A 74-year-old man was admitted for chest infection with acute exacerbation of chronic obstructive pulmonary disease. He was incidentally found to have an increased serum level of cardiac troponin I, despite the absence of symptoms and electrocardiographic evidence of ischaemic heart disease. Troponin I became undetectable after the serum was treated with polyethylene glycol, which removed any interfering antibodies. Serum cardiac troponin T was also undetectable after this treatment. Interference of the cardiac troponin I assay by heterophilic antibodies was thus confirmed. Because of the possibility of false-positive results due to immunoassay interference, clinicians should be alerted whenever laboratory findings are incompatible with the clinical picture, and should be ready to perform additional laboratory tests.

Introduction

A consensus document published by the European Society of Cardiology, the American College of Cardiology, and the American Heart Association in 2000 redefined the criteria for the diagnosis of myocardial infarction (MI).1 Either the cardiac troponin level or the myocardial band fraction of creatine kinase (CK-MB; preferably the CK-MB mass) is required for establishing the diagnosis of acute, evolving, or recent MI. Apart from being crucial diagnostic markers, cardiac troponins have also been shown to be prognostic indicators in acute cardiac events. 2-4 Higher troponin levels found in patients at presentation to the emergency department are predictive of poorer outcomes, and high troponin in this setting may help to identify a subgroup of patients who are at higher risk of experiencing subsequent cardiac events. Implementation of early intensive management strategies to this group of patients may be beneficial.

Despite the usefulness of analysing cardiac troponins, numerous reports have been published on erroneous cardiac troponin I (cTnI) results because of the presence of various interfering substances which affect the analytical procedures.5-7 For example, the presence of heterophilic antibodies, rheumatoid factor, or unidentifiable substances. A study showed that the prevalence of false-positive cTnI results was 14.8% among patients with positive cTnI test results.8 In another study, heterophilic antibodies interfered in approximately 2.0% of cases.9 In this article, we report on a patient who had increased cTnI levels, despite the absence of other features of myocardial injury.

Case report

A 74-year-old man presented with a sudden onset of breathlessness to the
Queen Elizabeth Hospital in March 2002. He lived in a home for the elderly and had a history of benign prostatic hyperplasia and chronic obstructive pulmonary disease (COPD); a few years previously, he started receiving long-term oxygen therapy at a rate of 3 L/min and had been homebound ever since.

The patient presented with cough and yellowish sputum, but he had no fever or chest discomfort. Physical examination revealed dyspnoea with increased expiratory breath sounds and occasional rhonchi. The chest X-ray showed a hyperinflated chest with a bulla in the lower-left lung and no pneumothorax. Electrocardiography (ECG) revealed sinus tachycardia, right-bundle branch block, and prominent P waves.

Routine blood tests were performed on the day of hospital admission (day 0). The white blood cell count was 14.0 x 10⁹ /L (reference range, 3.7-9.2 x 10⁹ /L). Analysis of arterial blood gas while the patient was receiving oxygen showed acute-on-chronic respiratory acidosis—namely, pH 7.25; partial pressure of carbon dioxide, 9.8 kPa; hydrogen carbonate level, 31 mmol/L; and partial pressure of oxygen, 22.0 kPa. He was treated for chest infection and an acute exacerbation of COPD. Cefuroxime, salbutamol, ipratropium bromide, and hydrocortisone were given. However, the cTnI level, as measured with salbutamol, ipratropium bromide, and hydrocortisone, showed no serial increase in subsequent blood tests. However, the cTnI level remained high, and reached 65.9 ng/mL on day 6. The level remained high, and reached 65.9 ng/mL on day 6. The patient did not have any clinical signs of congestive heart failure. Echocardiography revealed trivial aortic and mitral regurgitation, but no other abnormality. He had not recently received any vaccinations, undergone medical procedures involving the injection of animal antibodies, or made any contact with animals. He did not have any known allergies to drugs or food. His rheumatoid factor status was positive.

