**Macrotroponin - analytical anomaly or clinical confounder.**

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The term macrotroponin is usually applied to a complex of cardiac troponin (cTn) with an immunoglobulin(1). The resulting complex is cleared differently from uncomplexed cTn. The presence of macrotroponin can cause artefactual elevation of cTn values during routine measurement. Such a result is a true false positive cTn elevation. This complex may be with cardiac troponin T (cTnT), cardiac Troponin I (cTnI) or a complex with troponin C (I-C) or cTnT (T-I-C)(2-4).

Typical presentation is a call from a confused clinician(5). A patient with an elevated troponin, usually high, has undergone a range of imaging studies and no evidence of a myocardial infarction or anything consistent with the cTn has been found. A detective story unfolds, the laboratory consults a Clinical Chemistry Poirot who knows the relevant steps. Macrotroponin is revealed as the analytical villain.

The prevalence of macrotroponin has been a mystery. Typically, they are reported as case reports but to date the prevalence and clinical impact have not been systematically investigated. In this issue of Clinical Chemistry Lam and colleagues have addressed this evidence gap(6). Building on a previous study they have systematically screened a community population with a cTn value above the upper reference limit (URL) for macrotroponin. Their results are somewhat startling. 99/188 participants (52.6%) patients were found to have a macrotroponin. In a previous study from this group the prevalence was also found to be comparably high, 123/223 (55.2%)(4) when a similar community population was screened, although patients were not selected for troponin elevation.

The prevalence of anti-troponin antibodies (which may not necessarily produce macrotroponin) has been reported as 0.0 -12.7% in healthy individuals and up to 13.4% post myocardial infarction(7). Other studies have estimated the prevalence by examining discordance between different troponin methods and reported the prevalence of macrotroponin as 54/1074 (5.0%) for Abbott Architect hs cTnI vs Beckman Access hs cTnI (8) and (3-12)/184 (1.6-6.5) for Abbott Architect hs cTnI vs Vitros cTnI ES(9). The higher rate of macrotroponin detection reported here probably reflects differences in approach, although subject selection may also play a role. Identification of macrotroponin by screening discordant results between methods will be systematically biased to under detect compared to primary sample screening for macrotroponin.

In the current study (6), 4641 cTnI measurements were performed with 311 elevated results of which 99/188 (52.7%) were confirmed as macrotroponin. Elevation due to macrotroponin which manifests as a troponin value exceeding the URL can be estimated at 163/4641 (3.5%) overall. In an unselected population(4) 54/223 (24.2%) were below the URL, 160 (71.7%) from 1-10 times the URL (median approximately twice the URL and 9 (4.0%) above the URL.

The main impact of macrotroponin is to produce elevation of troponin values. The impact of macrotroponin on assay performance will be on assay specificity rather than sensitivity. The other factor to consider is the temporal stability of values. Macrotroponin typically present with persistent elevation and seems to show low short and medium term variation(5;8;9).The effects of macrotroponin will be influenced by these two factors.

Analytical effects.

Different cTn methods have different rates of macrotroponin detection. There is also a significant difference when cTnT is subsequently measured on the same sample. Typically when a cTnI macrotroponin is detected, this is not reflected in the cTnT result although there may be a positive for a T-I-C complex macrotroponin(2). The discordances between cTnI and cTnT has an immediate and useful consequence. If a cTnI macrotroponin is suspected rapid exclusion is possible by measurement with a different cTnI assay but ideally by measurement with cTnT. The corollary is probably also true, but a systematic study has not been performed.

Measurement of troponin is affected by haemolysis, phosphorylation, oxidation reduction, troponin fragmentation and epitope masking. Macrotroponin is another to add to the list of potential analytical interferences for method evaluation studies. Discordance between assays has been used to estimate the frequency of macrotroponin occurrence but is not a direct measure. Assay manufacturers should attempt to obtain a panel of macrotroponin samples to estimate the degree of interference in their assay. The number of discordant results between assays, will be affected by the prevalence of macrotroponin, the type of macrotroponin and the ability of the assays under investigation to detect the type of macrotroponin present. In method comparison studies, discordance between methods will show as the scatter around the line of agreement and the number of outliers(10).

