Unexpected troponin elevation in a patient treated with Atorvastatin.

Dr Paul Collinson. Departments of Clinical Blood Sciences and Cardiology, St George’s University Hospitals NHS Foundation Trust and St George’s University of London, Cranmer Terrace London SW17 0QT, UK

Dr Patrick Kiely, Department of Rheumatology, St George’s University Hospitals NHS Foundation Trust and Institute of Medical and Biomedical Education, St George’s University of London, Cranmer Terrace, London SW17 0QT, UK.

Running title False positive cardiac troponin T

Words 1013 words

Figures 0

Tables 1

Keywords

Cardiac troponin I

Cardiac Troponin T

Macrotroponin

Assay interference

Correspondence to: Dr Paul Collinson. Departments of Clinical Blood Sciences and Cardiology, NHS Foundation Trust, Cranmer Terrace, London SW17 0QT, UK.

Tel 0208 725 5934 Fax 0208 725 5838

Email: [paul.collinson@stgeorges.nhs.uk](mailto:paul.collinson@stgeorges.nhs.uk)

Introduction.

The measurement of the cardiac troponins cardiac troponin T (cTnT) and cardiac troponin I (cTnI) represent a paradigms shift in the ability to diagnose myocardial infarction compared to the conventional cardiac enzymes creatine kinase (CK) and its MB isoenzyme (CK-MB). A particular problem with CK and CK-MB is the ability to detect myocardial injury in the presence of skeletal muscle injury. Although early problems were documented with the first-generation cTnT assay, the problem of cross reaction with skeletal troponin T was removed in subsequent generations of the cTnT assay. It has therefore always been considered that measurement of cTnT using the current essay is not subject to interference by skeletal muscle injury.

Presentation: A female Asian age 65 years was referred for statin intolerance. She had previously been taking Atorvastatin 40 mg daily for secondary prevention following triple vessel coronary artery bypass grafting 7 years previously (2011). She had noted some generalised muscle weakness most marked in her quadriceps complex prior to presentation. When going on holiday she was transferring from the airport to her hotel but found herself unable to get out of a car on reaching her hotel. She has no cardiac symptoms at the time of attendance and no shortness of breath.

Her past medical history was of type II diabetes mellitus (for the past 8 years) and asthma. Medication at first consultation was Metformin 500mg bd, Zafirlukast 20mg od, Vitamin D3 (Cholecalciferol) 1000 units daily, Clopidogrel 75mg od, Omeprazole 20mg od, Losartan 50mg od, Ezetimibe 10 mg od, Tiotropium inhaler 18µg, Spiro base inhaler.

Investigations: Both troponin assays were high sensitivity (hs). All measurements were performed on a Roche Diagnostics Cobas system (Roche Diagnostics) except Cardiac Troponin I (hs cTnI, Architect, Abbott Diagnostics LOD 1.1 ng/L, 10% CV 4.7 ng/L, reference interval (RI) female15.6 ng/L) with analytical range, coefficient of variation (CV) and RI as follows: CK,7-2000 U/L, 0.5-1.4%, <200 U/L; Alanine transaminase (ALT), 5-700 U/L, 2.9-3.6% ,<40 U/L (female); hs cTnT, fifth-generation assay (3-10000ng/L, 15-1.3%, 10% CV13 ng/L, <14 ng/L; Total 25-OH vitamin D 7.5-175 nmol/L, 6.8-2.2%, >50 nmol/L.

On attendance (June 2017) she was noted to have an elevated CK (2853 U/L) with an elevated cTnT (55 ng/L).Total 25-OH Vitamin D was acceptable at 60 nmol/L. Genetic testing (next-generation sequencing) excluded familial hypercholesterolemia with intermediate risk of polygenic hypercholesterolemia and the Rs4149056 genotype T/C (SLCOB1 gene, intermediate risk of myositis). A rheumatological opinion was sought and clinically confirmed the diagnosis of myositis. A screen for mysositis specific and myositis associated antibodies was performed and found to be negative for Isoleucyl tRNA (OJ), Glycyl tRNA synthetase (EJ), Alanyl tRNA synthetase (PL-12), Threonyl tRNA synthetase (PL-7), Signal recognition particle (SRP), Histidyly tRNA synthetase (Jo-1), Pm-Scl75, Pm-Scl100, Ku, Small ubiquitin-like modifier 1 activating enzyme (SAE), NXP-2, Melanoma differentiation associated gene (MDA5), TIF1γ/α, Helicase protein (Mi-2) and Ro52 but positive for an antibody to HMGCoA reductase. The conclusion was an auto immune myositis induced by statin therapy. Her symptoms resolved with statin cessation with a fall in CK. Her serial biochemical results are summarised in Table 1.

