Overestimation of Serum κ Free Light Chain Concentration by Immunonephelometry

To the Editor:

Measurement of serum free light chains (sFLCs)\(^1\) has become an established method in the diagnosis and monitoring of monoclonal gammopathies (1, 2). Accurate quantification of sFLCs is important because it is one of the prognostic indicators at disease presentation. Moreover, the International Myeloma Working Group has recently incorporated concentrations of sFLCs in the response criteria of multiple myeloma and related monoclonal gammopathies (1).

sFLC quantification with nephelometric and turbidimetric immunnoassays is subject to substantial over- or underestimation (3). A strong nephelometric overrating of sFLC concentrations was reported in a patient with a λ light chain M-protein concentration of 23 g/L determined by serum protein electrophoresis (SPE). The λ-sFLC concentration quantified by nephelometry was 344 g/L and was overestimated more than 10-fold owing to the reaction of trimolecular aggregates of the monoclonal λ-sFLCs with the antibody (4). In our institute, a similar phenomenon was observed in a patient with a κ light chain M-protein concentration of 3.1 g/L by SPE and 32.2 g/L by nephelometry. To further study this discordance in κ-sFLC concentrations, we compared 2 methods, nephelometry and ELISA. When possible, κ-sFLC concentrations were also quantified by SPE.

We carried out the nephelometric technique on a BNII analyzer (Siemens) using Freelite\(^\circ\) reagents (The Binding Site) according to the manufacturer’s instructions. For the ELISA technique, we modified a reported assay (5). Briefly, we added calibrator (pooled urine samples containing polyclonal FLCs) and diluted serum samples to microtiter plates coated with polyclonal free κ antibody (A0100, Dako). Bound free κ was detected using polyclonal peroxidase-labeled total κ antibody (P0129, Dako). The dose response of the calibrator reacted in parallel to that of the serum specimen. For this κ-sFLC assay-comparison study, we included samples of 49 individual patients, both at diagnosis or during follow-up, with monoclonal intact and/or free κ gammopathies.

κ/λ-sFLC ratios measured with nephelometry ranged from 0.8 to 77 250. An increased sFLC ratio was found in 45 of 49 patients. A large difference was observed for the κ-sFLC concentrations measured by the 2 methodologies, with a median κ-sFLC concentration measured by nephelometry of 0.41 g/L vs 0.051 g/L measured with ELISA. The method comparison of κ-sFLC concentrations measured by nephelometry and ELISA (Fig. 1) showed an exponential correlation: [ELISA] = 0.63 [nephelometry]\(^{0.76} (R^2 = 0.94).

Because of this exponential discordance, the discrepancy between the 2 assays most strongly affects samples with high κ-sFLC concentrations. Overall, we observed a qualitative disagreement between the 2 methods in 5 of 49 samples (Fig. 1, triangles). All 5 samples had a borderline increase in κ-sFLC concentrations when measured with nephelometry but were considered normal by ELISA. One of these 5 had a normal sFLC ratio, and 4 had increased sFLC ratios of 3.5, 7.8, 6.8, and 15.5, respectively.

In our cohort, 10 of 13 patients with nephelometric κ-sFLC concentrations >1 g/L were expected to be quantifiable by SPE because the FLCs formed monoclonal bands that migrated in the γ region. However, only 4 of these 10 samples (Fig. 1, circles) had a quantifiable monoclonal band by SPE, of 2.3, 2.9, 3.1, and 3.3 g/L. The κ-sFLC concentrations in these samples by nephelometry were 22.4, 20.4, 32.2, and 34.1 g/L, and with the ELISA, 1.5, 1.9, 3.1, and 2.6 g/L. The other 6 samples (Fig. 1, squares) were below the quantifiable detection limit of SPE (<1 g/L), although the nephelometric κ-sFLC concentrations were quantified between 1.7 and 7.9 g/L. The ELISA method did quantify the κ-sFLC concentrations in these 6 samples as <1 g/L. These 10 samples demonstrate that sera with high κ-sFLC concentrations cannot be accurately quantified using nephelometry. In contrast, κ-sFLC concentrations measured by ELISA do correspond with the concentrations quantified by the monoclonal band in SPE.

