Clin Chem Lab Med 2011;49(6):1079–1080 © 2011 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.163

Letter to the Editor

Evidence on the cause of false positive troponin I results with the Beckman AccuTnI method

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Keywords: false positive; insoluble fibrin; troponin I.

False positive troponin I (TnI) results with the Beckman AccuTnI method have been reported since 2002 (1). Recent studies from my own laboratory have demonstrated that false positive TnI results occur with both serum and lithium heparin sample types with BD tubes (2) and Greiner tubes, at a rate of 14/1000 samples on both the Access 2 and DxI800 analysers (3). These and other publications since then have attributed the errors to residual fibrin (1, 4, 5). The discrepant results are rarely reproducible. The strategy employed to minimise erroneous results from being reported has been to utilise aliquots of serum or plasma and re-centrifuge the aliquot at centrifugation settings routinely used or at high speeds (6). In our laboratory, the strategy is to utilise the analyser's reflex function and analyse samples in duplicate when the result is >0.04 and <5.00 μg/L. Results outside these limits have been very rarely observed to produce false positive results and thus are not run in duplicate. If the duplicate results exceed a difference of 20% or more, which is considered clinically significant and needs investigation, an aliquot is re-centrifuged and the supernatant re-analysed. The third (and fourth replicate if produced) are reviewed in conjunction with other pathology results and clinical information before a result is reported. It is by no means a fool proof process, and on very rare occasion false positive results continue to be reported at a rate of one result in approximately 4000 samples. The downside with the process, as pointed out by Pfafflin, is that there can be delays in reporting of results and potential delays in diagnosis of myocardial infection (6). The delay in reporting of TnI results with this procedure is justified by minimisation of result inaccuracies, reducing the number of patients falsely diagnosed or being exposed to unnecessary stress with additional testing, treatment and admission. The cost of the reagent and other consumables are easily accounted for by preventing the inappropriate admission of a few patients each year, and preventing potential legal and professional implications.

The exact cause of the problem has not been identified nor has anyone shown residual fibrin or any other analyte being directly linked to the problem. Lithium heparin plasma samples stored at 4°C containing ''insoluble fibrin'' mesh were used to demonstrate the impact of sample quality resulting in non-specific binding and the lack of method robustness. Such insoluble fibrin is observed after 24 h of storage at 2–8°C in about 5% of samples in our laboratory. Insoluble fibrin is formed from fibrinogen, and fibrinogen is present in lithium heparin plasma. Fibrin formation and fibrin structure is stated to be influenced by a number of variables including fibrinogen and thrombin concentrations, pH, ionic strength, chloride ion concentration, presence of calcium ions, and polyphosphate, a potent anticoagulant (7). Disease state and medication can both diminish the efficacy of heparin activity and lead to increased fibrin formation, and this is enhanced when plasma is stored at low temperature (8).

In our laboratory, blood tubes (lithium heparin plasma and serum) are centrifuged for 10 min at 20°C and 3000 *g* (approx. 4200 rpm). Approximately 95% of samples are centrifuged within 1 h from the time the blood is collected. The total turn-around time (TAT) for TnI results from collection to result validation is <70 min for approximately 90% of samples, and the remaining samples have longer TAT mainly because they are requested at a later time. The sample mix for routine TnI analysis is 60% serum and 40% lithium heparin plasma. Routinely, three levels of control material are run and the mean CV values at the time of the study were 13.5% (0.058 μg/L), 9.6% (0.532 μg/L) and 8.3% (6.87 μg/L).

To determine if insoluble fibrin can lead to false positive TnI results, two experiments were performed. In the first experiment, a sample with TnI above the cut-off threshold and containing insoluble fibrin was analysed 12 times; 10 times with fibrin mesh intact and two times following re-centrifugation or using clean aliquot of the sample. Both clean sample TnI concentrations were 0.077 and 0.077 μg/L. The 10 replicates produced results ranging from 0.09 to 0.162 μg/L.

