Editorial

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Biotin interference in cardiac troponin immunoassay – where the wild things are?

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Interference in analysis, in particular in immunoassay, remains a perpetual headache for the laboratory and potential pitfall for the clinician. Interference has been documented for all types of immunoassay and includes analyte independent interferences (pre-analytical or analytical) and analyte dependent interferences [1]. Interference from drugs is fortunately rare but does occur.

Troponin measurement now defines myocardial infarction [2]. Treatment strategies for patients with non-ST elevation myocardial infarction (NSTEMI) are predicated by troponin measurement [3]. The development of high sensitivity troponin assays, defined as those with imprecision less than 10% at the 99th percentile and able to measure at least 50% of a reference population [4], has further changed the way cardiac biomarkers are used. Rapid diagnostic algorithms able to rule out NSTEMI by measurement of a single sample on hospital admission or following serial measurement over 1–2 h have been developed and extensively validated. Such strategies have been endorsed by the UK health technology assessment programme ([5] guidance soon to be updated) and by The European Society of Cardiology [3]. They depend on the ability to measure very low values of troponin reliably and that repeat measurements have low imprecision. There was therefore alarm when it was reported that there was a risk of false negative troponin results in patients taking biotin supplementation. https://www.fda.gov/medical-devices/safety-communications/fda-warns-biotin-may-interfere-lab-tests-fda-safety-communication#:~:text=The%20FDA%20has%20received%20a,would%20interfere%20with%20lab%20tests.

The majority of discussions following improvement in troponin assay sensitivity and the introduction of high sensitivity assays has been around "false positive" results. Much of this has been ill informed, with troponin elevation outside the spectrum of acute coronary syndromes (ACS) being labelled as "false positive" and given labels such as "troponinitis". Much of this confusion is due over requesting of troponin in patients who do not have ACS or even suspicion of ACS with requests made "just in case" [6]. Although true false positive troponin results can occur they are very rare and have the same causes as in other immunoassays, although there are false positives unique to troponin assays. Troponin elevations outside ACS indicate myocardial injury and are associated with a worse prognosis, whatever the cause [7, 8]. The main role of clinical testing for troponin is exclusion of myocardial infarction and of significant myocardial injury from any cause. In this regard, high sensitivity troponin assays are clinically excellent and this exquisite sensitivity is the basis of rule out algorithms. No troponin elevation means an excellent prognosis and safe discharge from hospital, with minimal hospital stay. This is an important attribute in the times of COVID 19. The documentation of a potential cause of false negative results is therefore worrying.

Steptavidin-biotin based assays have the potential for immunoassay interference. This was first documented in 1996 [9]. However the likelihood of encountering high levels of biotin outside of specific treatment regimens was considered unlikely [10]. Such interventions would also be expected to be documented as part of the drug chart. However, the current enthusiasm for biotin supplementation to improve hair and nails (with accompanying celebrity endorsement) means that a large number of people are taking biotin supplements, with a typical starting dose of 10 mg, for which there is little evidence of benefit [11]. Analytical interference has been demonstrated at the 10 mg level of supplementation [12] although the interference is variable between assay type and formulation [13].

Is this a real problem? An earlier study from the Mayo clinic suggested that 7.4% of patients attending the Emergency Department (ED) had biotin concentrations exceeding 10 μg/L [14]. This group subsequently estimated a clinical risk of 0.8% for the ED population. There have been other publications which have examined the rate of biotin elevation. These have variously estimated the

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proportion exceeding 10 μg/L as 0.8% (ED population) [15] and 0.2% (routine laboratory requests) [16].

