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How Well Do Laboratories Follow Guidelines on Cardiac Markers? The Cardiac Marker Guideline Uptake in Europe Study

To the Editor:

Evidence-based medicine is recognized as underlying good clinical practice. Similarly, evidence-based laboratory medicine should be used to guide and monitor laboratory testing. One area where laboratory testing has undergone a revolutionary change is in the diagnosis of acute coronary syndrome (ACS)[9], as underscored in the American College of Cardiology (ACC) and the European Society of Cardiology (ESC) consensus document on the definition of and diagnostic approach of myocardial infarction in 2000, updated in 2007. Since the original reports, the measurement of cTnT and cTnI has been recommended in many guidelines, *(1, 2)* and they have formed a part of the redefinition of myocardial infarction *(3)*.

The CARdiac MArker Guideline Uptake in Europe study (CARMAGUE) was a pilot study to provide baseline data and validate a web-based audit tool to assess compliance of cardiac marker testing in current clinical practice. The study was sponsored by the European Federation of Clinical Chemistry and Laboratory Medicine (EFCC) in conjunction with the national societies in 8 participating countries (Austria, Croatia, Denmark, Finland, Germany, Hungary, the Netherlands, and the U.K.). Laboratories were invited to participate by emailing a weblink to an electronic questionnaire. The questionnaire developed by the CARMAGUE study group comprised 123 questions covering all aspects of cardiac marker testing, concentrating on choice of markers and clinical decision making. The questionnaire can be viewed at www.carmague.fi/1. In a first analysis, we focused on the application of cardiac markers of myocardial necrosis used in ACS.

Responses were received from 220 laboratories (22% of those invited), with response rates varying from 12% to 80% of potential participants in each country.

The majority of the hospitals (94%) used a cardiac troponin (cTn), either cTnT (51%) or cTnI (49%), as their preferred test for diagnosis of acute myocardial infarction. All the other participants used creatine kinase MB isoenzyme (CK-MB) but were planning to change. Although troponin was the preferred marker, it was used in combination with a second marker in 34% of the laboratories, usually CK, CK-MB mass, or CK-MB activity, whereas only 2 laboratories used myoglobin. Of the 58.9% of laboratories with a written protocol, 90% used the same protocol in the emergency department and for inpatients. The protocols were developed jointly with clinicians in 80% of cases.

Turnaround time was reported as $<$60 min in most cases of urgent tests. Seven percent of laboratories did not participate in external quality assessment for cTnI and 11% for cTnT. The clinical decision limits chosen by the laboratories were split between 10% CV (39%), 99th centile (35%), and others. Information was obtained from assay package by more than half (52%), with only 4% of laboratories independently assessing manufacturers' claims. Peer-reviewed literature was cited by 16%. Actual guidelines were directly cited as the source by only 9%.

The study shows that guidelines are used less consistently than expected. Although cTnT and cTnI are the preferred markers, as recommended in all of the guidelines, they are combined with other markers. A defined protocol for cardiac biomarkers was one of the earliest recommendations. The absence of such a protocol in a large number of laboratories is a cause for concern, as is the lack of clinician involvement in protocol development in 20% of cases. Thus there is a clear need to improve the dialog between clinicians and laboratory.

Considering laboratory aspects, reported turnaround time was good and in accordance with recommendations. However, this may not be truly representative, as the questionnaire requested analytical turnaround time and not time from sample draw to the result being acted on. This will be addressed in the next audit. A serious concern is the fact that external quality assessment was not 100%. The reason for the selection of 10% CV or 99th centile was arbitrary, as it was seen for both troponin T and troponin I. The study also indicated that it was mainly the information given by the manufacturer that guided the selection of decision limits, not any in-house verification or guidelines.

In conclusion, the CARMAGUE study demonstrates the rapid transformation to using troponin measurement as standard. It also reveals some challenges, such as lack of dialog between the laboratory and clinicians and failure of laboratories to participate in external quality assurance schemes. A more extensive survey is required to confirm and extend these findings. A policy of encouragement and education, combined with further audit, should help to encourage more evidence-

[9] Nonstandard abbreviations: ACS, acute coronary syndrome; CARMAGUE, Cardiac Marker Guideline Uptake in Europe study; cTn, cardiac troponin; CK, creatine kinase; CK-MB, creatine kinase MB isoenzyme.

based practice and implementation of guidelines *(4, 5)*.