For the second experiment, lithium heparin plasma from two samples containing insoluble fibrin were pooled. The sample was centrifuged at 100 rpm for 1 min to concentrate the fibrin, but not destroy the fibrin mesh. The clear supernatant (plasma) was removed and replaced with an equal volume of normal saline. The sample was gently inverted and re-centrifuged at 100 rpm for 1 min. The saline wash

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Previously published online March 24, 2011

Table 1 Effects of insoluble fibrin from lithium heparin plasma on the Beckman AccuTnI assay.

Fibrin sample	cTnI, μg/L	Actual time of analysis
Fibrin aliquot 1	0.061	17:45
Fibrin aliquot 1 (reflex)	0.016	17:59
Fibrin aliquot 2	0.050	17:46
Fibrin aliquot 2 (reflex)	0.023	18:00
Fibrin aliquot 3	0.038	17:47
Fibrin aliquot 4	0.035	17:47
Fibrin aliquot 5	0.034	17:48
Centrifuged aliquot	0.015	18:00
Centrifuged aliquot	0.013	18:14

step was repeated two more times and the sample tube filled with saline to the original plasma level in the tube to resuspend the fibrin. An aliquot was also re-centrifuged and the clear supernatant analysed in duplicate. Five aliquots of the sample containing fibrin were analysed for TnI. The results are summarised in Table 1.

The results indicate fibrin is likely to have crossed linked the assay antibodies to produce false positive TnI results. The results also show that as fibrin settles out in the saline, interference was reduced as indicated by the reflex results. Additionally, the false positive TnI results are higher when the fibrin concentration is higher in the sample, before it settles. In plasma, insoluble fibrin does not settle to the same extent and stays dispersed throughout the sample either as result of networks with other proteins or binding/anchoring to the tube wall.

The mechanism suggested by Er et al. is that the antibody either binds non-specifically to fibrin or the indicator enzyme may be physically trapped by fibrin in the separation matrix (4). This is unlikely to cause false positive TnI results. For the false positive to occur, both antibodies (capture and signal) have to be bound by the fibrin and be retained in the cup following the wash to cause the error. To overcome this problem, method redesign is essential. This may involve a combination of factors, such as modification of reagent pH to minimise non-specific binding, introducing an additional wash step after the sample and capture antibody-paramagnetic particle have been incubated and bound, and the second wash step occuring after the signal antibody has been incubated to form the sandwich. Additionally, increasing the wash time and rigor needs to also be considered. The data represents direct evidence of non-specific binding leading to false positive TnI results, and confirms that insoluble fibrin, if present in the aliquot aspirated for analysis, is capable of causing false positive results.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Fleming SM, O'Byrne L, Finn J, Grimes H, Daly KM. False-positive cardiac troponin I in a routine clinical population. Am J Cardiol 2002;89:1212–5.
2. Dimeski G, Masci PP, Trabi M, Lavin MF, de Jersey J. Evaluation of the Becton-Dickinson rapid serum tube: does it provide a suitable alternative to lithium heparin plasma tubes? Clin Chem Lab Med 2010;48:651–6.
3. Ungerer JP, Pretorius CJ, Dimeski G, O'Rourke PK, Tyack SA. Falsely elevated troponin I results due to outliers indicate a lack of analytical robustness. Ann Clin Biochem 2010;47:242–7.
4. Er TK, Tsai LY, Jong YJ, Chen BH. Falsely elevated troponin I attributed to inadequate centrifugation using the access immunoassay analyzer. Clin Chem Lab Med 2006;44:908–9.
5. Hejl CG, Astier T, Ramirez JM. Prevention of preanalytical false-positive increases of cardiac troponin I on the Unicel DxI 800 analyzer. Clin Chem Lab Med 2008;46:1789–90.
6. Pfäfflin A. Doubt on prevention of false-positive results of cardiac troponin I by recentrifugation. Clin Chem Lab Med 2009; 47:892–3.
7. Smith SA, Morrissey JH. Polyphosphate enhances fibrin clot structure. Blood 2008;112:2810–6.
8. Cameron CL, Fisslthaler B, Sherman A, Reddigari S, Silverberg M. Studies on contact activation: effects of surface and inhibitors. Med Prog Technol 1989;15:53–62.