Data Processing in CO-Oximeters That Use Overdetermined Systems

To the Editor:

CO-oximeters are specialized spectrophotometers that automatically determine hemoglobin (Hb) derivatives by measuring absorbance at selected wavelengths [1]. We believe that a good understanding of the relevant theory may allow users to avoid many pitfalls during operation of these instruments. The mathematical basis of their operation has not, however, been fully explained by the manufacturers apart from Instrumentation Laboratory (Lexington, MA) at the introduction of their first CO-Oximeter® [2]. Here, we discuss what mathematical methods for data processing might be used in commercial CO-oximeters, particularly in those models that use an “overdetermined” system.

CO-oximeters depend on the observation that Hb solutions obey the Lambert–Beer Law; thus, the absorbance measured at a given wavelength is the sum of the absorbance of each Hb derivative at the same wavelength [2]. When we measure n wavelengths to determine the m Hb derivatives \( \chi_i \), we get n equations:

\[
A_j = \sum_{i=1}^{m} \epsilon_{ij} C_i \lambda_i \quad \text{for } 1 \leq j \leq n, \tag{1}
\]

where \( A_j \) is the absorbance at wavelength \( \lambda_i \), \( C_i \) is the concentration of derivative \( \chi_i \), and \( \lambda_i \) is the pathlength. \( \epsilon_{ij} \) is the molar absorptivity at wavelength \( \lambda_i \) for derivative \( \chi_i \).

When \( n = m \), we can solve Eq. 1 to get \( C_i \). This is termed an “exactly determined” system [3] and has been implemented in the IL 482 CO-Oximeter (Instrumentation Laboratory), the IL 282 (its predecessor), and the Radiometer OSM3 Hemoximeter® (Radiometer, Copenhagen, Denmark). The IL 482 uses four wavelengths for four Hb derivatives, whereas the OSM3 uses six wavelengths for six unknowns: five Hb derivatives plus one for noise (attributed to “turbidity”). The report by Steinke and Shepherd [4] illustrates how the full exposition of the algorithms used in specific instruments is useful not only to users but also to manufacturers. After measuring absorptive spectra of Hb derivatives at three different temperatures, Steinke and Shepherd applied their data to simulate the effect of temperature variation on the accuracy of the IL 482, according to the published mathematical formula. They found that significant errors occurred if the apparatus was not temperature-controlled. The marketed IL 482, however, precisely controls the temperature of the measuring cell.

When \( n > m \), the set of equations for Eq. 1 has been described as an “overdetermined” system [3]. Overdetermined systems have been utilized in the Corning 270 CO-oximeter (Ciba Corning Diagnostic Corp., Medfield, MA), the Corning 2500 (its predecessor), and the AVL 912 CO-Oxylite® (AVL Scientific Corp., Roswell, GA). The Corning 270 uses 7 wavelengths for five Hb derivatives, whereas the AVL 912 uses 17 wavelengths for five Hb derivatives.

We suspect that these CO-oximeters with overdetermined systems use the least-squares method for data reduction. In such cases, errors \( d_j \) are defined as the difference between observed value \( A_j \) and the predicted values

\[
d_j = A_j - \sum_{i=1}^{m} \epsilon_{ij} C_i \lambda_i \quad \text{for } 1 \leq j \leq n, n > m. \tag{2}
\]

The sum of the squared errors \( S \) is then given by:

\[
S = \sum_{j=1}^{n} (\omega_j d_j)^2 \tag{3}
\]

where \( \omega_j \) is the weighting factor, and \( p_{ik} \) is the partial equations

\[
p_{ik} = \sum_{j=1}^{n} \omega_j^2 \epsilon_{ij} \epsilon_{jk} \tag{4}
\]

and \( r \) is a constant independent of \( C_i \). The value \( S \) is minimized when the partial equations \( \partial^2 S/\partial C_i = 0 \) (for \( 1 \leq k \leq m \)) hold true. By solving these simultaneous equations, we can get \( C_i \):

\[
C_i = \sum_{j=1}^{n} t_{ij} A_j \quad \text{for } 1 \leq i \leq m, m < n, \tag{4}
\]

where

\[
t_{ij} = \sum_{k=1}^{m} \omega_j^2 p^{-1}_{ik} \quad \text{for } 1 \leq j \leq n,
\]

and \( p^{-1}_{ik} \) is the element for the inverse matrix of the square matrix \( [p_{ik}] \).