Overestimation of nephelometric measured intact monoclonal immunoglobulins could be explained by monoclonal immunoglobulin polymerization and by the differences in the immunoreactivity of monoclonal versus polyclonal immunoglobulins. To date, 1 report has described an overestimation of a sample with a high λ-sFLC concentration caused by λ-sFLC aggregates in the nephelometric assay (4). We speculate that the intrinsic capacity of monoclonal κ-sFLCs to polymerize directly influences nephelometric quantification. On the other hand, strong polymerization of FLCs could hide epitopes, which could theoretically lead to some underreading of the FLC concentrations in both the ELISA and the nephelometric assays. Future research is warranted to investigate the exact mechanisms that cause the consistent overestimation of κ-sFLC concentrations using nephelometry.

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\(^1\) Nonstandard abbreviations: sFLC, serum free light chain; SPE, serum protein electrophoresis.
Our data demonstrate that κ-sFLC concentrations can be measured more accurately by ELISA than by nephelometry. Our data further suggest that the κ-sFLC concentrations measured by nephelometry are constitutively and exponentially overestimated, explaining the large error in patients with high serum κ-sFLC concentrations.

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References


Corrie M. de Kat Angelino2
Reinier Raymakers3
Maria A. Teunesen2
Joannes F.M. Jacobs2,4,5
Ina S. Klasen2*

2 Department of Laboratory Medicine
Laboratory of Medical Immunology
4 Department of Tumor Immunology
and 5 Department of Medical Oncology
Radboud University
Nijmegen Medical Centre
Nijmegen, the Netherlands
3 Department of Hematology
University Medical Centre Utrecht
Utrecht, the Netherlands

*Address correspondence to this author at:
Radboud University Nijmegen Medical Centre
Department of Medical Immunology (469)
Geert Grooteplein 10
6525 GA Nijmegen
The Netherlands
Fax 31-24-3619415
E-mail I.klasen@abti.umcn.nl

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Circadian Secretion Pattern of Copeptin, the C-Terminal Vasopressin Precursor Fragment

To the Editor:

Copeptin, the C-terminal peptide of provasopressin, is stoichiometrically released with arginine vasopressin (AVP). In contrast to AVP, it is stable ex vivo (1) and reflects the AVP system, as shown in diabes insipidus or the syndrome of inappropriate antidiuretic hormone secretion (2). Copeptin is a reliable marker of severe stress, with increased concentrations found in cases of critical illness, sepsis, hemorrhagic shock, and stroke (2). The serum copeptin concentration is profoundly and immediately stimulated after myocardial infarction (3). The absence of such stimulation within the first hours after the onset of symptoms has recently been proposed as an important negative predictor for excluding the likelihood of infarction in patients with unspecific chest pain (3).

Any further use of the peptide as a marker critically depends on clear cutoffs between health and disease. To better characterize the diagnostic accuracy of copeptin, we studied its physiological pulsatile and circadian variation in healthy individuals and compared copeptin rhythms with those of cortisol in these patients.

Blood for copeptin analysis was sampled every 20 min for 24 h (0900 to 0900) in 7 healthy individuals (1 female, 6 males; age range, 18–37 years; mean body mass index, 22.6 kg/m²). Sera were separated and immediately frozen at –80 °C. The study was approved by the local research ethics committee.

Serum copeptin was measured with a chemiluminescence sandwich immunoassay [lower detection limit, 0.4 pmol/L; functional assay sensitivity at <20% interassay CV, <1 pmol/L; see (1)]. Cortisol was measured immunometrically (Bayer Immuno 1™ System, Bayer Corp.) according to manufacturer’s instructions. The maximal inter- and intraassay CVs were 6.5% at a copeptin concentration of 4.1 pmol/L and 7.9% at a cortisol concentration of 88 nmol/L (4).

We used a clustering algorithm to analyze attributes of the copeptin concentration profiles (fixed CV of 10%; \( \text{r statistic for an upstroke/downstroke} = 1; \) cluster size for test peak/nadir = 1) provided optimal peak detection (>/=90% sensitivity and positive predictive accuracy) (5).

Copeptin concentrations showed no consistent circadian rhythm.

