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High incidence of macrotroponin I with a high-sensitivity troponin I assay

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Abstract

Background: Cardiac troponin is the preferred biomarker of myocardial injury. High-sensitivity troponin assays allow measurement of very low levels of troponin with excellent precision. After the introduction of a high-sensitivity troponin I assay the laboratory began to receive enquiries from clinicians about clinically discordant elevated troponin I results. This led to a systematic investigation and characterisation of the cause.

Methods: Routine clinical samples were measured by the Architect High Sensitive Troponin-I (hsTnI) and the VITROS Troponin I ES assays (VitrosTnI). Results that were elevated according to the Architect but not the VITROS assay (Group 1) or results elevated by both assays but disproportionately higher on the Architect (Group 2) were re-analysed for hsTnI after re-centrifugation, multiple dilutions, incubation with heterophilic blocking reagents, polyethylene glycol (PEG) precipitation, and Protein A/G/L treatment. Sephacryl S-300 HR gel filtration chromatography (GFC) was performed on selected specimens.

Results: A high molecular weight complex containing immunoreactive troponin I and immunoglobulin (macro-troponin I) was identified in 5% of patients with elevated hsTnI. Patients with both macrotroponin and myocardial injury had higher and longer elevation of hsTnI compared with VitrosTnI with peaks of both macrotroponin and free troponin I-C complex on GFC.

Conclusions: Circulating macrotroponin I (macroTnI) causes elevated hsTnI results with the Architect High Sensitive Troponin-I assay with the potential to be clinically misleading. The assay involved in this investigation may not be the only assay affected by macrotroponin. It is important for laboratories and clinicians to be aware of

and develop processes to identify and manage specimens with elevated results due to macrotroponin.

Keywords: false-positive troponin; high-sensitivity assay; macrotroponin; macrotroponin I.

Introduction

Cardiac troponin is the preferred biomarker of myocardial injury and is central to the universal definition of myocardial infarction (MI), relying on a rise and/or fall of troponin with at least one value above the 99th percentile in patients with characteristic symptoms, imaging or electrocardiographic evidence of myocardial ischaemia [1]. The evolution of cardiac troponin I (cTnI) assays has led to the availability of methods which measure cTnI with excellent precision at the low concentrations found in healthy individuals. These high-sensitivity assays have been defined as having a CV of $\leq 10\%$ at the 99th percentile of a cardiac-healthy population and the ability to measure cardiac troponin above the limit of detection in at least 50% of this population [2]. The hope is that high-sensitivity troponin assays will lead to earlier diagnosis of acute coronary syndrome and, with their enhanced sensitivity and sex-specific cutoffs, may improve patient outcomes, especially in women. However, there is insufficient evidence yet to conclude whether these outcomes have been achieved [3–6].

Since the 1960s, circulating macroanalytes have been known to cause misleadingly elevated clinical laboratory results. The majority of macroanalytes are believed to consist of an analyte bound to analyte-specific autoantibodies resulting in high molecular weight complexes which are cleared more slowly from the circulation than the free analyte resulting in persistently elevated levels. Cases have been described for cardiac troponins [7, 8], prolactin, thyroid-stimulating hormone and numerous routinely measured enzymes [9–15].

In general, autoantibodies which form macroanalyte complexes are not believed to be disease-causing although cases associated with autoimmune disorders have been described [16, 17] and reports suggesting an association between the presence of autoantibodies against cTnI and cardiomyopathy have been published [18–20]. Based on

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current evidence, the main problem caused by a circulating macroanalyte is that it leads to multiple, possibly invasive, investigations in search of a cause.

Circulating troponin autoantibodies are not rare, having been identified in 2%–20% of individuals, with or without cardiac disease, in various studies [20–24]. They may be directed towards cTnI [19, 22] or cardiac troponin T [20, 21].

Historically, autoantibodies to cTnI have caused negative interference in some cTnI immunoassays [24, 25]. Investigation of the epitope specificity of cTnI autoantibodies has shown that the cTnI midfragment (amino acids 30–100) is the commonest target of these antibodies [26, 27]. The desire to standardise or harmonize cTnI measurement led to a recommendation that cTnI assays use antibodies specific to epitopes in the midfragment as this central portion is the most stable, being least likely to be affected by posttranslational modifications to the molecule, proteolytic degradation and circulating troponin complexes [2, 28]. However, it has been suggested that this recommendation be re-evaluated in the light of evidence about troponin autoantibody predilection for the midterminal region [27].

