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Letter to the Editor

Pre-analytical variables affecting discordant results on repeat sample testing for cardiac troponin I

To the Editor:

Analytical false positives (i.e., high concentrations) of cardiac troponin I (cTnI) can occur due to pre-analytical factors such as fibrin clots, hemolysis, etc. [1] or analytical factors such as carry-over, the presence of heterophile antibodies, autoantibodies, rheumatoid factor interferences, and macrocomplexes [1–4].

Within our hospital network (two hospital sites: A and B), the cTnI assay is measured using the Beckman Access AccuTnI reagent on the Unicel DxI800 or the Access2 analyzer (Beckman Coulter Diagnostics) depending on the workflow requirements at the main hospital site (A) or on two Beckman Access2 analyzers at hospital B. An incident whereby a false positive cTnI result was reported occurred in 2008, which prompted an internal review and search for discordant repeat measurements for cardiac troponin. At this time, it was recognized that the occasional "flyer" can occur when heparin plasma samples have not been well-mixed on blood drawing which may lead to the formation of microclots [5].

Falsely high cTnI concentrations, possibly from fibrin clots, for the Beckman AccuTnI assay have been reported since 2002 [5]. Many laboratories have employed a re-centrifugation step using high speed centrifugation to remove this potential interference [5]. Our hospital network, however, implemented a filtering process using TipTop® Blood Dispenser Caps manufactured by labcon, as a strategy to minimize error. The filter cap is an integral porous filter that removes fibrin from the heparin plasma sample. A policy was created that only those samples with initially high cTnI concentrations > 0.06 μg/L (the previously established 10% CV level) [6] with sufficient sample volume, would be filtered followed by reanalysis for cTnI. The filtered results were reported. Although this process will increase cTnI turn-around-time of at least 30 min, resulting in a possible delay in the diagnosis of myocardial infarction, it ensures the quality of cTnI results and may reduce exposing patients to unnecessary treatment and/or admission.

Over the last eight years (2010–2017), we reviewed 24,520 cases of paired pre-filtered and post-filtered cTnI concentrations across the two hospital sites (A and B). Cases without any recorded pre-filtered cTnI results or incorrect data entry were excluded. Overall, 5.3% (n = 16,494/311,301) and 3.8% (n = 8092/215,529) were filtered at hospital A and B, respectively. We speculated that the similar percentage of sample filtration between the hospital sites was probably due to the similar patient population being evaluated. Indeed, over 80% of the cTnI tests were ordered by the emergency department (ED) physicians at both hospital sites.

To identify discordant results we used two published approaches: i) a method that included both an absolute and percent change criteria; ii) the proposed total error goal of < 35% [7–9]. Specifically, for the first approach, an absolute difference of > 0.03 μg/L for cTnI in concentrations less than or equal to 0.10 μg/L or percent difference of > 20% at cTnI > 0.10 μg/L between pre-filtered and post-filtered cTnI results were considered to have exceeded analytical variation and could be considered as a significant change in concentrations [7]. Using the above criteria, a discordant rate of 10.95% (95%CI: 10.45–11.46; n = 1799/16,436) was found at hospital A which was significantly higher than the discordant rate at hospital B: 6.30% (95%CI: 5.77–6.88; n = 509/8075) (p < .001) (Fig. 1A). Applying the proposed total error goal for cardiac troponin (i.e., < 35%) [8,9] as the second approach, the proportion of discordant results were similar to the first approach with the discordant rate in hospital A being 10.3% (1693/16,436) and in hospital B being 5.9% (480/8075) (Fig. 1B).The majority of discordant paired results in cTnI was found to have higher pre-filtered concentrations, consistent with fibrin clots leading to these erroneous cTnI elevations (Fig. 1A, B). Moreover, the difference in concentration between the pre-filtered and post-filtered cTnI in hospital A (average cTnI difference = 0.128 μg/L) was significantly higher than hospital B (average cTnI difference = 0.078 μg/L; p < .05, Wilcoxon). Of the discordant paired results, the pre-filter results were higher in 94.9% and 92.3% for concentrations ≤0.10 μg/L and 91.1% and 94.8% for concentrations > 0.10 μg/L in hospital A and B, respectively. The discordant rates between the two platforms (DxI and Access2) were also comparable.

