Identification of Macro Troponin T

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Auckland City Hospital
Overview:

- Description of an unusual case encountered with elevated troponin T
- Methods to identify interference
- Autoantibodies to Troponin
- Concept of irregular interference
Conflict of interest

– No Conflict of interest to declare
Unexpected persistent elevation in Troponin T

- 22 year-old male from in-patient psychiatry unit
  - Episodes of light headedness
  - Chest discomfort
  - Coryzal symptom
  - No known renal dysfunction or myopathy

- Investigations:
  - Equivocal ECG without obvious abnormality
  - Normal Echocardiogram which was not suggestive of peri/myocarditis
  - Troponin T 73ng/L (<14 ng/L) at the time, also noted troponin T at time of admission also elevated
  - Troponin I testing at North Shore Hospital (Siemens Vista) < 15ng/L (Ref < 40ng/L)
What are some possible causes for discrepant troponin T and I?

- Biological/Pathology causes:
  - Cardiac causes, renal failure, myopathy, old age
- Analytical causes
  - Discrepant Troponin T and Troponin I
  - Haemolysis/ Icterus/ Lipaemia
  - Antibody mediated
    - Heterophile antibody, Anti-reagent antibody, paraprotein, rheumatoid factor etc...
  - Biotin
  - Calibration drift

How can we exclude interference in the laboratory

- Test by a different method
- Dilution series
- Heterophile blocking tube
- PEG (typically used for macro-prolactin)
  - Precipitates large molecules such as immunoglobulin
  - The supernatant is free from large interfering molecules
- Protein A Plus
  - Remove immunoglobulins
- If immunoreactivity drops after PEG or Protein A, this suggests interference
Does interference explain these findings?

- Heterophile Blocking tube:
  - No interference detected
- Low PEG Recovery 15-36% (RI: 61-111%; n=30)
- Low Protein A recovery 8% (RI: 30-137%; n=30)
- Findings suggestive of interference caused by antibodies
Gel Filtration Column

What is the explanation?
Can macro-troponin T cause inaccurate measurements?

- The patients serum was spiked with recombinant Troponin T
- Significant under-recovery
  - Which suggests results are inaccurate

![Graph showing recovery percentage against amount of Troponin T spiked in, with a clear trend indicating inaccurate measurements.](image-url)
 Persistent elevation in troponin

? Analytical problem
- HIL
- Antibody mediated
- Other (pre)-analytical problems

Low PEG/ Protein A recovery
- Rheumatoid factor
- Paraprotein
- Heterophile antibody
- Autoantibodies
- Anti-animal antibodies
- Anti-reagent antibodies

Gel filtration chromatography
- Autoantibodies

Standard addition identified inaccurate results

Summary so far….

- Where interference is detected, the result can be inaccurate.
  - Hence, we do not often report numerical results after treatment with heterophile blocking tube
What has happened happening?

- The patient has autoantibodies to troponin T
  - Troponin is usually very low in circulation
  - Presence of autoantibodies lead to formation of an antibody-troponin T complex
  - This complex clears very slowly in the body leading to accumulation
  - Troponin T in the serum is actually elevated
  - However, antibody-troponin T complex, does not behave identically as troponin on the Roche assay, leading to falsely low results.
Significance of autoantibodies to Troponin

While there is a suggestion that auto-antibodies are associated with cardiomyopathy it is also present in a significant number of healthy patients. Incidence estimated by Warner et al, interference from macro-troponin affects 5% of all positive results using a high-sensitivity Abbott Architect troponin I assay. Interference due to auto-antibodies to Troponin T has not been clearly described.

Table 1
Anti-troponin antibodies in healthy individuals.

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Age</th>
<th>M:F</th>
<th>Method of detection</th>
<th>% Positive (n)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATIA</td>
<td>750</td>
<td>38.8 (18–77)</td>
<td>1:1.5</td>
<td>CMI</td>
<td>12.7 (95)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>40</td>
<td>1:1</td>
<td>CMI</td>
<td>9.3 (28)</td>
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<tr>
<td></td>
<td>98</td>
<td>55 ± 11</td>
<td>4.44:1</td>
<td>SPRA</td>
<td>4.1 (4)</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>33.7</td>
<td>1:2.23</td>
<td>ELISA</td>
<td>0 (0)</td>
</tr>
<tr>
<td>ATIA</td>
<td>467</td>
<td>39.7 (18–72)</td>
<td>1:1.13</td>
<td>CMI</td>
<td>9.9 (46)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>52 ± IQR 14</td>
<td>10:0</td>
<td>ELISA</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

ELISA: enzyme-linked immunosorbent assay; CMI: chemiluminescent microplate immunoassay; SPRA: surface plasmon resonance analysis.
Macro Troponin an example of irregular interference

- Irregular analytical errors are due to intrinsic differences in the patient’s sample
  - These errors bypass conventional internal and external quality controls
- No single method can detect every type of interferences
- All analytical procedures are potentially susceptible to this
- Many assays can be affected adding to the confusion
  - E.g. biotin or anti-streptavidin antibodies

Vogeser and Seger, CCLM, 2018
What can we do about irregular interferences?

– From the laboratory:
  – Consider risk of interferences in evaluating and selecting methods
  – Raise awareness to laboratory professionals and clinical colleagues
  – Measure the occurrence

– When there is a suspicion of irregular interference
  – Consider characterizing mechanism of interference
  – Look for potential alternative ways to investigate patient
  – Look to see if other results are also affected
Take home message(s)

- Persistent elevation in Troponin T caused by macro-troponin
- Multiple causes of antibody mediated interferences requiring different techniques to detect and characterize
- Autoantibodies to Troponin is common in healthy population, however interference on the Troponin T assay is rare.
Acknowledgements

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What (else) are we measuring when we measure troponin?

What a Troponin T measurement include:
- Troponin T is a heterogenous measurement
- Two major reasons:
  - Formation of complexes
  - Proteolytic degradation

What can be causing an interference?
- Heterophile antibody
- Anti-reagent antibody
- Paraprotein
- Rheumatoid factor
  - No troponin present
- Autoantibody
  - Troponin Present
Troponin Complex

Breakdown due to necrotic myocyte Complexes e.g. I-C can also occur spontaneously in serum

Fragmentation of Troponins

Cytosolic release – peak within 24 hours
Second persistent cTnT elevation 7-14 days
* - Similar 16 and 18kDa also found in ESRD

Proteolytic degradation of Troponin occurs from necrotic myocytes

Tn: T
Tn: TIC
37kDa

Tn: T
Tn: TIC
29kDa

Tn: T fragments
14,16*,18*,27 kDa


Inaccurate troponin results from macro troponin; what next?

From pathologists
- Discuss options for alternative investigations
- Characterize cause of interference
- Does this interference affect other assays? Or previous test results
- How do we ensure appropriate tests are requested in the future

From clinicians/patients?
- Does this need monitoring
- Is this related to the patients symptoms
- Is there an underlying cause?
- Is troponin I affected?
Analytical interference in Troponin T

Haemolysis
- Variable effects, but on the Roche platform leads to lower results

Antibody mediated interference on troponin T has rarely been reported.
- We have previously demonstrated the potential for anti-streptavidin antibodies leads to falsely low Troponin T results (as well as all other sandwich immunoassays)
- Subsequent analysis by Berth et al, suggested a prevalence of 0.7% of anti-ccp

Macro Troponin T
- Circulating Troponin T antibody complex is proposed to have prolonged half life leading to falsely high results