While cTnI autoantibodies are typically associated with false-negative cTnI immunoassay results, two cases of macrotroponin causing elevated cTnI have been published [7, 8]. In these cases the presence of macrotroponin was proven by a panel of investigations. False-positive cTnI immunoassay results may be caused by factors other than macrotroponin, including interfering antibodies, fibrin microclots, carryover, random error and haemolysis [29–32].

When the clinical features of a patient and the cTnI results are discordant, investigation for preanalytical or analytical causes of a false-positive or negative result is mandatory. Laboratory investigations include analysis by an alternate method, re-centrifugation and re-analysis to exclude random error, treatment with heterophile antibody blocking agents, multiple dilutions of the specimen to identify non-linearity and polyethylene glycol (PEG) precipitation to remove a high molecular weight interferent [7, 8, 33]. Further evidence that an interferent is an immunoglobulin may be obtained by precipitation with an immunoglobulin-binding protein such as Protein A or G [7, 8]. Evidence for interference by a factor with a different molecular weight to cTnI can be obtained by gel filtration chromatography (GFC) [7, 26].

A few months after the introduction of the Architect high sensitive troponin-I (hsTnI) assay into our laboratory we noticed an increase in enquiries regarding possible false-positive troponin I results. Initial investigations

revealed that the false-positive results were likely to be due to a high molecular weight complex in the patients' plasma. We considered replacing the assay but our clinicians requested that we continue providing the high-sensitivity assay as it was so effective in a 2-h rapid rule-out protocol for MI. The relatively high frequency of this issue led us to set up a process to pre-emptively identify samples with spuriously high hsTnI results early in the diagnostic pathway and to characterise the nature of the interference. We also performed a panel of tests on the first 50 affected samples in order to determine the most reliable and efficient way of processing potentially false-positive results so that clinicians could be notified of the discrepancy in the shortest period of time.

Materials and methods

Routine lithium heparin plasma samples were analysed for cTnI by the Architect High Sensitive Troponin-I assay on the Architect i4000 SR analyser (Abbott Diagnostics, Brisbane, Australia) and the VITROS Troponin I ES (VitrosTnI) assay on a VITROS 5600 analyser (Ortho Clinical Diagnostics, Sydney, Australia). The VitrosTnI assay was selected because of its availability with the laboratory's existing instrumentation. Passing-Bablok analysis of 6700 in-house concordant samples demonstrated a 25% positive bias of the VITROS assay ($y=1.25x+2.6$ where y =VitrosTnI, x =hsTnI). VitrosTnI results were not routinely reported to clinicians, but used as a screening tool by the laboratory to identify possible spuriously raised hsTnI results. Samples were analysed in real-time, with turnaround times <60 min for both results and no refrigeration or freezing. Both methods were verified and kept in control through standard laboratory procedures.

For hsTnI Australia adopted the manufacturer's 99th percentile reference limit of 16 ng/L for females and the overall reference limit of 26 ng/L for males. In this laboratory the total imprecision is 4% at both 16 ng/L and 26 ng/L. The limit of quantification is 2 ng/L. For the VitrosTnI assay the manufacturer's 99th percentile upper reference limit is 34 ng/L (CV=7%) and limit of quantification is 12 ng/L.

All hsTnI results above the sex-specific 99th percentile cut-offs were scrutinised for concordance between the two assays. Sample volume permitting, the first 50 specimens with elevated hsTnI but VitrosTnI results below the cut-off (Group 1) or with hsTnI at least 2x VitrosTnI (Group 2) were subjected to the following procedures and reanalysed by the hsTnI assay: re-centrifugation of an aliquot (7 min at 1800 g) followed by re-analysis (n=50), incubation in Heterophilic Blocking Tubes (Scantibodies Laboratory, Santee, CA, USA) according to the manufacturer's instructions (n=44), dilution 1/2, 1/5 and 1/10 with Architect Multi-Assay Manual Diluent (Abbott Diagnostics) (n=45), PEG precipitation (n=50), treatment with Protein A/G/L-Sepharose (BioVision, Milpitas, CA, USA) (n=38), and in selected cases GFC (n=21). Samples were only refrigerated or frozen prior to GFC or Protein A/G/L treatment.

Plasma specimens from patients with concordant hsTnI and VitrosTnI elevations were used as controls for the PEG precipitation, Protein A/G/L binding and GFC. Numerical results below the hsTnI limit of quantification of 2 ng/L were used to calculate the post-PEG hsTnI recovery.

