Macrotroponins cause discrepancy in high-sensitivity examination

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Aim. We present two cases with clearly discrepant results of clinical examination and cardiac troponin I (cTnI) and cardiac troponin T (cTnT) concentrations. In similar cases with discrepant results, the possibility of interference should be considered.
Methods. Due to the suspicion of the presence of macrotroponin I in both of the presented cases, the patients were invited to our laboratory and both cTnI (Architect i1000, Abbott) and cTnT (Cobas 8000, Roche) concentrations were analysed. The samples were treated by preincubation in a heterophilic antibodies blocking tube (HBT) and analysed. Precipitation with polyethylene glycol solution (PEG) and molecular weight separation by gel filtration on Sephadex G100 was performed and concentrations of cTnI were analysed.
Results. In the same blood sample, the cTnT and cTnI concentrations were 7 and 1782 ng/L, respectively, in Case 1, and 6 and 96 ng/L, respectively, in Case 2. Incubation of samples in HBT had no significant effect. CTnI concentrations after precipitation with PEG – presented as the percentage of initial concentrations – were 7.4% in Case 1 (and 26.8% in the control sample) and 1.4% in Case 2 (and 56.0% in the control sample). These results indicate a significant decrease in both cases, supporting presence of macrotroponin I. Finally, analyses of cTnI concentrations after gel filtration also supported the presence of macrotroponin I.
Conclusion. The present cases show that the presence of macrotroponin can lead to unnecessary investigation of the patient. When the possibility of interference is suspected, cooperation with laboratory staff to help with interpretation or to perform more detailed analysis is crucial.

Key words: cardiac troponin, interference, immunoassay, macrotroponin

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CASE REPORT 1

A 57-year-old woman who had not yet been treated internally, a smoker (5 cigarettes a day), was admitted to the Neurology Department of the Privamed Hospital in Pilsen for cognitive impairment with partial amnesia. A similar condition had affected her 2 years ago. On admission, subjective symptoms were relieved. The patient was examined by a neurologist, including a brain computed tomography (CT) scan, with a negative finding. Because of ECG changes (inferolateral ST segment depression) and chest pain that had occurred 4 days ago, cardiac troponin I (cTnI) was determined by a hypersensitive method (Architect i1000, Abbott Laboratories). The result was 1782 ng/L (99th percentile 13 ng/L). CTnI concentrations were re-examined repeatedly with elevated values each time (1741, 3520 and 3622 ng/L). Therefore, the patient was transferred to the intensive care unit (ICU) of the Cardiology Clinic of the University Hospital in Pilsen to perform an early coronary angiography to rule out inferolateral non-Q myocardial infarction. Coronary angiography showed only insignificant stenoses of both coronary arteries. Transthoracic and oesophageal echocardiography showed a normal result.

However, there was an unexpectedly striking discrepancy between the cTnI and cardiac troponin T (cTnT) values: the baseline value of cTnT in our laboratory at the University Hospital in Pilsen before coronarography was 7 ng/L (99th percentile 14 ng/L), while the cTnI concentrations were repeatedly, significantly increased during examination at Privamed Hospital.

METHODS

Due to unexplained discordant results in cTnT and cTnI, we decided to perform more detailed laboratory analysis.

1. First, we avoided frequently made errors such as wrong identification of the sample and made sure not to use poor-quality samples (fibrin clot in the sample, extremely high haemolytic, lipaemic or icteric samples, etc.).
2. We incubated the sample in heterophilic antibodies blocking tubes (HBT, Scantibodies) and then reanalysed.
3. We performed precipitation with a solution of polyethylene glycol (PEG) 6000, 250 g/L in phosphate-

buffered saline (PBS), adding it 1:1 to the blood serum. Therefore, the final concentration of PEG was 125 g/L. The mixture was stored in a refrigerator at 5 °C for 10 min and then centrifuged for 10 minutes at 9,500 g at room temperature. The cTnI concentration in the supernatant was then measured using a Troponin-I kit (Abbott Laboratories) on an Architect i2000 analyser from the same company. The result is expressed as a percentage of the original serum cTnI concentration. We performed the same procedure on a sample from a patient after a MI with a comparable cTnI concentration.