![Fig. 1. Individual 24-h copeptin rhythms for 7 healthy individuals.](image-url)
Peaks and troughs of the individual rhythms varied widely over the 24-h period with no evidence of synchronization among individuals or a clear relationship with the light–dark cycle [mean (SD) copeptin concentration, 4.3 (1.5) pmol/L; 11.4 (3.6) pulses/24-h period; mean pulse height, 5.1 (1.8) pmol/L] (Fig. 1). Interestingly, the expected large increase in cortisol during the second half of the night in these individuals [see (4)] is not related to copeptin. Because AVP is known to acutely stimulate cortisol via corticotropin release in stress situations, our present findings argue against both a role of AVP in the circadian release of cortisol and an important physiological role of AVP in the generation of cortisol pulses under nonstress conditions.

The highest copeptin concentration measured was 13.1 pmol/L; the mean maximum concentration for all individuals was 7.8 (2.9) pmol/L. Therefore, the variation in circadian copeptin concentration in healthy individuals remained well within the described reference interval (1). The timing of blood sampling did not appear as critical as for the interpretation of cortisol results. Our data will help to better define reference values for the use of copeptin measurements in predicting stress conditions. Reference values may be particularly important when a low copeptin concentration is used as a very early negative predictor in stress situations such as myocardial infarction (3), in which the physiological variation in copeptin has little influence on the interpretation of the results.

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**References**


Ken H. Darzy
Kashinath C. Dixit
Stephen M. Shalet
Nils G. Morgenthaler
Georg Brabant*

*Address correspondence to this author at:
Department of Endocrinology
Christie Hospital NHSFT
Manchester, UK

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**Evaluation of the Quo-Test Hemoglobin A1c Point-of-Care Instrument: Second Chance**

To the Editor:

We previously reported the evaluation of 8 different hemoglobin A1c (Hb A1c)\(^1\) point-of-care instruments (1). Two of 8 manufacturers withdrew from that study after initial unpromising results. One of the 2 instruments withdrawn was the Quo-Test A1c (Quotient Diagnostics), which was withdrawn because of a technical problem. The manufacturer claimed to have resolved the problem and asked us to reevaluate the instrument.

The Quo-Test method is based on affinity separation and the use of fluorescence quenching and gives results in 3 min. The instrument was certified by the National Glycohemoglobin Standardization Program (NGSP) as of September 2009 (2).

We used the same approach for evaluation as in the initial study, following the CLSI EP-5 protocol for imprecision and the CLSI EP-9 protocol for method comparison. Because the American Diabetes Association has recommended Hb A1c as the preferred test for the diagnosis of diabetes (3), we added an additional sample of approximately 6.5% Hb A1c in the EP-5 protocol. The EP-9 protocol was performed twice with 2 different lot numbers and compared nonstandard abbreviations: Hb A1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; SRM, secondary reference measurement procedure.

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**Nonstandard abbreviations:** Hb A1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; SRM, secondary reference measurement procedure.
with 3 IFCC and NGSP secondary reference measurement procedures (SRM): the Roche Tina-quant Hemoglobin A1c Gen.2 on Cobas Integra 800, immunoassay, IFCC and NGSP SRM (Roche Diagnostics); the Primus Ultra², affinity chromatography HPLC, IFCC, and NGSP SRM (Primus Diagnostics, a Trinity Biotech Company); and the Tosoh G8, cation-exchange HPLC, IFCC SRM (Tosoh Bioscience N.V./S.A.).

To check overall calibration and bias, we compared the EP-9 protocol results to the mean of the 3 SRM results and also used the EP-9 protocol results to calculate the NGSP certification criterion with 2 reagent lot numbers.

In monitoring therapy, the reproducibility of Hb A1c assays is critical. The total CV should be <3% (realistic goal) and for optimal clinical use <2% (desirable goal) (1). The total CVs in the EP-5 protocol for the Quo-Test at Hb A1c values of 5.0%, 6.2%, and 10.2% were 5.9%, 4.5%, and 2.9%, respectively.

Comparisons between the Quo-Test with 2 reagent lot numbers and the mean of the 3 SRM are shown in Fig. 1 with the individual EP-9 results and the NGSP certification calculations. The 95% CI of the differences between the SRM and test methods should fall within ±0.75% Hb A1c (total error) to pass the current NGSP criteria (4). The Quo-Test NGSP certification was granted in September 2009 (2) before the tightening of the NGSP criteria from ±0.85% Hb A1c to ±0.75% Hb A1c. To evaluate this method in the same way as the other methods in our previous study (1), we used the old criteria. The calibration of the first lot number appeared adequate, but with the EP-5 protocol we observed high variability reflected by a high total CV, and a high SE of estimates was still a matter of concern. The discrepancy with the second lot number may have been attributable to problems associated with upscaling of the production of cartridges.

