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Short Communication

Relationship between free hemoglobin (hemolysis), potassium and ionized calcium in lithium heparin blood gas samples collected intraoperatively

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ABSTRACT

Objective: Develop sample acceptability rules by determining the relationship between free hemoglobin level (hemolysis) and potassium or ionized calcium in blood gas samples collected intraoperatively.
Design and methods: Hemolysis was assessed visually or by H index for lithium heparin blood gas samples collected intraoperatively. During periods one and three this was done using two different rules for visual assessment of centrifuged lithium heparin plasma. During period two H index was measured for all visually hemolyzed samples on a Roche Cobas c501 analyzer to determine acceptability. Potassium and ionized calcium were measured in 75 lithium heparin whole blood samples on a Radiometer ABL90 to correlate H index and potassium or ionized calcium.
Results: During period one 35 of 5808 (0.6%) blood gas samples had visual hemolysis levels exceeding tolerance for reporting of potassium. By switching to measured H index using a laboratory-established threshold, during period 2 we estimate that 171 of 5396 (3.2%) blood gas samples exceeded the H index threshold for reporting of potassium. In 75 intraoperative blood gas samples with H index and whole blood potassium and ionized calcium measured; we observed no relationship between H index and potassium or ionized calcium. During period 3 we switched to visual assessment of hemolysis with a greater tolerance for hemolysis; with only 3 of 5345 (0.06%) samples exceeding the new visual hemolysis threshold.
Conclusion: For blood gas samples collected intraoperatively, there is no relationship between hemolysis and measured potassium or ionized calcium. The results suggest that only grossly hemolyzed intraoperative blood gas samples should be rejected for measurement of whole blood potassium and ionized calcium.

1. Introduction

Hemolysis is the most common cause for blood sample rejection in the chemistry laboratory, accounting for 40%–70% of all unsuitable samples. Hemolysis can occur during blood sample collection, transport, or processing; and minimization of hemolysis has been an ongoing goal in clinical chemistry. However, in approximately 2% of cases, hemolysis is found to have occurred *in vivo* [1–3]. In such cases, efforts to modify pre-analytical variables have negligible impact on free hemoglobin content of samples.

Many automated chemistry analyzers offer free hemoglobin detection, approximated by optical hemolysis (H) index, to assess sample acceptability [1,4]. In our practice plasma or serum samples with an H index above laboratory-defined thresholds are rejected for analysis, and most often successfully recollected. During cardiovascular surgery, however, levels of free hemoglobin will increase beyond the amounts seen in blood samples collected before or after surgery. Values peak between 15 and 120 min following termination of the cardiopulmonary bypass circuit, with average peak free hemoglobin values of 60–90 mg/dL [5–8].

We use visual assessment of hemolysis to determine whether potassium or ionized calcium, measured in lithium heparin whole blood during blood gas analysis, is acceptable for reporting. After blood gas measurement, samples are centrifuged and visually assessed for hemolysis using a chart provided in the standard operating procedure.

In this study we measured the H index among visually hemolyzed blood gas samples collected intraoperatively to determine free hemoglobin levels in these samples, and determined whether H index was related to potassium or ionized calcium concentration for intraoperative blood gas samples. The results were used to determine appropriate sample acceptability criteria for intraoperative blood gas samples prior to releasing potassium (K) or ionized calcium (iCAL)

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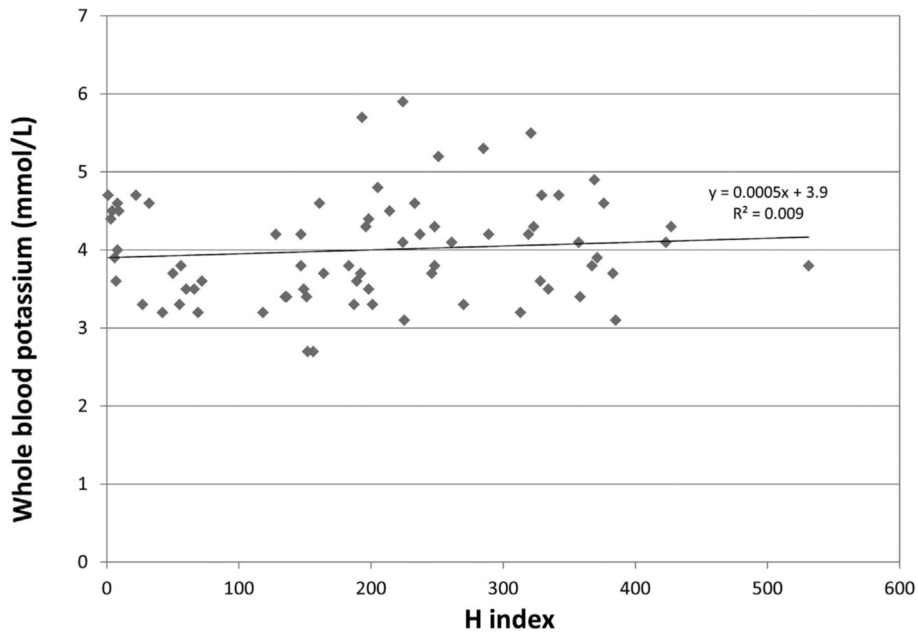

Fig. 1. Whole blood potassium *versus* hemolysis (H) index for 75 lithium heparin blood gas samples collected intraoperatively. Slope, intercept and correlation coefficient for the relationship between H index and potassium is included.

results.