4. We performed molecular weight separation by gel filtration on a Sephadex G100 column (column height 12 cm; sample volume 0.5 mL; mobile phase PBS, pH 7.4; fraction 0.25 µL). Subsequently, we determined the cTnI concentration in the eluate by using a Troponin-I kit (Abbott Laboratories) on an Architect i2000 analyser from the same company and albumin concentration using the Cobas system (Cobas 8000 Analyzer, Cobas c702 and e602 modules, Roche Diagnostics, Basel, Switzerland). The results measured in Case 1 are shown in Fig. 1.

RESULTS

After reanalysis to exclude the presence of heterophilic antibodies by preincubation in HBT, the cTnI levels using the Abbott kit remained unchanged. After precipitation with PEG, there was a more striking change in the sample concentration compared with the control sample (from a patient after an MI), 7.4% versus 26.8%. A large decrease in concentration after PEG precipitation (when concentrations are < 20% of the initial values) indicates a high probability of the presence of macrotroponin[1]. The results of gel filtration on Sephadex G100 of Case 1 are presented in Fig. 1.

Subsequently, we analysed cTnI concentrations by using kits from two other manufacturers with the following result: 5.6 ng/L (AU 480, Beckman Coulter, 99th percentile 17.5 ng/L) and 4 ng/L (ADVIA Centaur XP, Siemens, 99th percentile 47.3 ng/L).

After an acute coronary event had been ruled out, the patient was transferred back to the Privamed Municipal Hospital to examine the cause of impaired cognition. During the subsequent years, the patient was repeatedly invited for check-ups. The elevated cTnI concentration measured with the Architect device remained for an extended period of time. The cTnI and cTnT concentrations measured in Case 1 are summarised in Table 1.

CASE REPORT 2

A 13-year-old boy was examined for persistent increased fatigue after a 1-week sports camp. He competes in kayaking. He had a history of persistent foramen ovale from early childhood; otherwise, his history included common childhood illnesses. During the last year, he had had a viral illness and patellar tendinitis.

He had been examined by his paediatrician and no pathology was found on physical examination. A routine haematological and biochemical examination was performed with no significant abnormalities found. Sonographic examination of the abdomen and microbiological examination of the nasopharynx were performed without pathological findings. To rule out myocarditis, an ECG examination was performed and no pathology was found. His cTnI levels were measured using the high-sensitivity method with a concentration of 107 ng/L. Transthoracic echocardiography was performed, and no pathology was found. One week later, his cTnI levels were re-examined with a value of 835 ng/L. Both analyses were performed using the Abbott kit (Architect i1000, Abbott Laboratories).

Subsequently, he was examined by a sports doctor and then by a paediatrician at the University Hospital in Pilsen, where a blood sample was collected to determine the level of cTnT. The cTnT concentration measured using the high-sensitivity method was 8 ng/L (Cobas 8000, Roche). Due to these discrepant results, we invited him