According to standard mathematical textbooks, the usual way to fix the weighting factor \( \omega_j \) is to use the inverse of the standard error \( \sigma_j \) of absorbance \( A_j \) at wavelength \( \lambda_j \) (\( \omega_j = 1/\sigma_j \) for \( 1 \leq j \leq n \)), if we can determine \( \sigma_j \). Although we cannot determine the standard error for blood samples because of the heterogeneity in their content of Hb derivatives, we can determine the standard error for any (essentially) pure Hb derivative by experiment. We therefore estimate the standard errors \( \sigma_j \) for a particular blood sample by assuming that \( \sigma_j \) at a given wavelength is the total of the standard error due to each Hb derivative at the same wavelength:

\[
\sigma_j = \sum_{i=1}^{m} \sigma_{ij} (C_{ij}) \cdot C_i/C_{ij} \quad \text{for } 1 \leq j \leq n, \tag{5}
\]

where \( \sigma_{ij} (C_{ij}) \) (for \( 1 \leq i \leq m, 1 \leq j \leq n \)) is the standard error at wavelength \( \lambda_i \) for a pure Hb derivative \( \chi_i \) having the concentration \( C_{ij} \), and \( C_i \) is the concentration of the Hb derivative \( \chi_i \) in the blood sample. The problem with these formulas is that they include \( C_i \), which is the very unknown to be determined. This
problem can be avoided by using the following algorithms:

1) Fix all the initial weighting factors as 1 \([\omega_j(1) = 1\), for \(1 \leq j \leq n\).
2) Calculate the provisional concentration \(C_j(1)\) from the measured absorbance \(A_j\) \((1 \leq j \leq n)\) by using Eq. 4.
3) Calculate the provisional standard error \(\sigma_j(1)\) from \(C_j(1)\) by using Eq. 5.
4) Replace \(\omega_j(1)\) with \(\omega_j(2) = 1/\sigma_j(1)\).
5) Calculate the next provisional concentration \(C_j(2)\).
6) Calculate the next provisional standard error \(\sigma_j(2)\) from \(C_j(2)\).
7) Repeat 4), 5), and 6) until \(\omega_j\) and \(C_j\) converge.

Clearly, there may be other possibilities.

Brown [2] attributed a primary cause of the measurement error to the drift in the wavelength of the emitted light (“wavelength shift”). This suggests that the standard error \(\sigma_j\) may be linear with respect to the slope (first derivative) of the absorbance spectrum \(dA/d\lambda\) (where \(\lambda = \lambda_j\)). If we accept this assumption, we notice that Eq. 5 overestimates the standard error \(\sigma_j\) for a blood sample because the standard error of each Hb derivative in the sample could cancel out each other (the slope of the absorbance spectrum for the mixture of Hb derivatives is the total of the slope for each Hb derivative in the mixture only when the directions of the slope for all Hb derivatives are the same). On the other hand, we can measure absorbance \(A_j\) accurately when \(A_j\) falls in a given range (the background noise due to any turbidity produces large errors when \(A_j\) is too small, and the upper limit for \(A_j\) depends on the linearity of the phototoolphic system). Considering all of these factors, we suggest that the standard error is a function of the absorbance and of its first derivative:

\[ \sigma_j = F(A_j, dA/d\lambda) \] (where \(\lambda = \lambda_j\)). However, speculation as to the form of the function \(F\) is beyond our ability.

We ask the two companies who use overdetermined systems (Cornig and AVL) to comment on this approach—in particular, as to whether the least-squares method is, in fact, used for processing an overdetermined data set. If it is, we hope the manufacturers would explain how they fixed their weighting factors \(\omega_j\), i.e., how they have weighted the redundant wavelengths to achieve more accurate results.