A review of laboratory practices at both hospital sites was initiated to identify the possible root cause for the difference in discordant paired result rates. At both hospital sites, whole blood specimens were collected using PST gel separator tubes, transported to the core laboratory either by hospital staff or by a pneumatic tube system. Even though the phlebotomy practices in mixing samples are standardized, we have observed through audits and incidences that there were some periods where this was not being followed, especially at hospital B, leading to possible increase in fibrin strands and microclots interferences at this site. In agreement with possible minor differences in pre-analytical handling of the blood samples between the hospitals, we have also observed that the hemolysis rate was higher at hospital B (8.2%) as compared to hospital A (5.4%). This difference in hemolysis rates was mainly attributed to samples arriving from the ED (hemolysis rate at hospital B from ED = 8.7% versus hemolysis rate at hospital A from ED = 5.7%) and not from other critical care units (i.e., hemolysis rate at hospital B from CCU = 4.6% versus hemolysis rate at hospital A from CCU = 5.0%). The difference in hemolysis rates between the EDs and CCUs may reflect the fact that nurses draw blood samples in the ED whereas blood collection teams draw blood samples from the CCUs. Notwithstanding the fact that blood sample handling may be more problematic at hospital B, it nevertheless had a lower cTnI discordant rate, suggesting another pre-analytical factor being the source for the higher cTnI discordant rate at hospital A.

At both hospital sites, once samples are received in the laboratory they are centrifuged immediately, with approximately 95% of samples being centrifuged within 45 min from blood collection. Routine centrifugation is on the Power Processor Sample Handling System (PPSHS,

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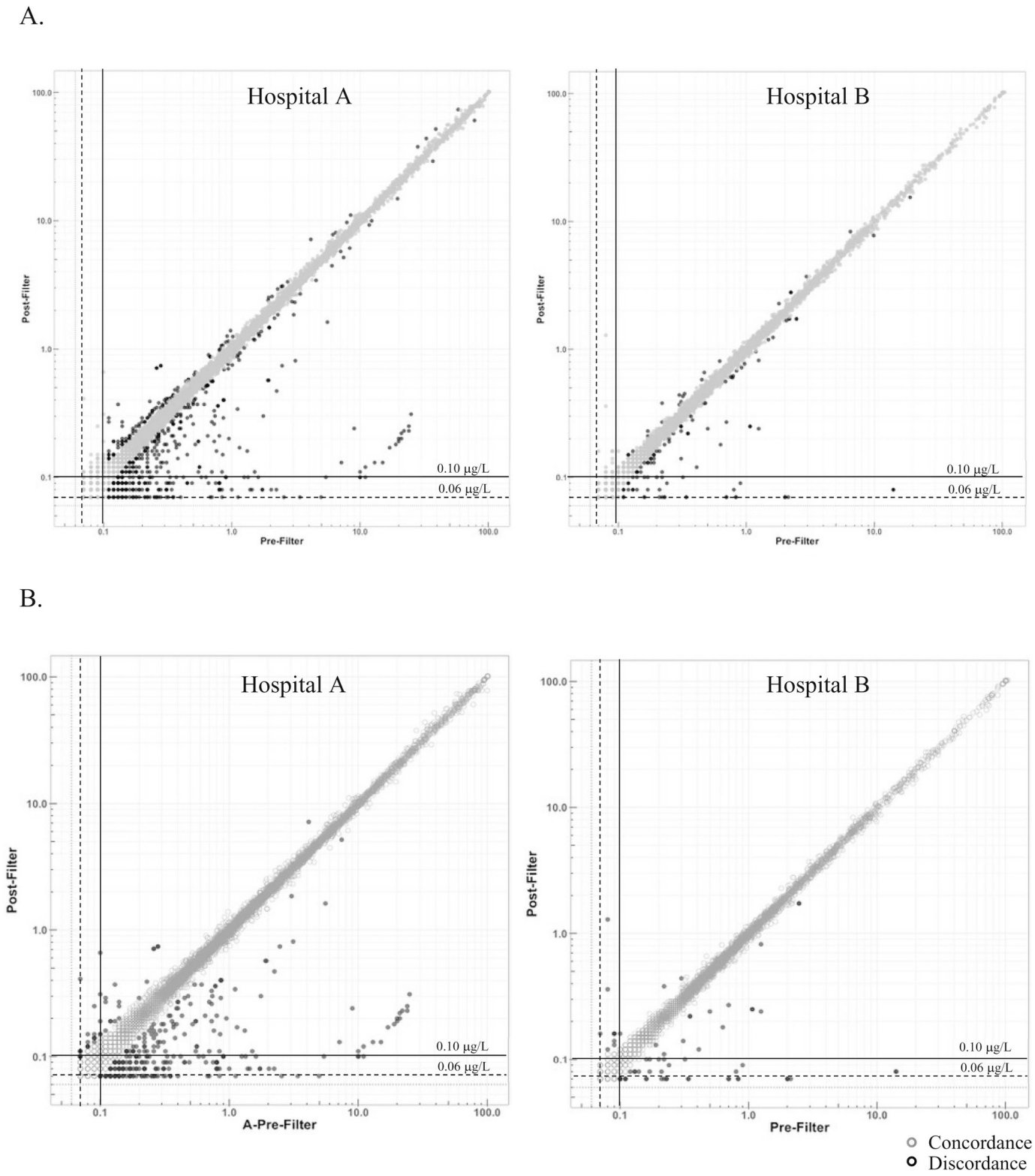