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Reverse Pseudohyperkalemia in a Leukemic Patient

To the Editor:

Hyperkalemia is a potentially fatal electrolyte abnormality that must be differentiated from pseudohyperkalemia, which can occur when potassium is released from ruptured platelets or blood cells during the clotting process in serum specimens *(1, 2)*. We describe a case of "reverse" pseudohyperkalemia in a patient with chronic lymphocytic leukemia (CLL) in whom potassium concentrations in plasma specimens exceeded concentrations observed in serum by more than 1.3 mmol/L *(3)*.

A 49-year-old woman with stage IV CLL was admitted for chemotherapy. Her white cell count (WBC) was 364×10^9 cells/L (96% lymphocytes) and 100×10^9 platelets/L. The patient was treated with rituximab, cyclophosphamide, and fludarabine and received bicarbonate and allopurinol. After therapy, the potassium concentration obtained from a lithium-heparin specimen with separator gel was 10.7 mmol/L on a Beckman LX-20 chemistry analyzer (Beckman Coulter). A second lithium-heparin plasma specimen yielded a potassium concentration of 11.2 mmol/L. Results were similar on a different analyzer. Specimens were transported by pneumatic tube, and the time from phlebotomy to analysis was approximately 60 min. No hemolysis was observed. All specimens were obtained using 5-mL Griener Vacuette tubes (GrienerBio-One) unless otherwise noted.

The patient exhibited no acidosis, renal failure, or tumor lysis syndrome; phosphate, calcium, and uric acid were not increased. Calcium chloride, albuterol, dextrose-insulin, furosemide, and sodium polystyrene sulfonate were administered to decrease the patient's potassium concentrations. Emergent dialysis was considered as a treatment option. The patient denied symptoms attributable to hyperkalemia (fatigue, muscle weakness, or palpitations), and an electrocardiogram revealed no abnormalities associated with hyperkalemia.

The possibility of pseudohyperkalemia was investigated. Additional venous blood specimens were simultaneously obtained 2 h after potassium-lowering treatment (Table 1). These specimens were lithium-heparin tubes lacking (specimen A) or containing (specimen B) a separator gel; a heparinized blood gas syringe (Portex® Line Draw Plus blood sampling kit; Smiths Medical) (specimen C) for immediate potassium measurement in whole blood (Rapid Laboratory 1200 series, Siemens); and a red-top tube without anticoagulant (specimen D). All specimens were immediately transported to the laboratory via pneumatic tube (<5 min transport time), promptly centrifuged (7 min at 1915*g*, samples A, B, and D), and analyzed within 5 min of centrifugation.

Both the whole blood (C) and serum (D) specimens indicated severe hypokalemia, and the plasma specimens (A and B) exhibited significantly higher potassium concentrations (Table 1). Examination of

Table 1. Measured potassium concentrations obtained in different specimens drawn simultaneously.[a]

Specimen	Potassium concentration, mmol/L	Specimen type	Analyzer (method)
A	4.9	Plasma lithium-heparin without separator gel	Beckman LX-20 (indirect ion selective electrode)
B	4.0	Plasma lithium-heparin with separator gel	Beckman LX-20 (indirect ion selective electrode)
C	2.6	Whole blood lithium-heparin specimen	Rapid Lab 1200 (direct ion selective electrode)
D	2.7	Serum	Beckman LX-20 (indirect ion selective electrode)

[a] All specimens were kept at ambient temperature during transport, centrifugation, and analysis. The laboratory reference interval for adult plasma potassium concentration is 3.5–5.0 mmol/L.

specimens A and B revealed a distinct buffy coat layer of packed WBCs on top of the erythrocyte layer. In specimen B, the buffy layer appeared to span the separator gel. No buffy layer was apparent in specimen D. To eliminate the possibility of potassium from lysis of residual platelets and WBCs present in the serum or plasma supernatant, cellular counts were performed using a Sysmex SE-2100 blood analyzer. Few remaining leukocytes or platelets were observed in the supernatant of specimens A, B, and D after centrifugation ($0.02–0.07 \times 10^9$ WBC/L and $<0.01–16 \times 10^9$ platelets/L).