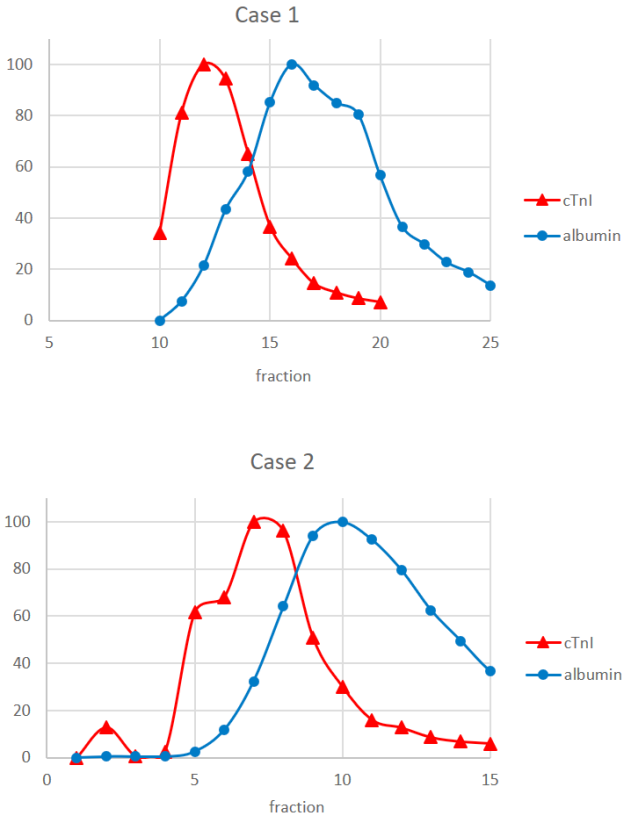

Fig. 1. Case 1 and 2 – Gel filtration on Sephadex G100 for separation of suspected macrotroponin I and albumin.
Albumin and cTnI concentrations are plotted as the relative value of the highest concentration of cTnI and albumin (y-axis) and the fraction number (x-axis) for a better presentation of the results in the graph. The cTnI peak before the albumin peak indicates the presence of macrotroponin I – larger molecules pass through Sephadex more quickly than smaller molecules.

Table 1. Summary of the cardiac troponin I (cTnI) and cardiac troponin T (cTnT) results using kits from different manufacturers.

	cTnI (ng/L) (Architect, Abbott)	cTnT (ng/L) (Cobas, Roche)
Case 1		
First sample (before hospitalisation)	1782	7
Samples during hospitalisation	1741, 3520 and 3622	34 (after coronary angiography)
After hospitalisation	395 (3 years after hospitalisation)	
	360 (4 years after hospitalisation)	
	536 (5 years after hospitalisation)	
Case 2		
June 25	107	–
July 2	835	–
July 28	–	8
August 25	439	
October 21	96	6

to our laboratory for further blood collection and more detailed analysis. We analysed both cTnI (Abbott) and cTnT (Roche) concentrations on the same day. All results up to that point are shown in Table 1.

MATERIALS AND METHODS

We followed the steps described for Case 1.

RESULTS

After reanalysis to exclude the presence of heterophilic antibodies by preincubation in HBT, the levels of cTnI using the Abbott kit remained unchanged. The cTnI concentrations before treatment with PEG were 96 ng/L in the patient's sample and 276 ng/L in control sample (from a patient after an MI). The cTnI concentrations after precipitation with PEG were 1 ng/L (1.4% of the initial concentration) in the patient's sample and 155 ng/L (56.0% of the initial concentration) in the control sample. A large decrease in concentrations after PEG precipitation (when concentrations are < 20% of initial values) indicates a high probability of the presence of macrotroponin[1]. The results after gel filtration on Sephadex are presented in Fig. 1.

DISCUSSION

Examination of cTnI and cTnT concentration is widely used in the diagnostic algorithm of myocardial damage[2]. Discrepant results of cTn assessment have been discussed by several authors and some theories have been published. Although cases with falsely elevated levels of cTn have been published, there are also cases with falsely negative interference in cTn analysis due to the presence of a complex of immunoglobulin and cTn molecule[3,4]. Furthermore, cases with falsely elevated levels of other molecules have been published[5].

Immunoassays in modern analysers are mostly based on the sandwich method. The first immobilised antibody binds to the target analyte, and the second antibody bound to a different part of the analysed molecule is able to generate detectable signal. Autoantibodies (heterophile antibodies) in a sample can create a bridge between the primary and secondary antibody and, consequently, increase the signal. Additionally, cTn-immunoglobulin complexes can lead to extended clearance from the bloodstream[6]. Some authors suggest that the prevalence of falsely positive results may be higher in modern laboratories when using high-sensitivity assays for cTn compared with older assays[7].