References

Nobuhiro Yukawa, Takashi Suzuoka, Takeshi Saito, Alexander R.W. Forrest, Motoki Osawa, Sanae Takeichi
1 Dept. of Forensic Med. Tokai Univ. School of Med. Isehara, Kanagawa 259–11, Japan
2 School of Computer Sci. Carnegie Mellon Univ. Pittsburgh, PA 15213
3 Dept. of Clin. Chem. Royal Hallamshire Hosp. Sheffield S10 2JF, UK

*Author for correspondence.

A manufacturer replies:

To the Editor:

We have read the letter of Yukawa et al., and believe that it clearly addresses some often misunderstood aspects of CO-oximetry. We offer what we believe to be some additional perspectives on the topic.

The technique of “least-squares” analysis of error is firmly grounded in the technical and analytical literature and can be a powerful tool in the simultaneous multicomponent analysis used in CO-oximetry. We believe, however, that the rationale for wavelength selection and the use of weighting factors is not quite so clear. In that light we offer the following discussion based on our experience over the several generations of CO-oximeters that Chiron Diagnostics (formerly Ciba Corning Diagnostics) has developed and manufactured.

Wavelength selection for a determined system is a conceptually simple process. One chooses a combination of absorption maxima and isosbestic points, with a total number of wavelengths equal to the number of components of interest. After experimental determination of the absorptivities, one can set up a matrix for solving for the various fractions of a test specimen.

The process for an overdetermined system is similar, except that one decides on an arbitrary number of additional wavelengths, then tests the system in the presence of typical interferents using the least-squares approach. When one has a choice of wavelengths, the process is repeated multiple times to determine the best set of wavelengths to combine measurement of fractions and minimization of the effects of interferents. As a part of the process, one might decide, on the basis of the results of the experimental findings, that either different wavelengths or a greater or lesser number are more robust than those originally selected.

The seven wavelengths used in the M270 CO-oximeter as used by Yukawa et al. have performed quite well for the vast majority of blood samples. However, after years of operation with many hundreds of field placements, it became evident that a small number of blood samples from apparently normal donors have significant deviations in their absorbance spectra in the region between 580 and 610 nm. These deviations
resulted in small changes in the reported Hb fractions as well. Although this region of the spectrum would appear to be useful for differentiating MetHb from the other fractions, it introduced unwanted variability in the results. Avoiding this variable region of the Hb spectrum is one of the reasons that led us to select a different set of 10 wavelengths for the new 800 Series CO-oximeter.

Although it is customary to use the standard error or variance of observations as weighting factors for least-squares analysis, it is often difficult to measure or estimate appropriate weighting factors to be used in real applications. Such is the case with CO-oximetry. As Yukawa et al. suggest, the standard error cannot be determined before the measurement because it depends on the actual concentrations of the components being measured. As they also suggest, the standard error could be estimated from measurements on donor samples and then applied to unknown samples in some iterative scheme. However, such an approach can account for only systematic errors in the measurement of absorbances and the variability in wavelength.

Our experience shows that for the measurement of typical blood samples, i.e., samples that are fresh and free of interfering substances and contamination, weighting factors of 1.0 yield both precise and accurate results. The systematic errors that affect all measurements in some measurable or estimatable manner have been minimized. It is the errors resulting from exceptional conditions such as interfering substances and improper preanalytical sample handling that cause the majority of inaccuracy and imprecision. Thus, our approach has been to select wavelengths that avoid many common interferents and minimize susceptibility to minor variations in wavelength.

Of course, it is not possible to avoid all interferents, so detection and correction algorithms have been devised for some known interferents such as lipid, SulHb, CNMetHb, and methylene blue. For samples that contain uncharacterized interferents, the overdetermined measurement affords a “quality-of-fit index” that is used to detect the presence of unknown interferents and flag the samples with the message, “If blood, question data.”

