Large Variation in Measured Cardiac Troponin T Concentrations after Standard Addition in Serum or Plasma of Different Individuals

Table 1. Tiered test features and ordering process in laboratory formulary.

	Tier 1	Tier 2	Tier 3 (nonformulary)
Type	Common	Specialized	Uniquely subspecialized/rare
Clinical utility	Well-proven clinical utility	Limited or narrow clinical utility	Unclear, controversial, poorly proven, or very limited clinical utility
Requirements for provider access/ordering	No restrictions; available to all providers	Subspecialty faculty appointment or privileges at URMC and affiliated hospitals	Subspecialty faculty appointment or privileges at URMC and affiliated hospitals and LDC[a] approval
Percent of test ordering volumes	87%	11%	2%
Examples	1. Most in-house testing	1. 1,25-Dihydroxy Vitamin D	1. Inflammatory bowel disease panel[b]
	2. Cystic fibrosis 32 mutations	2. Fecal pancreatic elastase	2. Pancreatitis panel[b]
	3. TSH receptor antibody	3. Disaccharidase analysis	3. α_1-Antitrypsin genotype
	4. Serum-free cortisol	4. Insulin antibodies	4. Hepatitis C RIBA
	5. Zinc	5. BMT Panel[b]	5. β-Thalassemia gene
			6. Biotinidase

[a] LDC, Laboratory Diagnostic Committee; TSH, thyroid-stimulating hormone; RIBA, recombinant immunoblot assay; BMT, bone marrow transplant.
[b] Panel test components vary by institution. The inflammatory bowel disease panel includes *Saccharomyces cerevisiae*, immunoglobulin A (IgA) and IgG antibodies, and neutrophil-specific antibodies. The pancreatitis panel includes next generation sequencing of *PRSS1*, *SPINK1*, *CTRC*, and *CFTR*. The BMT Panel includes hepatitis B surface antigen with reflex to confirmation; Hepatitis B core antibody; HIV 1/2/0 antibody; human T-lymphotropic virus (HTLV) 1/2 antibody with reflex to immunoblot; CMV total antibody; Epstein-Barr virus (EBV) IgG; varicella zoster virus (VZV) IgG; herpes simplex virus (HSV) 1/2 antibody; syphilis IgG; HIV/HBV/HCV NAT (HIV/hepatitis B virus/HCV nucleic acid amplification testing); hepatitis C antibody and Chagas antibody (Ab) IgG.

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Large Variation in Measured Cardiac Troponin T Concentrations after Standard Addition in Serum or Plasma of Different Individuals

To the Editor:

During acute myocardial infarction (AMI),[1] cardiac troponin T (cTnT) is released from the damaged myo-

[1] Nonstandard abbreviations: AMI, acute myocardial infarction; cTnT, cardiac troponin T; CK-MB, creatine-kinase MB isoenzyme; hs-cTnT, high-sensitivity cTnT; LoD, limit of detection; CV_g, intersubject CV.

cardium. One would expect a strong correlation between concentrations of cTnT measured in the blood after AMI and the extent of myocardial damage. However, various studies have shown a rather moderate correlation between infarct size and cTnT concentrations *(1, 2)*. Frequently considered explanations for this phenomenon are methodological or focused on physiological factors associated with differences in release and elimination of cTnT *(2)*. In contrast, less attention has been paid to the possible presence of inherent factors in the blood, such as (auto)-antibodies or proteases, that might modify cTnT or interfere with the assay. We hypothesized that these factors may be ubiquitously present in the general population and might affect the measured concentrations of cTnT in individuals.

We measured cTnT, cTnI, creatine kinase MB isoenzyme (CK-MB), and myoglobin concentrations in sera from 24 healthy volunteers (age range 22–54 years) before and after the addition of serum from a patient suffering from AMI (age 59 years, cTnT concentration approximately 19.5 μg/L). All participants gave written informed consent and leftover material was used in accordance with the code of proper secondary use of human tissue in the Netherlands. Spiking of AMI-patient serum (40 μL in 760 μL of volunteer serum) was performed in triplicate. cTnT [high-sensitivity (hs)-cTnT: limit of detection (LoD) 5 ng/L; 10% CV at 13 ng/L], CK-MB (LoD of 0.3 μg/L, 20% CV at 1 μg/L), and myoglobin (LoD 21 μg/L) were determined on the COBAS (Roche Diagnostics), and cTnI (hs-cTnI: LoD 1.1–1.9 ng/L, 10% CV at 4.7 ng/L) on the Architect (Abbott Diagnostics) (information based on product inserts). Biomarker concentrations were corrected for their baseline concentrations, and outliers (Grubbs test) were excluded. To make the results of different biomarkers easily comparable, the subject-specific deviation was calculated. The subject-specific deviation was defined as the percentage of deviation of a measured biomarker concentration in an individual subject compared to the mean concentration of that biomarker across all samples after spiking of a fixed amount of material. The subject-specific deviation of cTnT after spiking varied between −54% and +22% (Fig. 1). Next, the intersubject CV (CV_g) was calculated for cTnT (14.6%), which was significantly higher than the CV_g for cTnI (6.9%), CK-MB (2.6%), and myoglobin (3.0%) (all $P < 0.001$) measured in the same samples. In contrast, no significant difference was shown in the CV between the triplicate measurements of the different cardiac biomarkers (± 1.5%, not shown). Interestingly, when this experiment was repeated in serum from the same individuals that was deproteinated by perchloric acid and neutralized to pH 7.4, the CV_g for cTnT concentration significantly decreased from 14.6% to 6.5% ($P < 0.001$). These findings were confirmed in an independent duplicate experiment using purified human cTnT (HyTest LTD) instead of AMI-patient serum.