There are a variety of antibodies that can cause false elevation of cTn concentrations. Heterophilic antibodies are capable of binding to immunoglobulins of other species – for example, to antibodies used in immunoassays in modern analysers. These antibodies are not highly capable of causing interference. However, human anti-animal antibodies like human anti-mouse antibodies (HAMA) are highly specific and can falsely positive or negative interference during immunoassay[8].

According to a study by Pettersson et al.[5], patients with positive cTn autoantibodies have higher levels of cTnI and these levels persist longer after an MI. This phenomenon can lead to a different interpretation of patient results. The prevalence of autoantibodies in their study was relatively high at 13–20%; however, the authors added that they did not include a control group[6]. Another study showed that IgG molecules bound to cTn can be present even in blood donors[9]. Furthermore, some authors have published that elevated cTnT levels can be caused by re-expression of cTnT in skeletal muscles[10]; however, other authors have argued that cTn elevation can be caused by myocardial involvement as part of a systemic disorder[8].

When the presence of a falsely elevated or decreased concentration is suspected, the presence of fibrin clots, a high concentration of rheumatoid factor, analyser failure and common interferents (haemolysis, lipaemia and ictericity) should be excluded[8,11]. These causes can be excluded in daily routine practice without delay and additional expenses, including the measurement of serum indices[12]. Subsequently, the presence of heterophile autoantibodies should be rule out; this is usually done by

incubating the sample in a heterophile blocking tube and then reanalysing it.

As presented in Case 1, discrepancies in cTnI concentrations measured using kits from different manufacturers can help to find suspected analytical interference. While cTnI levels measured using an assay from one manufacturer can lead to falsely elevated results, assessment using an assay from another manufacturer does not. This can be explained by the different antibodies used by different manufacturers. Usually, antibodies employed in cTnI analysis are made to bind to epitopes in the central part of the troponin molecule. Some authors advocate that to minimise interference, cTn should be evaluated by using two primary antibodies to anchor the cTn molecule and two secondary antibodies to produce the signal[8].

In our cases, due to multiple results with elevated cTnI levels measured using plasmatic tubes, and automatic clot detection in our analysers, we presume that the clot was not the cause of the elevated levels. In addition, measurement of serum indices did not suggest this kind of interference. Furthermore, cTnI levels remained elevated after using HAMA tubes; thus, we presumed there was another kind of interference. We treated the samples with PEG and gel filtration chromatography (GFC). Macrocomplexes of analytes can be precipitated with PEG and the free analyte is measured in the supernatant. PEG precipitation is not completely specific for immunoglobulins and Ig complexes, PEG also precipitates less common forms of non-Ig macro-complexes. The procedure is fast and easy to perform and generally suitable as a screening method for the detection of macro-complexes of various analytes[4]. In both cases, the results were highly suggestive of so-called macrotroponin I. However, according to the used methods, we could not directly identify the molecular structure of the macro-complexes causing the interference in cTnI analysis. Recently, some authors suggest using algorithms when discordant results between clinical presentation and laboratory results are present[13]. It can help to avoid unnecessary investigation and possible harm of the patient especially when invasive procedures as coronary angiography are performed[13,14].

CONCLUSION

Falsely positive cTn results can lead to unnecessary intervention, as in the cases reported here, and to more side effects. In addition, these investigations can also harm the patient psychologically. When the presence of unexpected result is suspected, we recommend contacting a local laboratory specialist for consultation regarding the results and subsequent laboratory examination, but a certain level of experience and additional equipment are needed for laboratories to help identify possible interferences. However, most of the tests described in this report are time consuming rather than expensive and can be performed in smaller laboratories.

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