Yukawa et al. have clearly outlined the mathematics behind CO-oximetry measurements with overdetermined systems. However, we feel that the wider system issues of wavelength selection and detection of atypical samples also play an equally vital role in the performance of CO-oximeters. Hopefully this discussion has helped to enlighten users in regard to the characteristics and analytical limitations of present-day CO-oximeters.

Jacques A. Brunelle
Robert F. Moran*
Chiron Diagnostics Corp.
Medfield, MA 02052

* Author for correspondence.

Chronic Increased Serum Lipase in a Patient with Suspected Pancreatic Adenocarcinoma

To the Editor:

Donnelly et al. [1] described in this journal a patient similar to one studied by us. Our patient, a 57-year-old man, presented with cutaneous lesions in his legs, characterized by lobular panniculitis (PN) with fat necrosis and ghost cells. His serum lipase was 12,255 U/L at 25°C (by a turbidimetric method from Boehringer Mannheim, Mannheim, Germany; upper reference limit, 200 U/L). Surprisingly, his serum and urine amylase activities were not increased, and hepatic and renal function appeared to be normal upon admission. Three weeks later, his serum lipase was 11,141 U/L, and amylase remained within reference limits. Ultrasonography and computed tomography (CT) revealed a nodular lesion in his liver (13 × 10 × 15 cm). These features, combined with high lipase activity, led us to consider a pancreatic carcinoma with liver metastasis. However, two additional CT scans >2 weeks later showed no pancreatic abnormality. Histopathological analysis of needle aspirate from a liver nodule showed a papillary differentiated adenocarcinoma. Intestinal cancers were searched for but not found. Concentrations of carcinoembryonic antigen, CA 19.9, and CA 19.5 were within reference limits. Only α-fetoprotein was high (1075 μg/L; upper reference limit: 20 μg/L), consistent with hepatoma (although it can also be increased less dramatically in pancreatic carcinoma) [2]. Lipase activity was still increased 5 weeks after hospitalization (24,800 U/L), and activities of hepatic enzymes increased until the patient’s death 3 months after presentation. Two weeks before his death, ultrasonography revealed a small, hypoechoic solid node (2.3 × 2.8 cm) in the body of the pancreas, and the patient was sent to our reference center for treatment. Postmortem examination could not be performed.

To account for the high lipase activity, we tested for several possibilities in blood samples obtained from the patient during hospitalization and stored at −20°C until assay: (a) analytical or physiological effects of drug origin; (b) the presence of macroamylase; and (c) the presence of lipase isoforms (and their pattern). Drug effects [3] were excluded by history. The diagnosis of macroamylasemia was not pursued because <2% of the activity in the supernatant was lost (decreasing from 15,268 to 14,960 U/L) after precipitation with polyethylene glycol as described for macroamylase screening [4]. Sera of patients with acute pancreatitis submitted to the same procedure showed a decrease in lipase activity of <20%. A decrease of >73% of amylase activity is generally required before the presence of macroamylase is considered. The precipitation of immunoglobulins after treatment with polyethylene gly-
tracts, and all acute pancreatitis sera, pancreatic juice and tissue, duodenum excreted. For controls, we used sera from patients with acute pancreatitis, in which L1 and L2 isoforms are usually present in equal amounts [5]. Undiluted serum from our patient showed the presence of trace amounts of L1 and a large spot at the previously described L2–L3 position [1, 5] (Fig. 1). In diluted serum from the patient, a clear band appeared in the L2 zone. Proximity between L2 and L3 (carboxyl ester lipase) has been described, but our band is not L3 because the quantitative turbidimetric method we used does not detect carboxyl ester lipase activity [6]. Accordingly, if only one band appeared in the L2–L3 zone, it could not be L3. Moreover, in our case, after incubation for 20 min at 56 °C, the patient’s lipase activity changed from 11 000 U/L to 42 U/L, in agreement with the previously described thermolability of pancreatic isoforms [7].