2. Methods

Electronic Medical Records and Laboratory Information Systems were accessed during three periods to assess number/% of intraoperative blood gas samples that had hemolysis comments added. The majority of intraoperative blood gas samples were collected during cardiovascular surgery. Blood gas samples were collected by OR staff using 3 mL Smith Portex blood gas syringes containing 23.5 IU/mL electrolyte-balanced lithium heparin. During period 1 (August–September 2017), following whole blood analysis, blood gas samples with requests for electrolytes (K or iCAL) were centrifuged for 15 s at 5000 rpm in an Eppendorff Mini-Spin for visual assessment of hemolysis; using a chart demonstrating the appearance of samples with free hemoglobin concentrations (roughly equivalent to H indices) of 50, 100, 250, 500 and 1000 mg/dL. Samples with no visual free hemoglobin were released without a comment. Samples with free hemoglobin present below 250 mg/dL had a comment added to the result indicating mild hemolysis. Samples with gross hemolysis assessed visually (greater than 250 mg/dL by the chart) were either recollected or electrolytes were not reported.

During period 2 (December 2017–March 2018), blood gas samples with orders for electrolytes continued to be assessed for visual hemolysis after centrifugation. Samples with visual hemolysis (any level) were sent to a stat laboratory for measurement of H index on a Roche Cobas 501 analyzer. Samples with H index above the laboratory-defined threshold for potassium (H index of 125, approximately 125 mg/dL free hemoglobin) or ionized calcium (H index 250), were either recollected or electrolytes were not reported. During period 3 (May–August 2018), blood gas samples with requests for electrolytes were again visually assessed for hemolysis. Samples with no hemolysis were released without a comment. Samples with visual hemolysis below 500 mg/dL were released with a mild hemolysis comment. Samples with greater than 500 mg/dL hemolysis (compared to visual chart) were either recollected or electrolyte results were not released.

We also measured H index, K and iCAL on 55 blood gas samples collected from 22 patients intraoperatively with visual hemolysis noted after centrifugation; and an additional 20 samples collected from 11 patients that did not have visual hemolysis. There were 1–5 samples analyzed from each patient, and 26 of 33 patients were undergoing cardiovascular surgery. Potassium and ionized calcium were measured on a Radiometer ABL90 blood gas analyzer using whole blood samples (before centrifugation). H index was measured on centrifuged plasma using a Roche Cobas c501 analyzer. Regression analysis was performed to determine the relationship between H index and K or iCAL concentration for blood gas samples collected intraoperatively. Median and interquartile range (IQR) H index values for visually hemolyzed and non-hemolyzed samples were calculated to determine the distribution of H indices in samples collected in the operating room (OR). GraphPad InStat version 3 was used to perform linear regression and determine whether the slope of the line between H index and K or iCAL differed significantly from zero. The study was performed as a quality improvement project and deemed exempt from Mayo Clinic Institutional Review Board Approval.

3. Results

During period 1, when visual assessment was performed by centrifuging blood gas samples to look for free hemoglobin, 154 of 5808 (2.7%) samples were commented as mildly hemolyzed (hemoglobin below H index of 250) to the extent that potassium could be falsely elevated or ionized calcium falsely decreased. 35 of 5808 (0.6%) samples had a gross hemolysis (H index over 250) comment that should have resulted in sample recollection or cancellation of electrolyte results. In total 189 of 5808 (3.3%) of OR blood gas samples (with orders for electrolytes) had visual hemolysis as assessed by testing personnel.

H index, potassium, and ionized calcium were measured for 55 samples collected in OR with visual hemolysis (any level) as assessed by testing personnel, and an additional 20 samples that did not have visual hemolysis. Median (IQR) H index among the visually hemolyzed OR samples was 237 (188–329), compared to 25 (7–55) for samples not visually hemolyzed. 53 of 55 (96%) of visually hemolyzed OR samples had H index above 125, the threshold for acceptability for releasing potassium results on serum or plasma samples. During this time period, all of these samples with H index over 125 (and potassium requested) should have been either recollected or had potassium results cancelled. Regression analysis using all 75 samples showed no relationship