The Quo-Test passed the NGSP criteria compared with 1 SRM (Tosoh G8) with 1 lot number but failed the NGSP criteria for all the other comparisons (Fig. 1).

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**Fig. 1.** Hb A1c results for 2 different lot numbers from the Quo-Test point-of-care instrument compared to the mean Hb A1c results from 3 SRM procedures (individual EP-9 regression lines and NGSP certification criteria are shown below the graph).

The P value of the regression lines between the 2 lot numbers was <0.001, which confirmed the statistically significant difference between the 2 regression lines.
Tests performed by using Chow-statistics for the overall differences in slope and intercept per method for lot numbers 1 and 2 showed significant differences in analytical performance between the 2 lot numbers \( P < 0.001 \).

The manufacturer provided 2 controls with wide ranges; low control 4.2% to 7.5% and high control 10.5% to 15.3%. The manufacturer should narrow these ranges as was described recently (1).

Results of analysis of the analytical performance of the Quo-Test showed a high total CV, large bias with 1 lot number, failed NGSP criteria, and significant differences between lot numbers. The Quo-Test is officially NGSP certified and passed the NGSP criteria with only 1 lot number as tested at the manufacturer’s site (2). The results we report here demonstrate the large lot-to-lot variability in quality of the Quo-Test Hb A\(_{1c}\) point-of-care test.

Health care professionals should be aware of the clinical implications for an Hb A\(_{1c}\) value that is determined by using a point-of-care instrument (5). Moreover, to properly interpret the result, health care professionals must know the analytical performance of the Hb A\(_{1c}\) method used. This study and the previous study (1) prove that an NGSP certification does not guarantee the quality of results produced in the field and confirms the recommendation of the American Diabetes Association not to use Hb A\(_{1c}\) point-of-care assays for diagnostic purposes at this time (3).

Validation of a new method is always necessary and cannot be expected to be carried out by health care professionals. For this reason we think that point-of-care devices should be guided by and fall under the responsibility of a central laboratory.

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Erna Lenters-Westra\(^2,3\)*
Robbert J. Slingerland\(^2,3\)

\( ^2 \) Department of Clinical Chemistry, Isala Klinieken
Zwolle, the Netherlands
\( ^3 \) European Reference Laboratory for Glycohemoglobin
Zwolle, the Netherlands

* Address correspondence to this author at: Isala Klinieken, Department of Clinical Chemistry
Groot Wezenland 20
8011 JW Zwolle
the Netherlands
Fax +31-38-4242-676
E-mail w.b.lenters@isala.nl

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Could Susceptibility to Low Hematocrit Interference Have Compromised the Results of the NICE-SUGAR Trial?

To the Editor:

The recently published findings of the Normoglycemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation (NICE-SUGAR) trial have dramatically changed clinician attitudes toward the achievement of euglycemia in intensive care unit (ICU) patients (1). In defending the proof-of-concept studies that validated the efficacy of normalizing blood glucose in the ICU, Van den Berghe et al. pointed out numerous variances between their original studies and those of the NICE-SUGAR trial (2). They included differences in blood glucose targets, insulin administration, blood sampling, nutritional strategies, clinician expertise, and the relative accuracy of the glucose measurement devices. Recently, Clinical Chemistry presented a very interesting Q&A on the use of blood glucose meters to achieve tight glucose control in patients in the ICU (3). Because one of our ICUs participated in the NICE-SUGAR trial, we report here some interesting and relevant data that shed more light on the NICE-SUGAR trial, data that yield more questions than answers.

In our 30-bed general systems ICU at the University of Alberta Hospital, point-of-care glucose concentrations can be measured in 2 different ways: respiratory therapists measure arterial blood gases, hemoglobin, electrolytes, and glucose values with the Radiometer 800 blood gas system (BGA) and nurses measure arterial blood and capillary
blood glucose with the LifeScan SureStep Flexx blood glucose meter (BGM). Both the BGA and BGM glucose results are stored in a central laboratory data repository, and we retrieved Radiometer BGA and Lifescan BGM glucose results that were run within 15 min of each other for individual patients. The numeric differences between these paired values graphed against the date of collection (represented by the point data) are shown in Fig. 1. The numeric differences are represented by 6 different symbols, with each representing a different reagent strip lot. The lines represent lot-specific moving averages of the 237 differences (the dark line represents the moving average of the numeric differences; light line, the moving average of the relative [%] differences).