Our patient presented with an adenocarcinoma associated with high serum lipase activity, similar to the case reported by Donnelly et al. Nowadays, the diagnostic role for lipase isoforms is not clear [6]. In their series, Lott and Lu described the presence of L1 and L2 in pancreatic juice and tissue, duodenal extracts, and all acute pancreatitis sera, but not in healthy persons’ sera. L2 is also described in sera of patients with gastric, small bowel, or large bowel diseases and in 70% of patients with liver or biliary tract disease, in which the pancreas may be affected by disorders of nearby organs [5]. Our unusual serum isoform pattern shows only L2, differing from that described by Donnelly et al., who detected only L1. Lott and Lu described a pattern similar to ours in two patients of their series, but both patients had increased amylase and their lipase was not as high. In the only sample of pancreatic adenocarcinoma tissue they reported, Lott and Lu detected L3 alone, but we do not know the type of adenocarcinoma involved and it showed a low lipase activity of 2.83 U/g of tissue compared with 725 U/g in normal pancreatic tissue. To explain the presence of L1 in serum instead of the L1 and L2 isoforms detected in tumor tissue, Donnelly et al. proposed posttranslational modification. The uncertain origin of the primary tumor, with no pancreatic alterations, and the immunohistochemical reactivity of a retroperitoneal node to prostate-specific antigen (PSA) led Donnelly et al. to think that a prostatic carcinoma could be the source of tumor. However, this PSA reactivity has also been described in acinar cell carcinoma of the pancreas [8].

Interestingly, our patient developed PN as a paraneoplastic syndrome, in contrast to the case reported by Donnelly et al. Circulating lipase and other factors may be related to fat necrosis involved in pancreatic PN [9, 10], but not every patient with high serum lipase activities develops this and, as far as we know, no isoform pattern has been studied in tissue and serum of patients with pancreatic PN. Our patient presented with a sort of PN pathognomonic for pancreatic disease [9, 11]. This case presents many aspects similar to those described previously [10, 12, 13]. The presence of adenocarcinoma, presumably pancreatic, with large hepatic metastases, pancreatic subcutaneous fat necrosis, and very high lipase activities accompanied by normal amylase activity is consistent with acinar adenocarcinoma of the pancreas, an uncommon entity [13] but so aggressive that it can lead to death with disseminated metastasis before its detection in the pancreas. Thus, the biochemical profile may be one of the first findings of this adenocarcinoma.

We thank Beckman Instruments (Brea, CA) and Johnson & Johnson Clinical Diagnostic Systems (Rochester, NY), who provided the reagents Paragon isoCK kit and lipase dry chemistry slides, respectively, for electrophoresis of the lipase isoforms. We also thank Luis Rivas for assistance in the preparation of this manuscript.

References

![Fig. 1. Lipase electrophoretogram of acute pancreatitis serum (A and D) and patient’s serum, undiluted (lipase 24 800 U/L) (B) and diluted 20-fold (C).](image-url)
transport system maintains the reverse cholesterol metabolism and atherogenesis. Recently, greater attention has been given to acute-phase reactants, which acute-phase reactants are not useful marker for viral infections, in inflammatory disorders. Serum amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration increases up to 1000-fold in inflammatory disorders. To the Editor:

Serum Amyloid A Remains at Physiological Concentrations in Coronary Atherosclerosis

To the Editor:

Serum amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration in serum increasing up to 1000-fold in inflammatory disorders. Recently, greater attention has been given to acute-phase reactants, which acute-phase reactants are not useful marker for viral infections, in inflammatory disorders. Serum amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration increases up to 1000-fold in inflammatory disorders.

To the Editor:

Serum Amyloid A Remains at Physiological Concentrations in Coronary Atherosclerosis

To the Editor:

Serum Amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration in serum increasing up to 1000-fold in inflammatory disorders. Recently, greater attention has been given to acute-phase reactants, which acute-phase reactants are not useful marker for viral infections, in inflammatory disorders. Serum amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration increases up to 1000-fold in inflammatory disorders.