Fig. 1. Distribution of all duplication results. Pre-filtered (x-axis) and post-filtered (y-axis) cTnI results from the hospital A and B. The grey dots represent cases of concordance and the black dots represent cases with discordant results. The dashed lines indicate the 0.06 µg/L decision limits as positive cTnI results. The lines indicate the 0.10 µg/L cut-off when calculating the absolute or percent differences. A) Discordant determined by both an absolute and percent change criteria; B) the proposed total error goal of < 35% regardless of the cTnI concentration.

Beckman) at the main hospital site A, with a centrifugation time of 6 min at 1912 *g* (a maximum speed), with only the DxI analyzers being connected to the PPSHS, with the Access2 analyzer being a standalone instrument. However, at hospital site B, there is no automation line and all blood samples are manually centrifuged at 2500 *g* for 10 min and then tested on the Access2 instruments. The cTnI assay imprecision is similar between the analyzers and the sites, with three levels of quality control materials measured daily with CV < 11% across all analyzers

and levels of QC.

The decision to reduce the centrifugation time to 6 min at hospital A was aimed to decrease the total turnaround time at the main hospital site, as historically the turnaround time for testing was longer at this site. However, this may have led to more fibrin clots and to the higher discordant rate for samples with cTnI > 0.06 μg/L at hospital A as compared to hospital B, as this represented the main pre-analytical difference between the sites. Thus, balance is needed between obtaining the optimal centrifugation protocol with the shortest turnaround time, so that the accuracy of the cardiac troponin results is not compromised.

In summary, falsely elevated cTnI concentrations have been an issue in clinical diagnostic testing for > 10 years. Our data has showed that the Beckman Coulter AccuTnI assay is still impacted by microclots, as outliers identified by using a proposed change criteria ranged from 5 to 10%, which if not identified by the laboratory might have been misinterpreted as a clinical change. Strategies that laboratories have employed to combat this issue include re-centrifugation, filtration and recently changing the blood collection tube [10]. It is unknown if the new Beckman high-sensitivity cardiac troponin I assay is affected to the same extent as the AccuTnI assay. This could be of particular concern if low concentrations below 10 ng/L are used alone for clinical decision making, as the total error in this range is < 3.5 ng/L and studies assessing Beckman's hs-cTnI here are required [9]. However, initial data have indicated Beckman's regulatory approved hs-cTnI assay exceeds precision goals for hs-cTn assays and is more robust than previous versions [3,11,12]. Nevertheless, before more data is available, laboratories adopting this new hs-cTnI assay may opt for a combination of higher relative centrifugal force (*g*), or longer centrifugation time and/or filtering to avoid these potential microclots.

Conflict of Interests: Dr. Kavsak has received grants/reagents/consultant/advisor/honoraria from Abbott Laboratories, Abbott Point of Care, Abbott Diagnostics Division Canada, Beckman Coulter, Ortho Clinical Diagnostics, Randox Laboratories, Roche Diagnostics and Siemens Healthcare Diagnostics. McMaster University has filed patents with Dr. Kavsak listed as an inventor in the acute cardiovascular biomarker field.

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