![Fig. 1. Glucose differences (BGM – BGA) vs time in any patient who had arterial blood glucose measured on the Radiometer BGA and arterial or capillary blood measured on the LifeScan BGM within 15 minutes.](image)

Six different strip lots were primarily used. The 15-point moving average of these numeric differences and the moving average of the relative differences are shown (relative difference data not presented). The numeric differences are represented by 6 different symbols, with each representing a different reagent strip lot. The lines represent lot-specific moving averages of the 237 differences (the dark line represents the moving average of the numeric differences; light line, the moving average of the relative [%] differences).

Because many BGM systems provide artifactually high glucose concentrations in patients with low hematocrits (4), we graphed the BGM/BGA differences against hemoglobin that was proportional to hematocrit and measured by using the Radiometer analyzer. [The mean (SD) hemoglobin concentrations were very similar over the 2 periods, 92.7 (16.9) vs 92.8 (18.1) g/L]. The first 3 glucose reagent-strip lots were more sensitive to the effects of hemoglobin compared to the next 3 lots [glucose difference = $-0.0195 \times$ hemoglobin (mg/L) $+2.41; r^2 = 0.108; P = 0.0001$ (first 3 strip lots); glucose difference $= -0.0103 \times$ hemoglobin (mg/L) $+1.09; r^2 = 0.0926; P = 0.0021$ (last 3 strip lots)]. It appears that many of the samples measured with the first 3 lots of strips would have artifactually increased glucose concentrations. Our hospital general systems ICU participated in the NICE-SUGAR study, and the time of the data collection for NICE-SUGAR coincided with the period during which we were using lots 1, 2, and 3. Of the glucose values reported by our ICU for the NICE-SUGAR patients, the LifeScan BGMs were the source of the most of the glucose values. In accordance with the NICE-SUGAR protocol, high glucose values would be treated. During the NICE-SUGAR study, our LifeScan BGMs were providing increased glucose concentration results on most of our
Letters to the Editor

The Effect of Hemolysis on Current Troponin Assays—A Confounding Preanalytical Variable?

To the Editor:

The universal definition of myocardial infarction stipulates the detection of an increase and/or decrease in cardiac biomarkers [preferably troponin I (TnI)1 or T (TnT)], with at least 1 value >99th percentile of the upper reference limit, together with evidence of myocardial ischemia (1). With the emergence of more sensitive troponin assays, what constitutes a genuine increase and/or decrease becomes critically important (2). For analytical values to be considered different, it has been suggested that such values should vary by >3 SDs of the variance of the measurement method and that a 20% change for troponins is greater than what would be expected from analytical variation (1). At low troponin concentrations, either definition translates into small absolute changes. Good analytical precision is therefore critically important, as is careful consideration of preanalytical variables, such as sample type and particularly hemolysis. Hemolysis is known to be more prevalent in the emergency department environment, with rates of up to 20% of samples (3). Hemolysis is known to cause interference in both TnT and TnI assays (4), although such effects become even more important with the emergence of more sensitive troponin assays and the increased reliance on interpretation of small absolute changes. We therefore evaluated the degree of analytical inter-

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References


George S. Cembrowski* 
David V. Tran 
Linda Slater-MacLean 
Dat Chin 
R. T. Noel Gibney 
Michael Jacka

University of Alberta Hospital 
Edmonton, Alberta, Canada

* Address correspondence to this author at: University of Alberta Hospital Site Lab Med/Rm 4B1.24, WCM Center 8440 112th St. Edmonton, Alberta T6G 2B7, Canada Fax 780-407-8599 Email cembr001@cha.ab.ca

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1 Nonstandard abbreviations: TnI, troponin I; TnT, troponin T; HI, hemolysis index.
ference from hemolysis with currently available troponin assays.

Erythrocyte hemolysates were prepared according to the procedure of Meites (5) and added to 4 unhemolyzed pools of heparinized plasma with TnI concentrations of 0.00, 0.03, 0.5, and 5 μg/L, as measured with the Architect (Abbott Diagnostics), to produce a range of hemolyzed plasma samples. The hemoglobin concentration was measured as a hemolysis index (HI) (calibrated in milligrams per deciliter hemoglobin) on the Abbott c8000 analyzer.