To the Editor:

Serum Amyloid A Remains at Physiological Concentrations in Coronary Atherosclerosis

To the Editor:

Serum Amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration in serum increasing up to 1000-fold in inflammatory disorders. Recently, greater attention has been given to acute-phase reactants, which acute-phase reactants are not useful marker for viral infections, in inflammatory disorders. Serum amyloid A (SAA), an apolipoprotein found in HDL, is a sensitive acute-phase reactant, its concentration increases up to 1000-fold in inflammatory disorders.
FABP concentration, one has to take into account not only its source and rate of release into plasma but also its elimination from plasma. It is obvious that any change in the clearance rate of FABP would affect its plasma concentration, and thus may lead to erroneous interpretation. Kleine et al. [8] reported a patient with acute myocardial infarction and severe renal insufficiency in whom the plasma FABP concentration remained increased for the whole course of blood sampling (25 h after the infarction), whereas in patients with normal kidney function it normalized in ~10 h after the infarction. Unfortunately, preinfarction data on plasma FABP in this patient were not available. Low-molecular-mass proteins such as FABP and myoglobin are cleared mostly by the kidney [9, 10]. As it remains an open question whether, and, if so, to what extent an insufficiency of the kidneys affects the plasma FABP concentration in patients with heart and skeletal muscles intact, we studied plasma FABP and myoglobin in patients with chronic renal failure.

Blood samples were obtained from 15 blood donors (males) and 27 chronically hemodialyzed patients with renal failure (18 males, 9 females, ages 17–66 years; period of dialysis 2–70 months). Their primary renal diseases were: chronic glomerulonephritis (n = 14), interstitial nephritis (n = 2), acute renal failure (n = 3), adult dominant polycystic kidney disease (n = 3), hypertensive nephropathy (n = 3), diabetes mellitus (n = 1), and amyloidosis (n = 1). The patients were clinically stable and free of any severe intercurrent illnesses. They had no clinical evidence of severe secondary hyperparathyroidism. Hemodialysis was performed three times a week with the double needle technique, with cuprophane capillary dialyzers, and with bicarbonate as buffer in the dialysate. The membrane allows the passage of low-molecular-mass solutes up to ~2 kDa. Vascular access was in all cases a Cimino-Brescia arteriovenous fistula. Blood samples were obtained immediately before and after dialysis.

Plasma FABP concentration was measured by a sensitive noncompetitive sandwich ELISA [4]. Plasma concentration of myoglobin was measured with a turbidimetric immunoassay (Unimate 3 MYO; Roche Diagnostic Systems, Basel, Switzerland) on a Cobas Mira Plus analyzer (Roche). The concentrations of urea and creatinine in plasma were measured by the urease method and Jaffe reaction, respectively.

The significance of the differences between the means was evaluated statistically by unpaired and paired Student t-tests, where appropriate. Correlations between plasma FABP and (or) myoglobin concentrations and the period of dialysis, and plasma urea and creatinine concentrations were determined by Pearson product-moment correlation, and the level of significance was taken at P <0.05.

Plasma creatinine and urea concentrations were high before dialysis and dropped markedly after dialysis (Table 1). The mean plasma concentration of FABP in the uremic patients before and after dialysis was 21 and 25 times higher, respectively, than that in the blood donors. The insignificant increase in plasma concentrations of FABP and myoglobin after dialysis may reflect removal of blood water during dialysis. In the patients, before dialysis the mean myoglobin/FABP ratio was five times lower than in the donors, and after dialysis six times lower (Table 1). Neither plasma FABP nor plasma myoglobin concentrations showed a correlation with the period of dialysis or urea or creatinine concentration in plasma.

The present data are the first to show that plasma FABP concentration is markedly increased in patients with chronic renal failure and normal heart function, similar to that found for myoglobin [11]. It is clear that a certain amount of each protein must be constantly removed either by the kidney or by other tissues, thus preventing progressive increase in the concentration with time of renal failure. Interestingly, the plasma FABP concentration is much higher (20–25 fold) than that of myoglobin (fourfold) despite the fact that these proteins have similar molecular masses (15 and 18 kDa, respectively) and show a similar plasma release curve in patients with acute myocardial infarction and normal renal function [3]. These findings suggest that the kidneys play a more dominant role in the clearance of plasma FABP than of myoglobin.