Sample volume permitting discordant specimens ($n=37$) were also analysed with the previous generation Architect Troponin I assay, the Architect STAT Troponin-I assay (manufacturer's 99th percentile 28 ng/L). Passing-Bablok analysis of a 300 specimen in-house comparison: $y=0.96x+1.90$ where $y=hsTnI$ and $x=STAT$ Troponin-I, $R^2=0.997$. Reagents were supplied without cost by Abbott Diagnostics.

PEG precipitation was performed using a 25% solution of 8000 mol wt PEG [34] in phosphate buffered saline (PBS) (both from Sigma-Aldrich, Castle Hill, Australia). Two hundred microlitres of PEG solution was mixed with 200 μ L patient or control plasma by vortexing. After incubation at room temperature for 10 min and centrifugation (5 min at 16,000 g) the supernatant was analysed and results multiplied by 2 to adjust for dilution by the PEG solution. The recovery of hsTnI was then calculated as the percentage of hsTnI in the supernatant divided by the original result from the patients' plasma. Patient and control recoveries were compared. Recovery below that of controls indicated the presence of high molecular weight interference.

Protein A/G/L-Sepharose treatment was performed in 2 mL screw cap microtubes (Quality Scientific Plastics, San Diego, CA, USA) containing 500 μ L Protein A/G/L-Sepharose slurry as provided by the manufacturer. The Protein A/G/L-Sepharose was prepared by washing and centrifuging (10 min at 16,000 g) twice with 1 mL binding buffer (0.05 M sodium borate, 0.15 M sodium chloride, pH 8.0). The supernatant was removed, 300 μ L patient or control plasma added and the contents mixed at ambient temperature on an orbital shaker (250 rpm) for 1 h. The plasma supernatant was removed from the tube after centrifugation (10 min at 16,000 g) and analysed. The recovery of hsTnI was then calculated as the percentage of hsTnI in the supernatant divided by the original result from the patients' plasma. Patient and control recoveries were compared. Recovery below that of controls indicated the presence of high molecular weight interference. Results were not adjusted to account for dilution due to residual binding buffer because, although as much supernatant as possible was removed, it was not possible to accurately estimate the residual buffer volume. Any dilutional effect would have been comparable for both control and discordant samples. The Protein A/G/L-Sepharose was regenerated by washing and centrifuging twice with elution buffer (0.1 M citric acid, pH 2.75) and once with distilled water. The microtubes of Protein A/G/L-Sepharose were stored refrigerated in 20% ethanol/H₂O until re-use.

Samples were selected for GFC either at random, to clarify equivocal PEG precipitation results or to investigate Group 2 samples or serial samples with a rise in hsTnI. Initially a 9/300 GL column (GE Healthcare, Australia) containing Sephacryl S-300 HR (Sigma-Aldrich; separation range 10–1500 kDa globular proteins) was used at a bed volume of 14 mL with a mobile phase of PBS. When the 9/300 column required replacement due to loss a 10/400 GL column (GE Healthcare Life Sciences, Australia) was purchased as the 9/300 column size had been superseded. A bed volume of 24 mL was employed with the new column in order to improve resolution to investigate an impression that two different molecular weight species of macrotroponin had been detected. Depending on the hsTnI concentration, 200–500 μ L of patient or control plasma was applied to the column. An initial effluent volume of 5.3 mL was discarded then 330 μ L fractions collected. As well as hsTnI, fractions were analysed for prolectin (Architect, Abbott Diagnostics, Brisbane, Australia) IgG, IgA and IgM (Image, Beckman Coulter, Sydney, Australia) to act as molecular markers (23 kDa, 150 kDa, 340 kDa and 900 kDa, respectively). In one

experiment plasma from a patient with elevated hsTnI post-MI was mixed (equal volumes incubated at room temperature for 10 min) with plasma from a patient with macroTnI and subjected to GFC.

To exclude the unlikely possibility that our findings were matrix-related, PEG studies were replicated on EDTA plasma and serum from three patients with discordant results.

Wilcoxon-Mann-Whitney tests were applied to compare differences in PEG and protein A/G/L recovery with controls due to non-normally distributed data and small sample sizes of some groups. Statistics were performed using Analyse-it® (Version 2.26, Analyse-it Software Ltd, Leeds, UK).

Mater Health Services Human Research Ethics Committee approval was granted to publish the findings.

Results

Incidence

Over a 4-month period (from 01/06/2015 to 30/09/2015) 3897 individuals had hsTnI and VitrosTnI measured in parallel by the laboratory. Of these, 1074 (582 males) had hsTnI results above the sex-specific 99th percentile cut-offs. Fifty-one individuals (5%) had elevated hsTnI results (range 16–683 ng/L) which were discordant with VitrosTnI results (all VitrosTnI results <30 ng/L) (Group 1). In another five patients (Group 2) both results were elevated but the hsTnI results (range 216–15,659 ng/L) were 2–10 times greater than the VitrosTnI results (range 96–1490 ng/L) which is unexpected given that the VitrosTnI is positively biased against the Architect assay.