There were no cardiac symptoms reported at any time so further cardiac investigations were not performed. In the absence of cardiac symptoms and in the presence of an elevated CK and cTnT, a false positive cTnT due to assay interference was suspected. A sample was therefore sent for simultaneous measurement of cTnT and cardiac troponin I (cTnI). The cTnT was 122 ng/L with a cTnI of 5 ng/L. This excluded myocardial injury as a cause of the cTnT elevation. Serial dilution of the sample showed linear dilution, excluding a heterophile antibody. A subsequent sample was diluted 1 in 2 with troponin free diluent and with Polyethylene glycol (PEG) (25% w/v). Samples were incubated at room temperature for 10 minutes and centrifuged at 14,000g for 5 minutes and cTnT was analysed in the supernatant. The original cTnT was 25 ng/L. Following a 1:2 dilution value with diluent a cTnT value of 13.59 ng/L was obtained. After 1:2 dilution with PEG a cTnT value of 15.21 ng/L was obtained. This gave a bioactive cTnT of 30.42 ng/L with recovery of 111.9% hence over-recovery. It was concluded that macrotroponin T not present as previous studies have shown that recovery of 5-10% occurs in samples containing macrotroponin T. The elevated cTnT with cTnI within the reference interval excluded myocardial injury. Polyethylene precipitation excluded a macrotroponin causing cTnT elevation. On follow up over the subsequent 15 months cTnT fell in parallel with CK (Table 1). She was not rechallenged with a statin.

Discussion

The first generation cTnT assay used a cardiac specific capture antibody but a non-cardiac specific detection antibody. Because of the assay format, an analytical interference occurred in the presence of high concentrations of skeletal muscle troponin T (sTnT)(1). The second generation assay used two cardiac specific antibodies and completely resolved the problem. The current high sensitivity (hs) cTnT assay uses fragment antigen binding (FAB) portions of 2 cTnT-specific mouse MAbs directed against epitopes in the central region of human cTnT. It is documented as having no assay interference from sTnT(2).

Elevation of cTnT but not cTnI has been reported in conditions with muscle damage and muscle regeneration, and specifically in patients with renal failure. However, there is controversy in the interpretation of the methodology used in the original studies(3). It is now thought that the elevation of cTnT seen in patients with renal failure is not artefactual and reflects myocardial injury(4).

The question of potential assay interference in patients with chronic muscle diseases remains an open question. There have been a number of reports of elevation of troponin T but not troponin I in patients with muscle disease (5-8). The mechanism is considered to be re-expression of a cardiac isoform of troponin T in regenerating skeletal muscle(9). A recent paper supports this with demonstration of mRNA to the TNN2 gene, a cardiac isoform of troponin T, plus detectable cTnT in inflammatory muscle disease (10).

Conclusion: Auto immune myositis secondary to statin therapy with skeletal muscle regeneration and likely re-expression of a cross reacting TnT isoform.

Takeaway points.

1. True false positive elevations of cTnT are rare but do exist.

2. When clinical features do not match the laboratory findings, discussion with the laboratory and further investigations are required.

3. Laboratories should have a protocol to identify potential false positive results including reciprocal arrangements to measure the analyte by a different methodology.