The ratio of the concentrations of myoglobin over that of FABP is lower in the heart (ratio ~5) than in skeletal muscles (20–70, depending on muscle type) [3]. The use of the ratio of the plasma concentrations of myoglobin over that of FABP to discriminate between heart and skeletal muscle tissue injury has been sug-

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Creatinine, mg %</th>
<th>Urea, mg %</th>
<th>Myoglobin µg·L⁻¹ (range)</th>
<th>FABP, µg·L⁻¹ (range)</th>
<th>Myoglobin/FABP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 15)</td>
<td>0.77 ± 0.14</td>
<td>25.1 ± 7.7</td>
<td>45.8 ± 20.7 (22.3–96.8)</td>
<td>3.0 ± 1.4 (1.4–5.0)</td>
<td>16.2 ± 4.1 (10.9–25.12)</td>
</tr>
<tr>
<td>Renal failure patients (n = 27)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.4 ± 3.3</td>
<td>118.0 ± 30.2</td>
<td>170.6 ± 61.8 (63.8–290.1)</td>
<td>62.8 ± 25.2 (12.1–118.2)</td>
<td>3.2 ± 1.8 (0.7–9.6)</td>
</tr>
<tr>
<td>A</td>
<td>4.6 ± 1.3</td>
<td>53.9 ± 18.6</td>
<td>181.3 ± 67.6 (70.2–297.6)</td>
<td>75.5 ± 28.9 (13.6–120.9)</td>
<td>2.7 ± 1.2 (1.2–6.7)</td>
</tr>
</tbody>
</table>

B, before dialysis; A, after dialysis.
gested [3]. Because of the relatively longer increase in plasma FABP compared with myoglobin, the ratio calculated for uremic patients (~3) is similar to that found in patients after heart infarction. Thus, with respect to the discrimination of myocardial infarction from skeletal muscle injury, the decrease of the ratio in chronic renal failure indicates the limitation of the use of this ratio for this purpose.

Serial monitoring of the plasma FABP concentration can also be used to estimate infarct size [6]. However, our results indicate that if the myocardial infarction occurred in a patient with chronic renal failure, the plasma FABP concentration would be relatively higher than in a patient with intact kidneys, thus leading to overestimation of infarct size. Since preinfarct values differ widely among patients, a judgment about infarct size cannot be made.

In conclusion, our data indicate that in patients with chronic renal failure the plasma concentrations of the biochemical markers FABP and myoglobin each are markedly increased. Thus, caution must be taken when using these marker proteins for early diagnosis of myocardial infarction, in case of renal insufficiency, as the preinfarct plasma concentration is very likely to be already high.

We thank M. Pelsers for expert technical assistance. This work was supported by the Polish State Research Committee, project number 6 P20705607 and the European Community, grant CIPD CT 940273.

References

Jan Górska*  
Wim T. Hermens  
Jacek Borawski  
Michal Mysliwiec  
Jan F.C. Glatz

*Author for correspondence.

Automated Immunoassay of Cardiac Troponin I in Serum Evaluated

To the Editor:
We evaluated the Opus Troponin I assay (Behring Diagnostics, Westwood, MA), a two-site sandwich, fluorogenic ELISA that uses two goat polyclonal antibodies directed against different protein segments unique to cardiac troponin I (cTnI) [1]. Pipetting, incubations, measurements, and data-reduction steps are performed on the Opus analyzer; the first test result requires 20 min. The assay measures concentrations of cTnI in serum as great as ~135 μg/L.

The calibration appeared to be stable for at least 4 weeks. Serial dilution of a human serum sample with a high concentration of cTnI showed no significant curvature when the curve obtained was tested for linearity [quadratic regression: $y = -0.25 + 96.1x + 6.31x^2$, with the coefficient of $x^2$ not significantly different from 0 ($P = 0.27$)] [2]. Linear regression analysis of these data confirmed the high linearity of the response ($r = 0.9998$). The minimum detectable cTnI concentration, assessed by 10 replicate measurements of a human serum containing no detectable cTnI concentration and defined as the cTnI value corresponding to the fluorescence signal 3 SD greater than the mean found for this serum, was estimated as 0.38 μg/L. The Opus analyzer, however, reports results <0.50 μg/L as “<0.5 μg/L.”