The current study(6) shows clear differences when analytical performance is compared by receiver operating characteristic curve analysis. Comparison of the area under the curve (AUC) shows a significant difference overall between the assays according to the degree of macrotroponin detection. Impact on AUC is inversely proportional to the degree of detection

of macrotroponin. When split into populations with and without macrotroponin, assays with a low rate of macrotroponin detection show a significant difference in the AUC, reflecting reduced specificity of the macrotroponin sensitive assay used to make the initial diagnostic selection. Analytical performance is not particularly affected by very high or very low values but is most affected by classification of results in the intermediate troponin range. The majority of elevations are in the low range (1-10 x URL) which will explain the impact on the AUC. The effect will also be exaggerated by using a population with a very low prior probability of ACS. To put these findings in context, diagnostic studies on cTn assays to date have not shown significant differences in diagnostic performance between assays in the ACS population but macrotroponin will undoubtedly contribute to the difference between them at low troponin levels.

The need to exclude samples containing macrotroponin is clearly important if harmonisation studies using sample pools is undertaken to avoid variable false positive elevation across methods. In reference range studies, troponin values are disproportionally affected by the tail around the upper end of the distribution. The likelihood that macrotroponin contributes to this appears to be high and this must be added to the criteria for exclusion. Pragmatically this would be best achieved by testing values in the upper 10% of the distribution. Figure 1 (6) shows lower cTnI values in the non-ACS group when assays are less affected by macrotroponin or macrotroponin is not present.

Clinical impact.

The findings from this paper need to be seen in the context of current clinical practice which mandates serial sampling for the diagnosis of myocardial infarction (MI). Patients with macrotroponin without MI are very unlikely to show a significant change in troponin (delta troponin) due the macrotroponin itself. Indeed it seems if anything the opposite is the case(8). Currently recommended rapid diagnostic algorithms combine admission testing with serial testing(11). Rule out admission testing uses a low threshold and in theory would be compromised by macrotroponin but in practice this does not seem to occur. Rule in however will be compromised as the thresholds are not as high as 10 times the URL so a significant proportion of immediately admitted patients might have a macrotroponin and no ACS. Indeed, the specificity of rule in algorithms is poor. This paper(6) supports the value of serial testing even when troponin is elevated and most importantly that the troponin result must always be interpreted as part of the clinical picture. It is often overlooked that current ACS management guidelines do not require immediate (less than 2 hours) intervention(11). There is always time to repeat the troponin. This paper(6) also may explain why there are so many patients with modest troponin elevations in the emergency department with a multifactorial or indeterminate cause of troponin elevation.

Outcome studies in the asymptomatic population support the role of cTn measurements as a prognostic risk marker for long-term risk(12). Depending on method, there is a risk of inappropriate classification of patients into a higher risk group(13). Methods selected for ooutcome studies need to avoid macrotroponin interference. Lam et al exmine clinical outcomes in the population studied based on a single sample case. No adverse outcomes were seen but hospital stay was longer in macrotroponin patients without ACS.

Recently a case based review on interferences on cardiac biomarkers was able to identify clinical implications in 45% of cases of true false positive elevations(14). The most common intervention was cardiac imaging including catheterisation and unnecessary interventions occurred in 10% of cases. The commonest causes where, in order, outliers (fliers), heterophile antibodies and macrotroponin (22/222 case reports, 10 with clinical implications). Again, repeat the measurement(15).

So, what is the implication of macrotroponin for the laboratory and clinician? A rubeum allec? As Zhou Enlai is (incorrectly reputed) to have remarked when asked to comment on the significance of the French Revolution (of 1789), it’s too early to tell. This paper underlies the importance of clinical assessment of the patient, serial sampling and liaison with the lab. At the moment analytically fascinating and potentially a confounder but apparently clinically inconvenient rather than catastrophic. The current rapid diagnostic algorithms require serial confirmation of elevated values but should be modified so that all elevated values require repeat.

**DECLARATIONS**

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