AOK Chan 陳安琪
JPS Chan 陳珮珊
KL Choi 蔡建霖
CC Shek 石志忠

A patient with an increased troponin level without evidence of ischaemic cardiac injury

無缺血性心臟病的病人血清測試呈高濃度心肌旋轉蛋白

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.

一名74歲病人因患上肺炎及慢性阻塞性肺病發作而入院，住院期間並無缺血性心臟病的病徵及心電圖改變，然而，他的血清測試呈高濃度心肌旋轉蛋白I。經聚乙烯乙二醇處理以去除干擾抗體後，病人血清中的心肌旋轉蛋白I測試變成陰性，其血清中的心肌旋轉蛋白T也呈陰性反應，從而確定心肌旋轉蛋白I的分析會受到heterophilic抗體的干擾。由於免疫分析法測試顯示的假陽性結果並非罕見，故此當醫生發現化驗結果與其臨床診斷出現分歧時，便應提高警覺，並向化驗室要求予以協助。

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

Key words:
Antibodies, heterophile;
False positive reactions;
Myocardial infarction;
Troponin

關鍵詞：
抗體，heterophile；
假陽性反應；
心肌梗死；
旋轉蛋白

Hong Kong Med J 2004;10:277-9

Queen Elizabeth Hospital, 30 Gascoigne Road, Kowloon, Hong Kong:
Department of Pathology
AOK Chan, BMedSc, MB, ChB
CC Shek, MB, BS, FRCPath
Department of Medicine
JPS Chan, MB, BS
KL Choi, FRCP, FHKAM (Medicine)

Correspondence to: Dr AOK Chan
(e-mail: chanok@ha.org.hk)

Table. Patient and control serum levels of cardiac troponin I and T

Serum sample	cTnI (ng/mL)*	cTnI after PEG† treatment (ng/mL)	cTnT (ng/mL)‡
Patient	54.1	<0.1	<0.01
Controls	1.2	1.8	0.17
	7.7	9.6	0.64
	21.0	15.7	0.39
	45.2	36.6	1.07
	171.8	143.0	2.16

* cTnI cardiac troponin I; reference level, <0.4 ng/mL
† PEG polyethylene glycol
‡ cTnT cardiac troponin T; reference level, <0.04 ng/mL

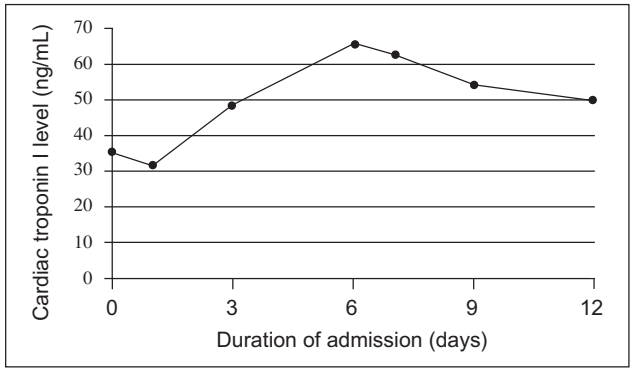

Fig. Changes in patient serum levels of cardiac troponin I

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 10^9 /L (reference range, 3.7-9.2 x 10^9 /L). Analysis of arterial blood gas while the patient was receiving oxygen therapy showed acute-on-chronic respiratory acidosis—namely, pH 7.25; partial pressure of carbon dioxide, 9.8 kPa; hydrogencarbonate 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 an enzyme immunoassay (AxSYM; Abbott Laboratories, Illinois, US) was very high, at 35.5 ng/mL (reference level, <0.4 ng/mL; the diagnostic cut-off for major myocardial injury is 2.0 ng/mL), although the patient did not complain of any chest discomfort. Subsequent ECG showed no serial changes or S1Q3T3 pattern. Levels of CK and lactate dehydrogenase were within normal ranges and showed no serial increase in subsequent blood tests. However, the cTnI 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 minutes[10]; 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.[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 autoantibodies 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.[11] Interference caused by rheumatoid factor could be specifically confirmed by using an appropriate agent that removes the factor.[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[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.[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 falsely 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

1. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959-69.
2. Galvani M, Ottani F, Ferrini D, et al. Prognostic influence of elevated values of cardiac troponin I in patients with unstable angina. Circulation 1997;95:2053-9.
3. Stubbs P, Collinson P, Moseley D, Greenwood T, Noble M. Prognostic significance of admission troponin T concentrations in patients with myocardial infarction. Circulation 1996;94:1291-7.
4. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342-9.
5. Dasgupta A, Banerjee SK, Datta P. False-positive troponin I in the MEIA due to the presence of rheumatoid factors in serum. Elimination of this interference by using a polyclonal antisera against rheumatoid factors. Am J Clin Pathol 1999;112:753-6.
6. Knoblock RJ, Lehman CM, Smith RA, Apple FS, Roberts WL. False-positive AxSYM cardiac troponin I results in a 53-year-old woman. Arch Pathol Lab Med 2002;126:606-9.
7. Fitzmaurice TF, Brown C, Rifai N, Wu AH, Yeo KT. False increase of cardiac troponin I with heterophilic antibodies. Clin Chem 1998;44:2212-4.
8. Fleming SM, O'Byrne L, Finn J, Grimes H, Daly KM. False-positive cardiac troponin I in a routine clinical population. Am J Cardiol 2002;89:1212-5.
9. Yeo KT, Storm CA, Li Y, et al. Performance of the enhanced Abbott AxSYM cardiac troponin I reagent in patients with heterophilic antibodies. Clin Chim Acta 2000;292:13-23.
10. Casesnoves A, Mauri M, Dominguez JR, Alfayate R, Pico AM. Influence of anti-insulin antibodies on insulin immunoassays in the autoimmune insulin syndrome. Ann Clin Biochem 1998;35:768-74.
11. Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem 1998;44:440-54.
12. Boscato LM, Stuart MC. Incidence and specificity of interference in two-site immunoassays. Clin Chem 1986;32:1491-5.
13. Burtis CA, Ashwood ER. Tietz textbook of cinical chemistry. In: Kricka LJ, editor. Principles of immunochemical techniques. 3rd ed. WB Saunders Company; 1999:205-25.
14. Baillard C, Boussarsar M, Fosse FP, et al. Cardiac troponin I in patients with severe exacerbation of chronic obstructive pulmonary disease. Intensive Care Med 2003;29:584-9.