Assay reproducibility was tested by assaying in duplicate, once a day for 10 days, two serum samples with concentrations distributed over the measuring range and the three kit controls containing human cTnI [3]. Analysis of variance showed within-run CVs between 3.4% and 7.2% and total CVs between 5.6% and 13.0%. No interferences were detected in assays of lipemic (triglycerides >2.5 g/L) or hemolyzed (hemoglobin >2.5 g/L) specimens; concentrations of bilirubin >50 mg/L spuriously increased the reported cTnI concentrations in serum.

To compare the Opus assay with the cTnI Pasteur immunoenzymometric assay (Sanofi Diagnostics Pasteur, Marnes-la-Cooqette, France; performed manually according to the manufacturer’s current protocol [4]), we assayed 85 unselected individual serum samples with detectable cTnI concentration (>0.04 μg/L as measured with the Pasteur assay). The correlation was good ($r = 0.959$), but the data showed considerable scatter ($S_{yx} = 10.6 μg/L$), the Opus results being relatively higher within the mid-range of values but equal to
the Pasteur results at concentrations outside the limit of linearity of the instrument. As a whole, the Opus assay led to approximately 10-fold higher values than the Pasteur assay (linear regression equation: Opus = 11.1 Pasteur + 1.5 \mu g/L). Differences in the calibration materials and in the ways their theoretical values are assigned in the two methods may partially explain this observation. When the three calibrators supplied with the Pasteur kit (S1, S2, and S3 with assigned cTnI concentrations of 0.13, 0.55, and 1.56 \mu g/L, respectively) were determined with the Opus assay, the results were as follows: 1.21, 4.39, and 11.3 \mu g/L, respectively.

We used the Opus procedure to measure the concentration of cTnI in sera from subjects in four groups: (a) 46 apparently healthy people, ages 26–82 years; (b) 21 patients with a typical history of myocardial infarction (MI) of <8 h duration, not treated with thrombolytic therapy; (c) 8 patients with severe skeletal muscle damage [total creatine kinase (CK) values 10 240 to 226 000 U/L] but no apparent cardiac injury; and (d) 39 consecutive dialysis patients with no evidence of myocardial injury. CK-MB mass concentrations were determined with the Magic Lite assay (Ciba Corning Diagnostic Corp., Medfield, MA). No cTnI was detected in the healthy subjects (all values <0.5 \mu g/L). In patients with MI, cTnI peaked at 20.8 ± 8.1 h (range, 8–33 h) after the onset of chest pain, reaching a mean peak concentration of 165 \mu g/L (range, 3.3–1674 \mu g/L). Comparing their upper reference limits, we noted a higher relative increase at peak in cTnI than in CK-MB mass concentration (mean, 330- vs 47-fold). Furthermore, unlike the short duration of increased CK-MB concentrations, cTnI remained diagnostic over all the period tested, i.e., from the arrival at the hospital until 96 h after onset of pain, being still >0.5 \mu g/L in 16 of 18 samples obtained at day 4 post-MI (Table 1).

Consistent with the manufacturer’s declared cross-reactivity of <0.007% with skeletal troponin I [1], cTnI was undetectable in all but one of the patients with severe skeletal muscle damage or chronic renal failure. That patient had a slightly increased cTnI (1.0 \mu g/L) while receiving continuous ambulatory peritoneal dialysis; clinical evaluation, however, revealed no evidence of myocardial injury.

We conclude that the Opus method has the potential to become a valuable aid in specific detection of myocardial injury.

We thank Istituto Behring, Milan, Italy, for supplying reagents for the cTnI assay.

References

Mauro Panteghini*  
Roberto Bonora  
Franca Pagani  
Clin. Chem. and Enzymol. Section  
Spedali Civili  
25125 Brescia, Italy

*Author for correspondence.