Re-analysis, dilutions and heterophile blocking

Re-centrifugation and re-analysis demonstrated that two of the discordant hsTnI results were due to random error in the Architect assay. Dilutions produced linear results in all cases. Incubation in Scantibodies tubes found no cases of heterophile antibody interference (recovery 85%–122%).

PEG precipitation

Recovery of hsTnI following PEG precipitation of 37 control samples (hsTnI range 6–815 ng/L) was 33%–61%. Recovery following PEG precipitation of Group 1 samples was 1%–15% (88% were $\leq 10\%$ of original results) ($p < 0.001$). Post-PEG recovery from Group 2 samples was similar (1%–19% of original values) ($p < 0.001$) which is consistent with the presence of immunoglobulin-bound troponin I. However, post-PEG recovery from two Group 2 samples

which had further elevation of hsTnI and VitrosTnI on serial testing was 21 and 29%.

Protein A/G/L

Recovery of hsTnI following Protein A/G/L treatment of 12 control samples (pre-treatment hsTnI range: 49–815 ng/L) was 40%–70%. Recovery of IgG, IgA and IgM was <4%, 11%–22% and 11%–18%, respectively (n=6).

Recovery of 35 Group 1 samples was 2%–30% (<15% in 28 samples) ($p < 0.001$). Samples from three Group 2 patients (hsTnI=15,659, 608 and 213 ng/L vs. VitrosTnI=1490, 127 and 96 ng/L, respectively) were treated with Protein A/G/L and recoveries were 6%, 7% and 37%, respectively ($p < 0.01$), consistent with the presence of immunoglobulin-bound troponin I. Carryover was not demonstrated at the concentrations in these samples except following the 15,659 ng/L specimen, after which carryover was observed to be 10 ng/L (0.06%).

Architect STAT Troponin-I

Plasma from 37 individuals with discordant troponin I results was analysed using the contemporary Architect STAT Troponin-I assay. Five results (18–26 ng/L) were below the manufacturer's 99th percentile cut-off and corresponded with hsTnI levels of 19–31 ng/L. The remaining 32 results ranged from 31–6320 ng/L corresponding with hsTnI concentrations 21–15,659 ng/L.

Gel filtration chromatography

Using the 9/300 column the peak elution volume of hsTnI was 4.0–5.0 mL in control (post-ST elevation MI) plasma (n=4), and 2.3–3.3 mL in Group 1 specimens (n=15). HsTnI in control plasma eluted before prolactin (23 kDa) and is presumed to be the 39 kDa binary troponin I-C complex (2). HsTnI in three specimens from Group 2, including the patient with 37% recovery following Protein A/G/L treatment, eluted at both 4.0–5.0 mL (troponin I-C) and 2.3–3.3 mL. Using the 10/400 column the peak elution volume of hsTnI was 10.6–11.9 mL in control plasma (n=3) and 6.6–7.6 mL in Group 1 patients (n=7, including two previously analysed on the 9/300 column). Peak elution volumes for IgG, IgA and IgM using the 9/300 column were 3.6–4.0 mL, 3.0–3.3 mL, and 1.0–1.3 mL, respectively (n=9). Peak elution volumes for prolactin, IgG, IgA and IgM using the 10/400 column were 12.5–12.9 mL, 9.2–9.6 mL, 7.9–8.3 mL,

and 5 mL, respectively (n=2). Figure 1 shows the molecular weights against elution volumes for both columns.

Figure 2 displays elution profiles from the 10/400 column of two individuals with either MI (troponin I-C) or macroTnI. macroTnI elutes between the IgM and IgA peaks and the troponin I-C complex elutes between the IgG and prolactin peaks.