TnI was measured with the Abbott Troponin I (840549/R6) assay on the Abbott Architect instrument (99th percentile, 0.028 μg/L; 10% CV, 0.032 μg/L) (2) and the Ortho Clinical Diagnostics Vitros ECI instrument (99th percentile, 0.034 μg/L; 10% CV, 0.034 μg/L) (2). TnT was measured with the Roche Troponin T STAT (04660307) assay (99th percentile, <0.01 μg/L; 10% CV, 0.03 μg/L) (2) and the Roche Troponin T hs (05092744/190) assay (99th percentile, 13 ng/L; 10% CV, 12 ng/L) on the Elecsys 2010 instrument (Roche Diagnostics) (2). The within-batch CV was <3% for all pools without added hemolysate.

In the calendar month of August 2009, we assayed 966 samples (788 patients) from our emergency department for Troponin I on the Abbott Architect and measured the HI on our Abbott c8000 analyzer. Nineteen percent of these samples were hemolyzed, and 786 samples (81%) were not hemolyzed (HI, 0–35 mg/dL). Of the hemolyzed samples, 111 (12%) were slightly hemolyzed (HI, 36–100 mg/dL), 43 (4%) were hemolyzed (HI, 101–250 mg/dL), and 26 (3%) were grossly hemolyzed (HI, >250 mg/dL). Of the 788 patients, 177 (22%) had at least 1 hemolyzed sample, and 112 (63%) of these patients had subsequent samples that were not hemolyzed.

Current troponin assays showed different susceptibilities to interference by hemolysis (Table 1). The Roche TnT assays both showed negative interference (up to 50% in low pool B for Troponin T hs) with increasing degrees of hemolysis, as previously described for the fourth-generation Roche TnT assay (4). The Vitros ECI TnI assay showed positive interference (up to 576% in low pool B), as previously documented for a prior generation of the same assay (4), whereas the Abbott Architect TnI assay appeared to be more robust to interference from hemolysis (<10% in low pool B).

These considerations are critically important when interpreting changes of 20% or 30% at low troponin concentrations, which translate into small absolute changes in concentration. Initial samples taken in the emergency department are more likely to be hemolyzed than samples taken later in other areas; thus, an increase and/or decrease in troponin concentration may be masked by hemolysis in either sample, depending on the assay. From our

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* Hb, hemoglobin.
data, the confounding effects of hemolysis on the interpretation of serial changes in troponins vary according to what assay is being used. Laboratories should be aware of the influence of hemolysis and should offer caveats regarding potential interference when appropriate.

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References

High-Sensitivity Cardiac Troponin: Seeing the Wood from the Trees

To the Editor:

The perspective provided by Jaffe and Apple (1) regarding the landmark publications in The New England Journal of Medicine offers an insightful commentary regarding the clinical utility of the so-called high-sensitivity cardiac troponin assays (2, 3).

We fully support the notion that many of the assays evaluated in these papers should be considered contemporary rather than high-sensitivity. The scorecard categorization is a valuable tool for any clinical laboratory to assess the analytical performance of their cardiac troponin assay (4). Jaffe and Apple incorrectly referenced the high-sensitivity cardiac troponin T (hs-cTnT) (Roche) in the study of Keller and colleagues. In reviewing the Keller et al. article (2) and the supplementary methods (available as an online appendix), we observed that the authors used the current fourth-generation cTnT assay rather than the hs-cTnT assay for comparison between methodologies.

It is interesting to note that the diagnostic utility of the Abbott cTnI, the Roche standard cTnT, and developmental cTnI assays is superior when using the 99th percentile cutoff rather than the 10% coefficient of variation, at the cost of specificity (3). Diagnostically, they perform as well as the hs-cTnT, calling into question the true sensitivity of this assay. Many clinical laboratorians still favor the 10% coefficient of variation cutoff value over the 99th percentile, presumably as this is within a margin of safety with which they are both familiar and comfortable and that is applied to other immunoassay tests.

The drive to use the 99th percentile is warranted, as demonstrated by the studies of Keller et al. and Reichlin et al. (2, 3); however, the real challenge to adequately assess clinical sensitivity and specificity will come from prospective studies of unselected chest pain patients presenting to the emergency department.