In this issue of the journal Mumma and colleagues [17] report a novel approach to assessing the risk of biotin interference. They combine estimation of prevalence of significant elevation with risk modelling in two different cohorts of patients. In the first cohort they used patients enrolled in a clinical study of suspected ACS and measured biotin levels in residual samples. In the second cohort they used randomly selected samples submitted for routine analysis to a US laboratory. In both cohorts they obtain the prevalence of an elevated biotin (defined as exceeding 20 μg/L). They then undertake a modelling exercise to determine the likelihood that an elevated biotin would result in misclassification of a patient with acute myocardial infarction (AMI) based on misclassification at the 99th percentile. In the second cohort they additionally attempt to model the potential impact of elevated biotin resulting in interference with a single sample rule out strategy by lowering the troponin below the rule out decision threshold. In the ACS population, the percentage of patients with biotin >20 μg/L was 0.13%. In the laboratory population it was 0.74%. In the ACS population the risk of misclassification of AMI (based on the 99th percentile) was 0.026%. For the general laboratory population the derived risk for misclassification of AMI was 0.025% and for the single sample rule out strategy it was 0.063%. How should these findings be interpreted in relation to the current literature in this field? The first problem is the prevalence of elevated biotin. The second is the question of the assay version, interference threshold selected, and the pharmacokinetics of biotin ingestion.

Biotin measurements are not standardised. In the original Mayo clinic publication measurement was by liquid chromatography tandem-mass spectrometry (LC-MS/MS) [14]. This technique was also used by two of the other groups documenting values above 10 μg/L [15, 16]. The authors have used a research immunoassay which detects total serum biotin. The argument for this is that it detects all biotin species including metabolites, so is more likely to detect the levels at which interference will occur. Although the assay is calibrated against LC-MS/MS biotin measurements the slope of the graph is not 1.0 suggesting under recovery, which is slightly surprising for an assay that measures total biotin (including other forms of biotin) rather than biotin alone.

The question of assay version, interference threshold, and the effect of biotin ingestion is much more complicated. Evaluation of the fifth generation troponin T assay reported no interference was observed with biotin concentrations upto 20 μg/L [18]. In examining the impact of biotin interference it was reported that concentrations of 15.6 μg/L or greater would generate significant interference [13]. The choice of 20 μg/L as the interference threshold might therefore be somewhat optimistic as it is derived from spiking experiments. In a volunteer study, five normal individuals ingested a supplement containing 10 mg biotin per day, a commonly available dose. The post ingestion levels of biotin detected would have been expected to generate a significant fall in measured troponin values [19]. This study also showed the variability in dose response between individuals following biotin ingestion. A further complicating factor is that the spiking experiments *in vitro* may not mimic the biological effect of biotin ingestion (alone or as a multivitamin preparation) with interference by biotin metabolites and other tablet constituents in addition to that of biotin alone. To add a further level of complexity, it is possible that measured biotin levels do not necessarily reflect the value that generates assay interference when biotin is taken as a supplement.

What may we conclude? The differences between the studies using LC MS/MS may represent different populations with Americans tending to take more biotin containing supplements. Comparing the prevalence in the two US studies, there are different methods for biotin measurement in different populations and a different cut-off has been used. The risk of interference in the assay would therefore seem to be between a pessimistic 0.8% and an optimistic 0.063–0.025% and is driven by the estimate of the percentage above the interference threshold. Based on UK figures for chest pain admissions this would mean between 3,600 and 28 missed cases annually. The authors make the point that the risk of a false negative from biotin assay interference is lower than the risks involved with rapid rule out strategies. These have an estimated misdiagnosis rate of ~0.5% [20], which is deemed clinically acceptable. This is a valid point and it is reassuring that the number of cases missed may well be small. It is also the case that rapid rule out protocols will become the norm hence there is only a single sample on admission and repeat sampling with physiological biotin fall will not apply. Although strategies have been suggested to mitigate risk in practice this will be difficult to implement in the context of rapid diagnostic strategies [21, 22].

Biotin interference remains an avoidable cause of false negative results. In medicine risks are frequently additive and often multiplicative. And in clinical medicine, Murphy's law applies with depressing frequency. It is therefore encouraging that a more recent formulation of the cardiac troponin T assay with a much higher biotin interference threshold has been produced.

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