In view of the spuriously high cTnI level, antibody interference in the assay was suspected. Interference studies were thus conducted during hospitalisation. A sample of the patient’s serum taken on day 9 was divided into three portions. The first portion was used to determine the cTnI level as usual, which was found to be 54.1 ng/mL (Table). The second portion (200 µL) was mixed with 200 µL of 25% (w/v) polyethylene glycol in deionised water (PEG 6000; BDH Chemical Ltd, Poole, UK), vortexed vigorously, and centrifuged at 3000 g for 10 minutes⁵; the supernatant was then assayed for cTnI. Results showed that cTnI was no longer detected in the patient’s serum; however, sera from five patients who had received a diagnosis of acute MI were also treated in the same way and yielded negative results for cTnI (Table). The last portion of the patient’s serum was tested for an alternative cardiac marker, cardiac troponin T (cTnT) by using an electrochemiluminescence immunoassay (Elecsys 1010; Roche/Hitachi, Mannheim, Germany) the result of which was negative. Hence, these findings supported the suspicion of antibody interference in the cTnI assay.

The patient responded well to antibiotic and bronchodilator therapy. On the basis of the information that the high cTnI was due to antibody interference, the patient was discharged home on day 12. No evidence of ischaemic heart disease was detected on follow-up (Fig).

**Discussion**

Heterophilic antibodies are antibodies detected in human sera against animal immunoglobulins. Although not leading to clinical diseases, these antibodies sometimes cause interference in immunoassays, because they can interfere with the binding of the capture antibody to the signal antibody, or rarely, they can have idiotypic antibody interactions.¹¹ People who have frequent contact with animals, such as farm workers or veterinarians, or those who keep domestic animals at home have an increased chance of developing heterophilic antibodies. The production of these antibodies can also be triggered by the injection of animal antibodies during radiological imaging or cancer therapy. The prevalence of heterophilic antibodies in the general population has been estimated to be as high as 40%, although only a minority of affected people have titres.
high enough to cause interference in immunoassays.\textsuperscript{12} The patient in our case had no recent history of vaccination, animal exposure, or special imaging that required the injection of animal antibodies.

Rheumatoid factor is a heterogeneous group of auto-antibodies that are directed against immunoglobulin G. It is sometimes found in the elderly, and was present in the patient in our case. Rheumatoid factor is a well-known interfering substance in immunoassays and its mechanism of action is similar to that of heterophilic antibodies.\textsuperscript{11} Interference caused by rheumatoid factor could be specifically confirmed by using an appropriate agent that removes the factor.\textsuperscript{5} Unfortunately, this agent was not readily available in our laboratory, and whether the antibody interference was specifically caused by rheumatoid factor could not be determined.

There are several ways of demonstrating the presence of antibody interference in immunoassays. Apart from repeating the test with an alternative assay, one can use polyethylene glycol—a high–molecular weight polymer—to precipitate large immune complexes in the serum\textsuperscript{13}; this method is simple and inexpensive, and is commonly used in many laboratories. Serum samples containing interfering antibodies will yield abnormal results but apparently normal ones after polyethylene glycol treatment. Although we do not know the exact nature of the interfering substance in the patient’s serum, the normal cTnT result, together with the undetectable cTnI after treatment, confirmed our suspicion of antibody interference in the cTnI assay. Such a phenomenon was not observed in the samples taken from control patients who had had an acute MI.

It has been reported that cTnI can rise to 4 times the cut-off level in approximately 18% of patients with acute exacerbation of COPD.\textsuperscript{14} However, in the patient in our case, the grossly elevated cTnI level, together with other findings in the interference studies, pointed towards the presence of antibody interference rather than a concomitant acute myocardial insult.

Clinicians should consider the indications carefully before requesting any investigation. On the other hand, with the increasing reliance of cardiac troponins in the diagnosis of acute MI or the spectrum of acute coronary syndrome, clinicians have to be aware of the possibility that heterophilic antibodies can falsly elevate a test result. When unexpected cTnI results are encountered, it is important to seek assistance from the laboratory to clarify whether antibody interference is occurring.

In a broader perspective, immunoassays are now widely used in all clinical specialties. Although the standard of medical care in general has significantly improved, and many types of immunoassay test are available, clinicians should be cognisant of the possibility of inaccurate results arising from antibody interference in these assays. Whenever a laboratory result is inconsistent with the clinical picture, clinicians should consult the laboratory before performing expensive or potentially invasive procedures to investigate the unexpected laboratory abnormality.

**Conclusion**

False-positive cTnI results can be due to antibody interference of the immunoassay procedure. Physicians should be aware of patients who have elevated cTnI levels without other clinical evidence of MI or injury. Consultation with the laboratory is advised to clarify the biochemical abnormality.

**Acknowledgements**

We thank Ms SH Cheng of the United Christian Hospital and Ms ML Yuen of the Queen Elizabeth Hospital for their technical assistance.

**References**