The newer hs-cTn assays in development may prove to be superior diagnostically. Analytically, both the contemporary and high-sensitive assays challenge the performance of current immunoassay technology. The development of other detection methods such as single-molecule-counting technology may be the appropriate alternative (5), if this technology can be adapted for large high-throughput analyzers with reduced turnaround time without compromising analytical performance.

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David C. Gaze*
Paul O. Collinson

Clinical Blood Sciences
St George’s Healthcare NHS Trust
London, UK

* Address correspondence to this author at: Clinical Blood Sciences
St George’s Healthcare NHS Trust
London, SW17 0QT UK
Fax +44 (0)20-8725-5868
E-mail david.gaze@stgeorges.nhs.uk

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Comment on “High-Sensitivity Cardiac Troponin: Hype, Help, and Reality”

To the Editor:

We thank Jaffe and Apple (1) for their interest in our study. In their perspectives article, they commented on details of our study and the study by Keller et al., which were both published recently in the New England Journal of Medicine (2, 3). Although we fully agree with several of their statements, we strongly disagree with others. It is detrimental to the much-needed interdisciplinary discussion on the best possible clinical use of sensitive cardiac troponin assays when the discussion is based on incorrect statements. Unfortunately, the Jaffe and Apple perspective contains 2 important errors.

First, and of most importance, Jaffe and Apple (1) asked, “How did these studies deal with the problem of increased [cardiac troponin] concentrations produced [by diseases other than acute myocardial infarction (AMI)]?” and stated, “Not very well. For the most part, the authors . . . used a [cardiac troponin] concentration above the cutoff value as the sole criterion for the diagnosis of acute coronary syndrome (ACS).” They also stated, “The presence of a changing . . . pattern, which is an essential part of the criteria for the diagnosis of AMI in all the guidelines, was used in only a subset of patients in the Reichlin et al. article . . . .” These statements are incorrect in 2 respects. On the one hand, AMI was defined in our study in all patients in full agreement with the current universal definition of AMI (4), which requires evidence of myocardial necrosis with a changing pattern associated with clinical signs of myocardial ischemia, as stated clearly in the Methods section of our report (2). Necrosis was diagnosed on the basis of a rising and/or falling pattern of the cardiac troponin concentration, with at least 1 value above the 99th percentile at a level of precision of <10% (2). On the other hand, noncoronary conditions that increased cardiac troponin concentrations according to conventional cardiac troponin assays were not called “true positives” but were included in the diagnostic group “cardiac symptoms from causes other than coronary artery disease” (2). Ninety-five (13%) of 718 patients were adjudicated in this group, and 16 of these patients had acute cardiac necrosis of an origin other than coronary artery disease (e.g., myocarditis, tachyarrhythmias, or acute heart failure).

Second, Jaffe and Apple (1) argue that the incidence of ACS is much lower in the US than the incidence reported in the 2 studies published in the New England Journal of Medicine (2, 3) and state that the incidence of ACS was 46% in our study. This statement is incorrect. The incidence of ACS in our study as reported in the Results section of the report was 33% (2). In addition, results from a recently completed multicenter study that included consecutive patients presenting to the emergency department with symptoms suggestive of AMI showed that the difference in the incidence of local emergency department diagnosis of ACS among centers in the US [range, 4%–40%; data on file at FAST-TRAC Data Management Center, San Diego, CA (Dr. Greg Shipp, Nanosphere, personal communication, January 15, 2010)] was greater by far than potential differences between the US and Europe. Therefore, we think that the disease incidence encountered in an individual emergency department is predominantly determined by the details of the local patient flow, such as a competing high-volume catheterization laboratory in a neighboring hospital that receives most high-risk and high-probability ambulance cases, or such as local hospital standards that guide chest pain patients only to the emergency department as long as they are perceived as low risk and low probability and that triage all high-probability patients directly to a coronary care unit.

We hope that these clarifications help to advance the important
discussion on the best clinical use of sensitive cardiac troponin assays.