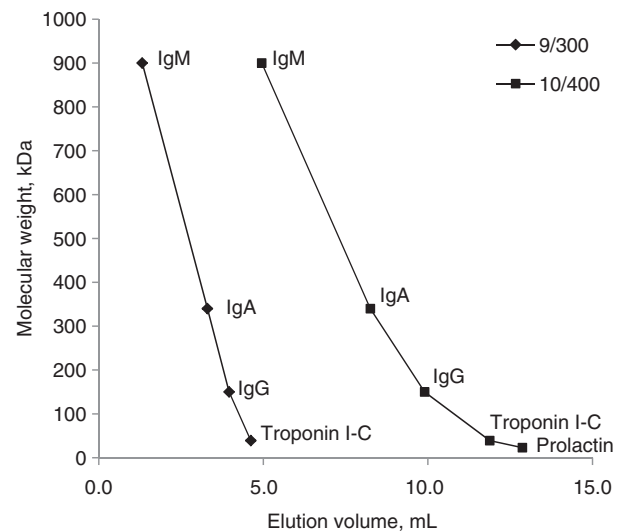


Figure 1: Elution volumes vs. molecular weights in post-MI plasma of the troponin I-C complex and internal molecular weight markers using a 14 mL Sephacryl S-300 HR 9/300 GFC column and a 24 mL Sephacryl S-300 HR 10/400 GFC column.

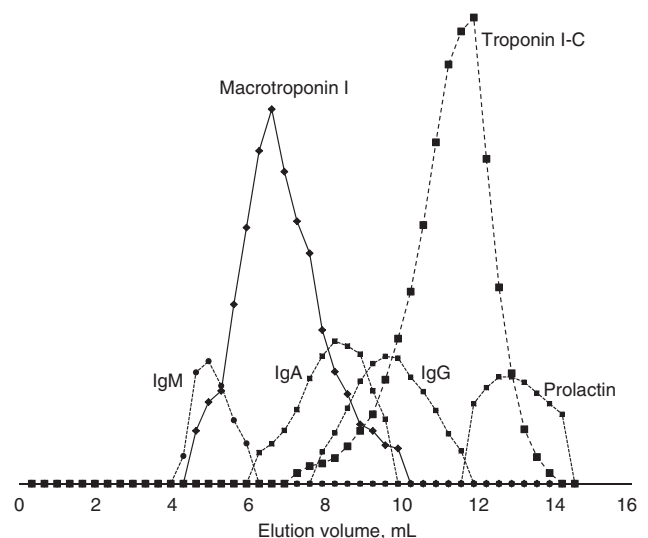


Figure 2: Elution profiles from the 10/400 column of 2 individuals, each with either troponin I-C (post-MI) or macrotroponin I. The immunoglobulin and prolactin profiles are representative of the elution profiles from this column.

EDTA plasma and serum

There was no difference in post-PEG recovery for EDTA plasma and serum specimens paired with lithium heparin plasma samples from patients with discordant cTnI results (n=3).

Serial testing

Twenty-seven percent of patients with macroTnI had hsTnI elevation on serial testing. GFC was performed on plasma from two such patients and demonstrated a rise in both the macroTnI fraction and troponin I-C complex (Figure 3).

Serial testing after MI or cardiac surgery (n=13) showed that, compared with VitrosTnI results, hsTnI in patients with macrotroponin rose disproportionately higher than expected, after taking the positive bias associated with the VitrosTnI assay into account. Figure 4 demonstrates this in four patients, one of which, (C), had hsTnI which persisted at very high levels for days after the VitrosTnI result had fallen and the patient was improving by all other parameters post-MI. HsTnI for patient (D) was high on admission with MI, fell in parallel with the VitrosTnI results but remains chronically elevated at 48 ng/L (post-PEG recovery=2%) 3 months after admission.

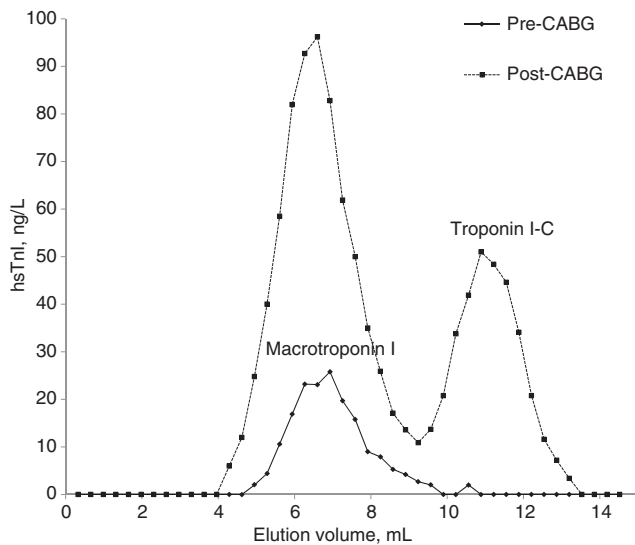


Figure 3: Elution profile (10/400 column) of hsTnI from a patient pre- and post-coronary artery bypass grafting (CABG) showing macrotroponin I in the pre-CABG specimen and macrotroponin I with troponin I-C complex post-CABG.