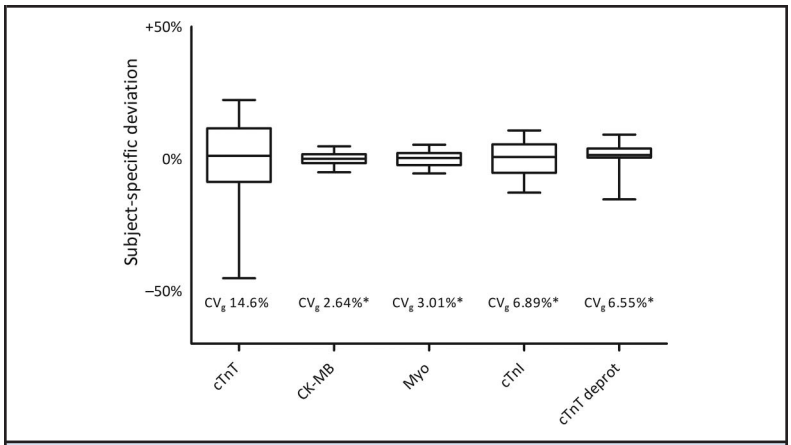

Fig. 1. Subject-specific deviation (%) of cardiac biomarkers added to serum samples of healthy individuals.

Box plots showing quartiles and range (minimum–maximum) of the subject-specific deviations for cardiac biomarkers in 24 healthy volunteers (mean of triplicates). We detected 2 outliers (1 for cTnT in deproteinated serum and 1 for CK-MB) within triplicate measurements and excluded these single measurements. * Significant ($P < 0.001$) difference in the intersubject CV of a biomarker compared to that of cTnT in unprocessed serum samples. Myo, myoglobin; deprot, deproteinated serum.

The wide intersubject variation in cTnT concentrations observed in these first experiments was validated in a consecutive experiment in which 2.5 μL of purified human cTnT (HyTest) was spiked into left-over heparin plasma samples (250 μL) from 119 patients. Again, concentrations were corrected for baseline values. In this population, we observed a subject-specific deviation between −39% and +37% that was normally distributed (D'Agostino-Pearson omnibus test, $P = 0.210$). The CV_g of 15.8% in this experiment was comparable to that in our previous experiment.

Thus, we showed a substantial and specific variation in cTnT concentrations after the standard addition of equal amounts of cTnT to both serum and plasma of several individuals. This intersubject variation decreased significantly when se-

rum was deproteinated. Currently, we can only speculate about the nature of the observed variations. Since the effect diminished after the removal of proteins from serum, a protein factor could be responsible. Individual differences in proteolytic degradation of cTnT cannot be excluded as the responsible factor but this seems to be unlikely since the cTnT present in the serum sample used for spiking was already fragmented and the observed pattern remained similar when purified human cTnT was used *(3)*. Also, the involvement of heterophilic antibodies, like human antimouse antibodies and rheumatoid factor, is unlikely since the variation is ubiquitously present. A remaining possible explanation could be the blocking of epitopes or alternative folding of cTnT due to the interaction with specific (auto)antibodies *(4)*. More research is needed to identify the exact factor(s) responsible for the observed variation, including their mechanism of action, to ascertain whether or not these differences also occur with nonforeign cTnT in vivo, and to determine the exact clinical implications including the determination of infarct size.

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Pseudohypocalcemia in Cancer Patients: A Recommendation for the Postanalytical Correction of Serum Calcium in Patients with Hypoalbuminemia

To the Editor:

Critical value reporting is a regulatory responsibility of all clinical laboratories. Efforts to reduce the number of calls that are not truly critical could have a significant impact on the clinical laboratory, medical team work flow, and patient care. At Memorial Sloan Kettering Cancer Center (MSKCC),[1] the clinical laboratory makes approximately 30 000 critical value calls a year with only 9 analytes accounting for nearly 75% of these calls. Calcium is one frequently called analyte, responsible for approximately 1000 critical calls a year.

We evaluated the findings of critical hypocalcemia [critical value: <6.5 mg/dL (<1.62 mmol/L); reference interval (RI): 8.5–10.5 mg/dL (2.12–2.62 mmol/L)] in cancer patients with albumin concentrations <4.0 g/dL (RI: 4.0–5.2 g/dL). At MSKCC between 2013 and 2015, 38% of albumin results from a comprehensive metabolic panel (CMP) were below 4.0 g/dL. There are several published formulae to correct serum calcium in patients with hypoalbuminemia. The Payne formula {corrected total calcium (mg/dL) = total calcium (mg/dL) + 0.8 × [4.0 − albumin (g/dL)]} is the most widely used and based on the observation that each 1-g/dL reduction in albumin <4.0 g/dL results in an approximately 0.8 mg/dL (0.2 mmol/L) reduction in total calcium *(1, 2)*.

[1] Nonstandard abbreviations: MSKCC, Memorial Sloan Kettering Cancer Center; RI, reference interval; CMP comprehensive metabolic panel.