4. Exclusion of macrotroponin is a straightforward procedure.

5. Identification of the false positive result prevented unnecessary invasive cardiac investigations.

Table 1 Serial results of investigations performed following the initial consultation and subsequent follow-up.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | [CRP](http://stg1epr05/epr/ie4.web#CRPH) (<3 mg/|L) | Creatinine (60-120 µmol/L) | [ALT (<40 U/L)](http://stg1epr05/epr/ie4.web#ALT) | [CK](http://stg1epr05/epr/ie4.web#CK)(<200 U/L) | Troponin T (<14 ng/L) | Troponin I (<30 ng/L) |
| 02-Jun-2017 | 1.0 | 44 | [55](http://stg1epr05/epr/ie4.web#ALT) | [2853](http://stg1epr05/epr/ie4.web#CK) | [55](http://stg1epr05/epr/ie4.web#TRO) |  |
| 09-Jul-2017 | 9.5 | 55 | [47](http://stg1epr05/epr/ie4.web#ALT) |  |  |  |
| 11-Jul-2017 | 108 | 48 |  |  |  |  |
| 31-Aug-2017 |  | [40](http://stg1epr05/epr/ie4.web#CRE) | [61](http://stg1epr05/epr/ie4.web#ALT) | [3904](http://stg1epr05/epr/ie4.web#CK) |  |  |
| 15-Sep-2017 | 0.8 | 47 | [74](http://stg1epr05/epr/ie4.web#ALT) | [3770](http://stg1epr05/epr/ie4.web#CK) | [122](http://stg1epr05/epr/ie4.web#TRO) | 5 |
| 16-Oct-2017 |  | [43](http://stg1epr05/epr/ie4.web#CRE) | 38 | [1120](http://stg1epr05/epr/ie4.web#CK) |  |  |
| 19-Mar-2018 | 1.5 | 49 | [44](http://stg1epr05/epr/ie4.web#ALT) | [1101](http://stg1epr05/epr/ie4.web#CK) |  |  |
| 06-Sep-2018 | 1.0 | 53 | [41](http://stg1epr05/epr/ie4.web#ALT) | [699](http://stg1epr05/epr/ie4.web#CK) | [25](http://stg1epr05/epr/ie4.web#TRO) | 7 |

Abbreviations: CRP, C reactive protein; ALT, alanine transaminase; CK, creatine kinase.

Reference List

1. Collinson PO, Stubbs PJ, Rosalki SB. Cardiac troponin T in renal disease [letter; comment]. Clin Chem 1995;41:1671-3.

2. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:254-61.

3. Fredericks S, Bainbridge K, Murray JF, Collinson PO, Carter ND, Holt DW. Measurement of cardiac troponin I in striated muscle using three experimental methods. Ann Clin Biochem 2003;40:244-8.

4. Ricchiuti V, Voss EM, Ney A, Odland M, Anderson PA, Apple FS. Cardiac troponin T isoforms expressed in renal diseased skeletal muscle will not cause false-positive results by the second generation cardiac troponin T assay by Boehringer Mannheim [see comments]. Clin Chem 1998;44:1919-24.

5. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS. Diseased skeletal muscle: a noncardiac source of increased circulating concentrations of cardiac troponin T. J Am Coll Cardiol 2011;58:1819-24.

6. Rittoo D, Jones A, Lecky B, Neithercut D. Elevation of cardiac troponin T, but not cardiac troponin I, in patients with neuromuscular diseases: implications for the diagnosis of myocardial infarction. J Am Coll Cardiol 2014;63:2411-20.

7. Dhir T, Jiang N. Misleading Elevation of Troponin T caused by Polymyositis. Int J Biomed Sci 2013;9:107-11.

8. Schmid J, Liesinger L, Birner-Gruenberger R, Stojakovic T, Scharnagl H, Dieplinger B et al. Elevated Cardiac Troponin T in Patients With Skeletal Myopathies. J Am Coll Cardiol 2018;71:1540-9.

9. Bose F, Renna LV, Fossati B, Arpa G, Labate V, Milani V et al. TNNT2 Missplicing in Skeletal Muscle as a Cardiac Biomarker in Myotonic Dystrophy Type 1 but Not in Myotonic Dystrophy Type 2. Front Neurol 2019;10:992.

10. Ciciliot S, Schiaffino S. Regeneration of mammalian skeletal muscle. Basic mechanisms and clinical implications. Curr Pharm Des 2010;16:906-14.