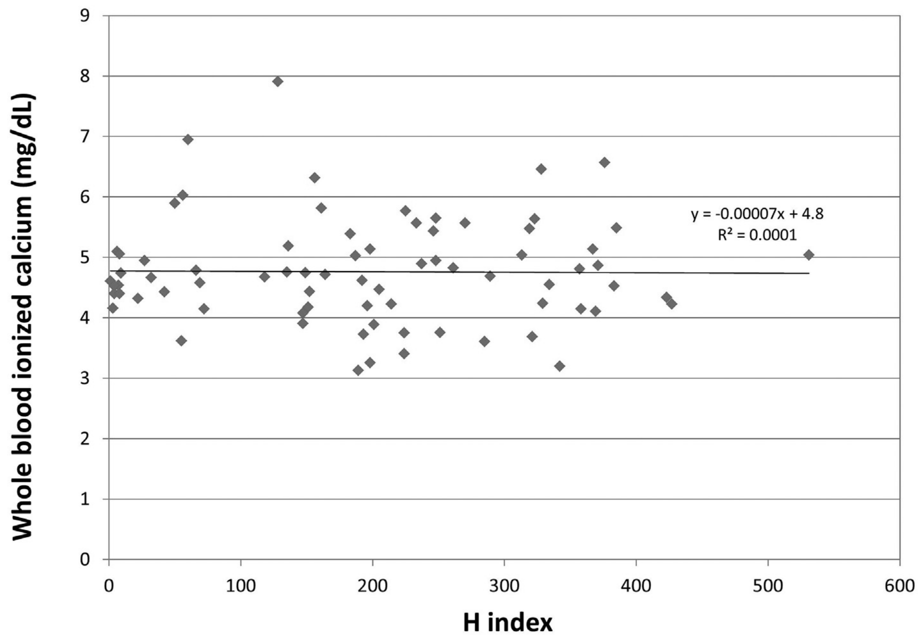

Fig. 2. Whole blood ionized calcium *versus* hemolysis (H) index for 75 lithium heparin blood gas samples collected intraoperatively. Slope, intercept and correlation coefficient for the relationship between H index and ionized calcium is included.

between H index and potassium ($r^2 = 0.009$) for these samples, with the slope of the line between H index and K not differing significantly from zero ($p = .41$) (Fig. 1). Regression analysis also demonstrated no relationship between H index and ionized calcium ($r^2 = 0.0001$), with the slope of the line between H index and iCAL not differing significantly from zero ($p = .93$) (Fig. 2).

There were 5396 blood gas with electrolyte requests from the OR during period 2. Assuming that (similar to period 1) 3.3% of OR samples were visually hemolyzed, and 96% of visually hemolyzed samples had H indices above 125, we estimate that 171 (3.2%) OR blood gas samples would have been recollected or had K results suppressed during period 2.

During period 3 we reverted to the previous system of visual assessment for gross hemolysis. Only samples with visual assessment of 500 mg/dL or more free hemoglobin were recollected or had electrolyte values suppressed. 44 of 5345 (0.8%) samples were released with a comment indicating mild hemolysis, while only 3 of 5345 (0.06%) had a gross hemolysis comment which would have resulted in recollection or suppression of electrolyte results.

4. Discussion

Hemolysis is the most common pre-analytical error encountered in clinical chemistry [1,3]. For lithium heparin plasma samples analyzed in a central laboratory setting, average K increases linearly as a function of free hemoglobin [9]. For this reason many laboratories use automated H indices to determine sample acceptability before releasing plasma K results [3]. In contrast for lithium heparin blood gas samples collected intraoperatively, there is no relationship between free hemoglobin level and potassium or ionized calcium concentration. This is likely due to increased free hemoglobin levels observed during cardiovascular surgery, from effects of the bypass circuit and use of cell salvage devices. These devices lyse cells and result in increased free hemoglobin levels, but under these circumstances free hemoglobin level is not related to potassium or ionized calcium concentration. The type and amounts of fluids given during surgery may also contribute to the lack of correlation between H index and K or iCAL.

We speculate that during washing of reclaimed blood in the cell salvage system, K and free hemoglobin are returned to the patient in concentrations that differ from relative concentrations observed during either intravascular or *in vitro* hemolysis; confounding the use of free hemoglobin as a marker of sample acceptability. Previous studies have found that the average peak free hemoglobin during cardiovascular surgery with use of cell salvage is 60–90 mg/dL; much higher than average free hemoglobin levels in serum samples collected by venipuncture in our practice (data not shown). One limitation of our study is that we did not identify what percent of hemolyzed samples came from patients during use of a cell salvage device. Another limitation is that we had few samples with H index between 400 and 500. Given the increased free hemoglobin in blood gas samples during cardiovascular bypass, it is not surprising that more intraoperative blood gas samples may have H indices higher than thresholds developed for serum or plasma electrolyte measurement. Thus our data suggest rejecting only grossly hemolyzed samples collected intraoperatively during cardiovascular surgery, in order to avoid frequent sample recollection and delays in analysis.

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