We hypothesized that potassium might be diffusing out of the packed buffy coat layer into the plasma supernatant. Because the serum and whole blood potassium values correlated with the clinical assessment of the patient, no dialysis was performed, and potassium supplements were administered. Specimens submitted 3 days later (WBC of 264×10^9 cells/L) also exhibited a similar reverse pseudohyperkalemic pattern.

Because of the potential of pseudohyperkalemia in serum, plasma has been recommended as the preferred sample type for determinations of potassium in patients with leukocytosis, thrombosis, or erythrocytosis *(1, 4)*. In this patient, the observed plasma potassium concentrations appeared to be falsely increased, and the use of serum or whole blood specimens provided results that were consistent with clinical findings. Previously observed relative increases of potassium in plasma specimens have been attributed to preanalytical factors including centrifugation delays or heparin-mediated cell membrane damage *(3, 5)*.

Susceptibility to heparin-mediated cell membrane damage during processing and centrifugation may have contributed to diffusion of potassium into the supernatant from ruptured leukocytes. The presence of gel separator material may have reduced the leeching of potassium from the buffy layer (specimen B). Heparinized whole blood (specimen C) was used in blood gas analysis, but testing was performed immediately after the blood was drawn.

We conclude that the potential for reverse pseudohyperkalemia exists in plasma specimens in the setting of leukocytosis. Further investigation of the frequency of this effect in different specimen containers may be warranted. Clinicians should be aware of this phenomenon to avoid inappropriate therapeutic measures.

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Measurement of Creatinine in Whole Blood Samples Supplemented to Achieve Increased Creatinine Concentrations

To the Editor:

During clinical trials of a new biosensor for measurement of whole blood creatinine, we were unable to obtain sufficient quantities of samples with naturally occurring high creatinine concentrations to validate performance of the sensor across the proposed reportable concentration range for the device [18–1326 μmol/L (0.2–15.0 mg/dL)]. Addition of creatinine to whole blood samples was required to validate performance of the sensor at the high end of the range. We noticed irreproducible results for samples that were supplemented with creatinine to increase the concentration. Like other direct-reading electrochemical biosensors *(1)*, creatinine sensors respond to the *molality* of creatinine in the sample (amount of creatinine per unit mass of water). It is known that molality of creatinine in erythrocyte fluid is equal to molality in plasma *(2)* and that creatinine is transported by passive diffusion through the lipid bilayer of the erythrocyte membrane *(3)*.

Heparinized blood from a healthy volunteer was centrifuged and the separated plasma supplemented with creatinine to target concentrations of 442 and 1061 μmol/L (5 and 12 mg/dL). We measured the creatinine concentrations in the plasma samples with and without added creatinine by use of the Vitros DT 60 II analyzer (Ortho Clinical Diagnostics). Red blood cells were added back to the supplemented plasma to obtain hematocrit values approximating 20%, 40%, and 60%. Actual hematocrit values by microcentrifugation are shown in Fig. 1. These samples were stored at room temperature with mixing. After 10 min, 1 h, 5 h, and 24 h, plasma was separated from the supplemented blood samples, and we measured creatinine concentrations in the plasma and in the original supplemented plasma stored for an equivalent time at room temperature without red cells; we also measured creatine concentration *(4)*. Measurement of creatinine, in duplicate, demonstrated pooled, within-run SDs of 6.8 μmol/L (0.08 mg/dL) and 13.5 μmol/L (0.15 mg/dL) at 442 and 1061 μmol/L, respectively. Measurement of creatine, in triplicate, demonstrated a pooled within-run SD of 1.5 μmol/L (0.02 mg/dL).