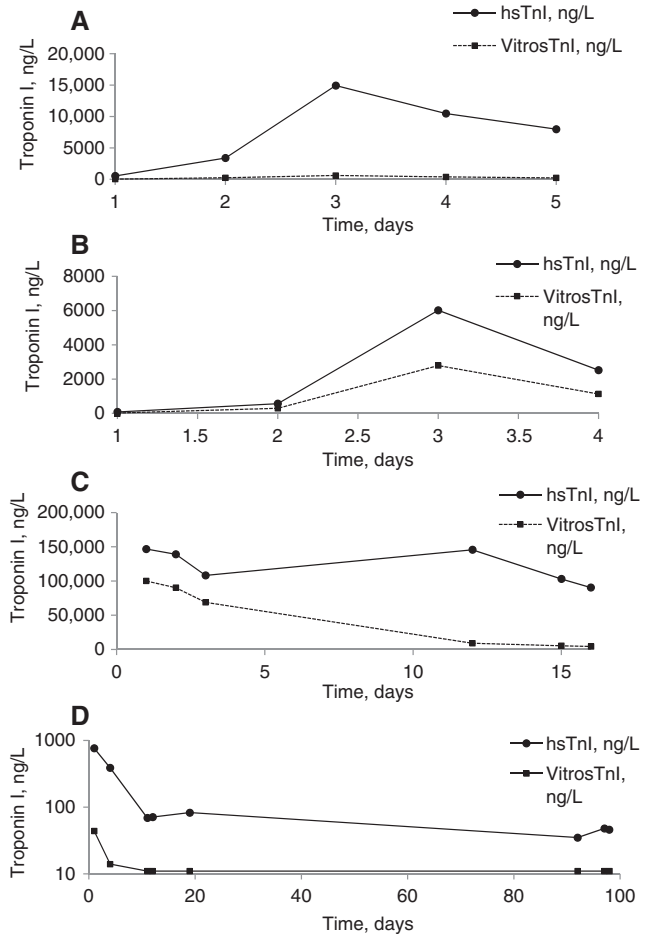


Figure 4: Time course of hsTnI vs. VitrosTnI results for patients with macrotroponin I (A) and (B) post-cardiac surgery, (C) and (D) post-MI.

Sample mixing

Plasma from a patient with elevated troponin I-C post-MI was mixed (equal volumes incubated at room temperature for 10 min) with plasma from a patient with macroTnI. GFC of 1) 200 µL post-MI plasma, 2) 200 µL macroTnI plasma and 3) 400 µL mixed post-MI and macroTnI demonstrated an elevation in the macroTnI peak with lowering of the troponin I-C complex peak in the mixed sample (Figure 5).

Subsequent cases and clinical information

At the time of writing 102 cases (53 female) of macroTnI have been detected since the first case in March 2015. These are defined as having hsTnI above the sex-specific cut-off, discordant with the VitrosTnI result, reproducible on repeat analysis and recovery post-PEG precipitation ≤15%

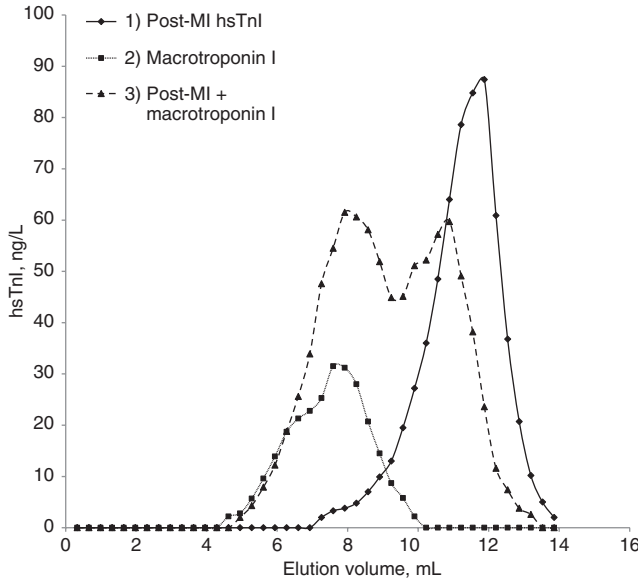


Figure 5: 10/400 column GFC of (1) post-MI plasma containing troponin I-C complex, (2) plasma from a patient with macrotroponin I and no myocardial injury and (3) a mixture of plasma from (1) and (2).

of the original result. Figure 6 shows the age distribution and hsTnI values for males and females.

