmol/L, epidemic hemorrhagic fever IgM antibody was positive. Other results including thyroid function, liver function, antinuclear antibody spectrum, antineutrophil antibody, RF, abdominal CT, B-ultrasound, gastroscopy and enteroscopy showed no abnormalities. Epidemic hemorrhagic fever IgM antibody was positive.

DISCUSSION

The staff of the laboratory department asked the clinical staff to take blood again for reexamination. The redrawn blood sample and the original sample were detected at the same time, and the results were consistent with the initial disease detection.

At the same time, we divided the patient's serum into three parts. One part was treated by PEG precipitation method; one sample was tested by Roche detection platforms, and the test results of one sample after dilution are as follows (Table 1).

At the same time, through communication with doctors, it was found that the patient lived in rural areas and had fever, thrombocytopenia, and abnormal renal function. After that, the patient was tested for epidemic hemorrhagic fever IgM antibody, and it was found that the antibody was positive. It was preliminarily determined that the patient had epidemic hemorrhagic fever.

In the experiment of antigen antibody detection, the main factors affecting the detection results are exogenous and endogenous interference factors. The main external interference factors include hemolysis and bacterial contamination of the specimen. None of them were present in the specimen, so endogenous factors were considered. Autoantibodies and RF were excluded one by one. After dilution, PEG precipitation, and replacement of the detection system, it was inferred that heterophilic antibody interference was present. At present, the interference mechanism of HA in an immunoassay reported in literature mainly occurs when using the sandwich or competitive immunoassay. Therefore, patients with HA in their bodies can interfere with immune serological tests by binding HA to reagent antibodies.

Our hospital uses Abbott's two position "one-step" chemiluminescence particle immunoassay, also known as "sandwich" analysis, to test CEA, as shown in Figure 1A. Our CEA detection antibodies come from mouse sources. The main reason for the impact of this result is that the patient was exposed to contaminated substances from mice, which resulted in the production of mouse derived heterophilic antibodies in the body. The sandwich method and competition method, which are commonly used in clinical practice, are the main interference methods. In the double antibody sandwich method, the HA antibody affects the immune response in two main ways: 1) acting as the tested antigen, while combining the captured antibody and labeled antibody, resulting in a false increase in the results. This patient belongs to this situation, as shown in Figure 1B. 2) It does not form a bridge, but binds to the captured antibody or labeled antibody separately to inhibit the recognition of the tested antigen by the reagent antibody resulting in a false decrease in the results (Figure 1C).

Tumor markers are increasingly widely used in health experiences and as auxiliary diagnostic indicators. Continuous observation of the dynamic interaction of tumor markers is clinically more meaningful. If a physical examination reveals a continuous and progressive increase in one or several tumor markers, vigilance should be increased, and further examination methods such as CT and ultrasound are needed. If there is only a single increase or no significant change in the results of each examination, there is no need to be so nervous.

In this case, when there were abnormal results, it was limited to reviewing the case and timely clinical communication. The initial examiner has also undergone corresponding follow-up procedures and issued a report. However, due to methodological limitations, the significantly elevated false results mislead clinical practice. As a laboratory doctor, one should not only be fully familiar with the expected use of project reagents, testing principles, sample requirements, interference factors, limitations of testing methods, and precautions. What is more needed is to utilize diverse knowledge structures, conduct comprehensive analysis, and help clinical interpretation of report forms. When there is a discrepancy between the test results and clinical practice in daily work, timely contact and communication with clinical practice should be made, and various techniques and

Table 1. CEA values using different detection methods.

	CEA (ng/mL)
Dilution	**50.2**
PEG precipitation	**45.2**
ROCHE	**4.66**
ABBOTT	**51.1**

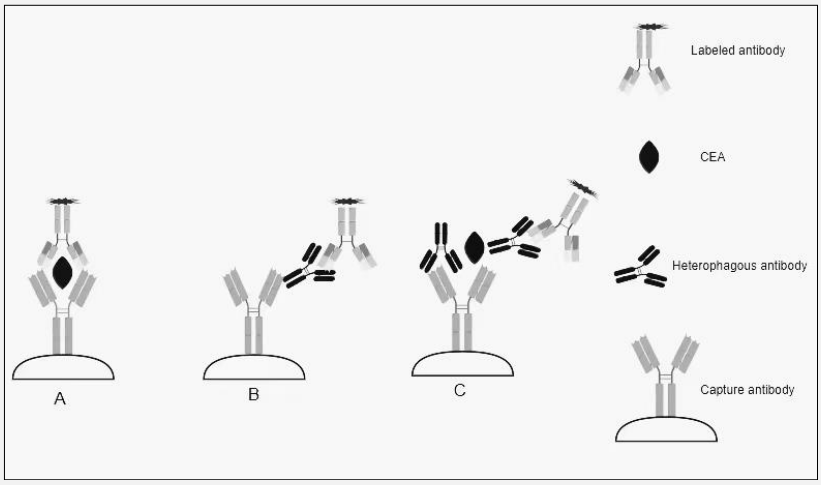

Figure 1. CEA detection interference mechanism diagram.

A. CEA Schematic diagram of normal detection mechanism.
B. CEA Schematic diagram of pseudo elevation mechanism.
C. CEA Schematic diagram of pseudo reduction mechanism.

methods should be used combined with clinical practice, to gradually uncover the truth of the matter.

Afterwards, the general methods for removing HA interference we encounter are:

1. Dilution method: Some immunoassay reagents have added components that absorb heterologous antibodies. If the concentration of HA in the sample is not very high and the interference caused by it is not significant, the dilution method can reduce its interference, but it cannot completely eliminate it [5].
2. Physicochemical technology: It can be achieved by ultracentrifugation, sample heating, gel filtration or chromatography, and polyethylene glycol (PEG) precipitation [6].
3. Blockers: Use non-specific and specific blockers to bind to HA to reduce interference. Special blocking agents are mainly Ig inhibitors and HA blocking agents, which are generally non-specific blocking agents of mouse monoclonal antibodies. High concentrations of non-specific Ig are used to block the interference of HA [7].
4. Utilize substances with low reactivity to HA: Non Ig affinity proteins, specific rabbit $F(ab)^2$ fragments, etc. can be used as solid-phase antibodies or enzyme-linked antibodies to reduce the chance of binding to HA [8].

CONCLUSION

In summary, the current living environment of patients and whether they keep pets may have some interference with clinical immunological test results. This case aims to remind clinical laboratory staff to be familiar with the testing methods and principles of testing indicators. If the test results do not match the clinical symptoms, a detailed medical history should be asked. In addition,

laboratory staff must also be familiar with and master common methods for eliminating interference.

Declaration of Interest:
All authors declare that they have no conflict.

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