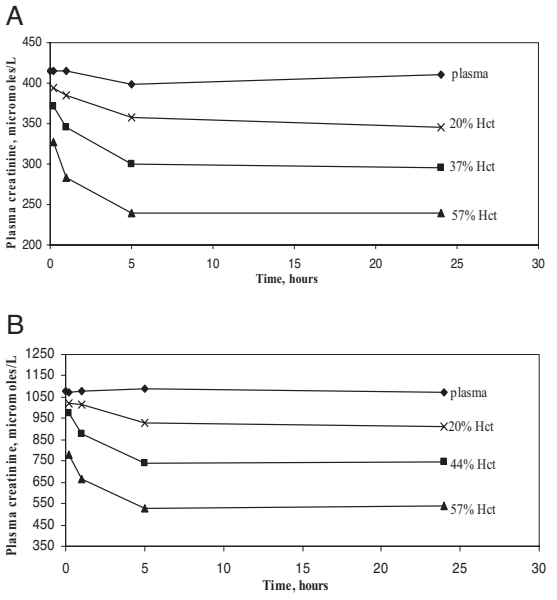

Fig. 1. Change in concentration of creatinine in plasma at various levels of hematocrit (Hct) and at different creatinine concentrations spiked into plasma: 442 μmol/L (A) and 1061 μmol/L (B).

Each point is the mean of duplicate measurements with pooled, within-run SDs of 6.8 μmol/L (0.08 mg/dL) and 13.5 μmol/L (0.15 mg/dL) at the 442 and 1061 μmol/L levels, respectively.

Fig. 1 shows the change in plasma creatinine as a function of time the plasma was in contact with red blood cells, at various hematocrits. Supplemented plasma samples with target creatinine concentrations of 442 and 1061 μmol/L (5 and 12 mg/dL) are shown in Figs. 1A and B, respectively. The plasma creatinine concentration decreased for up to 5 h after initial contact with red blood cells, after which no further decrease was seen. During this time the creatinine concentration of the creatinine-supplemented plasma samples to which no red blood cells were added was unchanged. The mean (SD) plasma creatine concentrations of the samples at the various hematocrits also remained unchanged [43.7 (1.4) μmol/L,

0.57 (0.02) mg/dL], indicating that conversion to creatine was not responsible for the decrease in creatinine. The decrease in plasma creatinine was greater at higher hematocrits, at both creatinine concentrations [442 and 1061 μmol/L (5 mg/dL and 12 mg/dL)]. At 5 h, the decrease in plasma creatinine concentration resulting from contact with red blood cells was statistically significant (95% confidence) at both concentrations and at all hematocrits tested.

It is possible to estimate the whole blood creatinine concentration at equilibrium, with knowledge of the water concentrations of plasma and red blood cells, hematocrit, the initial plasma creatinine concentration, and the plasma creatinine concentration after addition of creatinine. Assuming normal mass concentrations of water equal to 0.93 kg/L for plasma and 0.71 kg/L for red cell fluid, respectively *(5)*,

$$\mathrm{Creat_{est}} = \left[\left(\frac{0.71 * \mathrm{Hct}}{(0.71 * \mathrm{Hct}) + 0.93 * (1 - \mathrm{Hct})}\right)\mathrm{Creat_0}\right] + \left[\left(\frac{0.93 * (1 - \mathrm{Hct})}{(0.71 * \mathrm{Hct}) + 0.93 * (1 - \mathrm{Hct})}\right)\mathrm{Creat_1}\right] \quad (1)$$

where $\mathrm{Creat_{est}}$ is expected whole blood creatinine concentration after addition of creatinine, $\mathrm{Creat_0}$ is initial plasma creatinine concentration, and $\mathrm{Creat_1}$ is plasma creatinine concentration after addition of creatinine. In all cases, the expected concentrations are within 10% of the measured concentrations 5 h after spiking.

The concentration of creatinine in plasma of blood samples that are supplemented with creatinine will decrease for up to 5 h at room temperature, as molalities of creatinine in plasma and red blood cell fluid approach equality. Measurement at 3 h after addition of creatinine indicated that the process was not yet at equilibrium (data not shown).

If addition of creatinine to samples is to be used to validate performance of a device for measurement of creatinine in whole blood, sufficient time must be allowed after the addition to avoid misleading results. Samples diluted to obtain creatinine concentrations below the reference interval may show a similar effect. In our study, sufficient numbers of naturally occurring samples in the interval 27–70 μmol/L (0.3–0.8 mg/dL) were obtained to avoid the need for sample dilutions.

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