According to clinical information from pathology request forms or discussion with clinicians 65% of patients (aged 16–91 years) had presented to the emergency department with chest or abdominal pain, dizziness, collapse or dyspnoea. Of these, 40% had histories of ischaemic heart disease, congestive cardiac failure or cardiomyopathy. Most of the others had risk factors for

coronary artery disease such as hypertension, dyslipidaemia or diabetes mellitus. Ten patients had hsTnI requested either before or after an elective cardiac procedure. Eight patients (aged 14–87 years) had hsTnI measured following emergency or elective surgical procedures, either as a routine or for the investigation of possible perioperative myocardial damage. HsTnI was requested by general medical practitioners in the community for the investigation of chest pain in three patients. Three patients had hsTnI measured in the context of cardiotoxic chemotherapy for malignancy. Four patients, with known heart or lung disease, had hsTnI measured before elective surgery. One patient was pregnant with chest pain and no history of heart disease.

Discussion

Five percent of elevated hsTnI results in this laboratory appear to be due to circulating macrotroponin, as evidenced by precipitation with PEG clearly below that of controls, depletion by Protein A/G/L, lack of evidence of the presence of heterophile antibodies and elution profile on GFC. Most of these are low level elevations (<100 ng/L) but have the potential to be clinically misleading. While this has become apparent with the adoption of the Architect High Sensitive Troponin-I assay, it is possible that the contemporary Architect STAT Troponin-I assay was similarly affected given the majority of Group 1 samples were also above the 99th percentile for that assay. It is likely that the slightly increased rate of troponin I elevation with

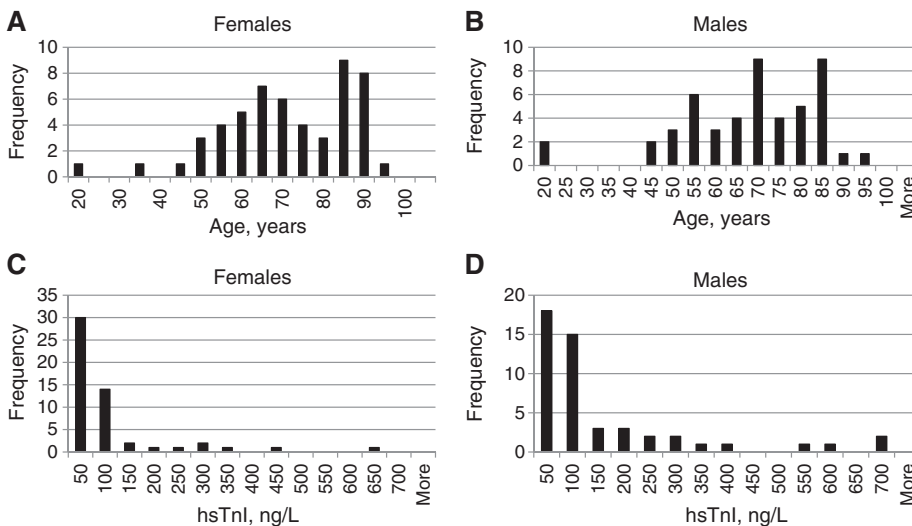


Figure 6: Age distribution of macrotroponin I in (A) 53 females and (B) 49 males. Range of hsTnI values in (C) 53 females and (D) 49 males with macrotroponin I.

the improved sensitivity of the new assay in combination with a change in units by our laboratory from $\mu\text{g/L}$ to ng/L , giving the impression of “higher numbers”, precipitated the clinicians’ enquiries which led to this investigation.

The GFC and PEG precipitation demonstrate that these elevated hsTnI results are due to high molecular weight complexes of troponin I. The Protein A/G/L data provide evidence that the complexes include immunoglobulins as IgG, IgA and IgM were shown to be depleted by Protein A/G/L treatment and, according to the product information, Protein A/G/L binds the Fc fragment of IgG and kappa light chains, thereby depleting IgG, IgA, IgM, IgD and IgE immunoglobulins [35]). macroTnI elutes from the GFC column between molecular weights 340 and 900 kDa indicating that the immunoglobulins involved could be IgG or IgA. The mixing studies and data from serial samples post-myocardial injury suggest that the complexes contain the troponin I-C complex but they could contain free troponin I or fragments of it. Further investigation is required to clarify this.

The mixing study and serial samples post-myocardial injury also suggest that circulating troponin I autoantibodies scavenge free troponin I-C complex so that in vivo it rises higher and is cleared more slowly from the circulation. The observation that autoantibody-positive patients have higher troponin I levels that persist longer post-MI has been reported [36]. Our data from serial measurements also suggest that antibody affinity and concentration vary from patient to patient as, compared with VitrosTnI results, the duration and degree of hsTnI elevation varies considerably.