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Tobias Reichlin2,3
Raphael Twerenbold2
Christian Mueller*

Departments of2 Internal Medicine
and 3 Cardiology
University Hospital Basel
Basel, Switzerland

* Address correspondence to this author at:
Department of Internal Medicine
University Hospital Basel
Petersgraben 4
CH-4031 Basel, Switzerland
Fax +41-61-265-53-53
E-mail chmueller@uhbs.ch

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In Reply

We are pleased to have the opportunity to clarify some issues that, due to word count restrictions, were not as clear as would have been ideal.

The issue of the use of a δ (changing pattern) is a good example. Deltas are important for lower cardiac troponin concentrations because most chronic increases (an occasional dialysis patient excepted) are modest. At low concentrations, the δ takes into account the imprecision of the assay and, if possible, biological variation. The δ for the cardiac troponin T (cTnT) assay used for the gold standard diagnosis was unstated. Clinicians think of a changing pattern as any alteration, and that is not the case. At the Mayo Clinic, we require a 0.03-μg/L change at low cTnT concentrations, which is 300% from 0.01 μg/L and 100% from 0.03 μg/L. At higher concentrations, lower δ values are used because the imprecision is less. No criteria were provided for the comparison assays or for the novel high-sensitivity assay (1). Table 3C in the supplementary material for the report of Reichlin et al. (1), where this matter is evaluated, provides no criteria. We have recently established that the conjoint biological and analytical variation with the high-sensitivity cTnT assay is 85% in the short term (2). Kavsak et al. used a 20% change on the basis of an imprecision profile with the Beckman assay (3). It appears that Reichlin et al. (1) allowed clinical judgment rather than criteria to be used, with the consequence varying from embracing a change of 0.01 μg/L (a 20% change at 0.05 μg/L) to something more reasonable. In addition, most cardiac troponin assay results are reported in hundredths, and rounding issues will thus alter percentage changes. It is not clear that these authors understand these issues, which affect clinical classifications and triage decisions.

Who was or was not included in the various diagnostic groups and what criteria were used are also unclear. Even now, how many patients had increases in cTnT is unclear. The authors say that 95 (13%) of 718 patients were in the group, but only 16 had necrosis. If indeed 13% had increases due to noncoronary causes, it would have been important to comment on the specificity of rising and/or falling cTnT concentrations for coronary disease in patients with chest discomfort because noncoronary increases were nearly as prevalent as acute myocardial infarction (AMI). If only 16 patients fit and the others had typical cTnT values but alternative diagnoses, that might be less necessary. The criteria for these decisions are not stated. For example, for patients who present late after AMI and the cardiac troponin concentration is near its peak, a changing pattern might not be seen. Was this possibility taken into account? If patients did not have acute lesions according to the angiography results but had severe coronary artery disease, were they then classified as
having a noncoronary etiology for chest pain, stable angina, or unstable angina? If patients had classic symptoms but did not have obstructive disease, were they diagnosed with acute coronary syndrome (ACS), AMI, or noncardiac pain? Some patients, especially women, present this way and likely have ACS (4). It is important to know whether these patients were included in the ROC curve analyses because they would influence the analyses.

The issue of incidence and how it affects the data are important. There are differences between the US and Europe in most of the chest pain studies, likely due to how patients are screened. In Europe, there often are cardiologists in the emergency department. In the US, that 46% of patients would have acute cardiac disease in a chest pain cohort would be unusual. This percentage includes AMI, unstable angina, and cardiac symptoms from other “noncardiac diseases” (page 861 of the authors’ report (1)). The issue is not the number but an important conceptual issue. If one includes all the patients with ST-elevation myocardial infarction (STEMI) and overt non-STEMI, the pretest probability that an increased cardiac troponin concentration will be associated with ACS is higher than if one includes only patients for whom the diagnosis is unclear. Such a group, judging from ROMICAT (Rule Out Myocardial Infarction Using Computer-Assisted Tomography) trial data, has an incidence of coronary artery disease of about 8%. This changes the pretest probability of disease and exaggerates the predictive accuracies of the approach. This is the point of the recent report of Januzzi and colleagues (5).

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References


Allan S. Jaffe2*
Fred S. Apple3

2 Mayo Clinic
Rochester, MN

3 Hennepin County Medical Center
and University of Minnesota
Minneapolis, MN

* Address correspondence to this author at:
Mayo Clinic
Gonda Building, 5th Flr.
200 First St. S.W.
Rochester, MN 55905
Fax 507-266-0228
E-mail jaffe.allan@mayo.edu

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