As the risk of misdiagnosis and patient mismanagement was high we thought that pre-emptive identification of macroTnI, in as close to ‘real time’ as possible, was warranted. Results from evaluation of the first 50 cases demonstrated that re-analysis and PEG precipitation were sufficient to clarify whether a discordant elevated hsTnI result was due either to random error or to high molecular weight interference. PEG precipitation does not distinguish between heterophile antibody interference and macroTnI but this can be clarified by GFC or treatment with heterophilic blocking reagents. Protein A/G/L treatment confirmed PEG precipitation results and is specific for immunoglobulin-bound troponin but was a slower, more laborious process, a more expensive reagent and is not practical for rapid detection of macroTnI. GFC however, once set up, was easy to use and valuable in interpreting PEG precipitation results above 15% in Group 2 patients.

While it is not feasible for many laboratories to routinely perform two troponin assays in parallel a suitable

compromise may be to reanalyse and PEG precipitate specimens when serial troponin results do not change or results are not consistent with clinical and other findings. Differences in numerical results and concordance are expected between troponin I assays on individual samples for a variety of reasons and using a second assay to “validate” the results of another does have limitations. While our approach was simple (investigate positive hsTnI, negative VitrosTnI) we consider it a pragmatic one that functioned well as a real time process in a routine clinical laboratory. Our goal was to intercept and investigate possible false-positive hsTnI results as expeditiously as possible, given that the hsTnI assay is used routinely in our laboratory. As such, samples with a high VitrosTnI but normal hsTnI, which were comparatively infrequent, were not given the same level of scrutiny and are not presented here. In the long term, as other high-sensitivity troponin I assays are released onto the market, as long as they are shown not to detect macrotroponin, the combination of rapid diagnosis of MI with low risk of false positive results may be found in one assay.

Australia’s 99th percentile for the hsTnI assay is lower than is used elsewhere for males (26 vs. 34 ng/L) and eight (16%) of the male macroTnI patients had hsTnI values between 26 and 34 ng/L . Therefore laboratories using the manufacturer’s male-specific hsTnI cut-off will report fewer elevated results due to macroTnI.

Detection of macroTnI by the Architect hsTnI assay could be advantageous if, for example, evidence accumulates that macroTnI has clinical significance (unproven) or contributes to the assay’s ability to precisely measure low levels of troponin (speculative). The corollary is that the VitrosTnI may be a disadvantage for not detecting this form of troponin, however, our study was not designed to answer this question. If the incidence of macroTnI in the general population is comparable to our findings, this would affect the current 99th percentile cut-off, as approximately 5% of control subjects would have some, albeit moderate elevation in cardiac troponin measured by hsTnI due to the presence of macroTnI. This would need to be confirmed, however, as macrotroponin may, for example, be more common after cardiac injury, so many of these individuals would be excluded from reference interval studies.

True cTnI elevation and the presence of macroTnI are not mutually exclusive. Their co-existence can result in equivocal (>15% recovery) PEG precipitation results requiring further investigation (GFC or alternate troponin method) to clarify, as we saw in Group 2 patients who had both macroTnI and true elevation of cTnI secondary to acute myocardial damage. It is reassuring that the

presence of macroTnI does not appear to mask a true cTnI elevation due to myocardial injury, but may exaggerate it.

In the hsTnI assay the capture antibody is directed toward amino acid residues 24–40 while the detection antibody targets amino acids 41–54 [37]. The VitrosTnI assay has two capture antibodies targeting amino acid residues 24–40, and 41–49 with the detection antibody binding to residues 87–91. We present evidence that the Architect assay either captures or detects macroTnI complexes that the Ortho assay does not. Whether this is because of steric hindrance (perhaps of the detection antibody) by the autoantibody in the Ortho assay or due to displacement of the autoantibody from the troponin I molecule by reagent antibodies or other components in the Abbott assay requires investigation. It is possible that the Ortho assay also over-reports TnI concentrations because of macrotroponin, but to a lesser extent. Given the similar antibody configuration of most assays (2), it is likely that that other assays, possibly also for troponin T, measure macrotroponin. Perhaps circulating macrotroponin should be added to the list of causes of persistent troponin elevation. A high index of suspicion and close liaison between clinicians and the laboratory are required to fully investigate the extent of this issue and determine a strategy for managing elevated troponin results due to macrotroponin.

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