Detection of Unexpected Isoforms of Human Chorionic Gonadotropin by Qualitative Tests

To the Editor:

Human chorionic gonadotropin (CG) is a heterodimeric glycoprotein hormone composed of noncovalently associated α and β subunits that are synthesized by trophoblastic tissue in pregnancy. Systemic modification and degradation of the intact CG molecule and subunits leads to molecular heterogeneity in the serum and urine (1). The β subunit is a component of nicked CG (CGn), CG β-subunit (CGβ), nicked CG β-subunit (CGβn), and CG β-core fragment (CGβcf).

Qualitative urine testing for CG is employed in point-of-care and laboratory settings because it is a rapid and effective pregnancy screen. These tests typically detect 20 to 25 IU CG/L; other properties of the devices are shown in Table 1. Each device was tested and interpreted 5–10 times with the prepared urines, according to the manufacturer's instructions.

As shown in Table 1, all qualitative CG devices detected dimeric CG isoforms (CG and CGn) but only the Osom Card Pregnancy test did so exclusively. The remaining devices detected CGβ and CGβn. CGβcf was consistently detected by the Clinitest.

Table 1. Characteristics of 6 qualitative CG devices and results of qualitative and quantitative urine tests using various CG isoforms.

<table>
<thead>
<tr>
<th>Capture antibody specificity, type</th>
<th>Label antibody specificity, type</th>
<th>4th IS-CG</th>
<th>CGn</th>
<th>CGβ</th>
<th>CGβn</th>
<th>CGβcf</th>
<th>CGα</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-α (u)</td>
<td>Anti-β (m)</td>
<td>10/10</td>
<td>10/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-CG dimer (u)</td>
<td>Proprietary (p)</td>
<td>10/10</td>
<td>10/10</td>
<td></td>
<td>10/10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QuickVue+</td>
<td>Anti-α (m)</td>
<td></td>
<td>10/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osom</td>
<td>Anti-β (m)</td>
<td></td>
<td></td>
<td>5/5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QuickVue+</td>
<td>Anti-α (m)</td>
<td></td>
<td>10/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osom</td>
<td>Anti-β (m)</td>
<td></td>
<td>10/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hCG Combo</td>
<td>Anti-α (m)</td>
<td></td>
<td>10/10</td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICON II</td>
<td>Anti-β (m)</td>
<td></td>
<td></td>
<td>10/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elecsys*, IU/L, pmol/L</td>
<td>Anti-β (m)</td>
<td>1220</td>
<td>2263</td>
<td>7800</td>
<td>2336</td>
<td>8800</td>
<td>630</td>
</tr>
<tr>
<td></td>
<td>Anti-α (m)</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data for the qualitative tests are shown as the number of positive test results per total tests performed.

<sup>a</sup> Results are the mean of 2 measurements.

<sup>b</sup> Molar concentrations are based on the ampoule unitage provided by the National Institute for Biological Standards and Controls.

<sup>c</sup> Data obtained from manufacturer’s package insert and/or technical support representative (m = monoclonal; p = polyclonal; u = unspecified).

<sup>d</sup> The 4th International Standard, 1999, Chorionic Gonadotropin, human, contains 650 IU lyophilized CG per ampoule.
hCG and hCG Combo SP, whereas it was inconsistently detected by QuickVue. No device detected CGα.

These data support the hypothesis that some qualitative CG devices detect nondimeric isoforms, despite being designed to detect only heterodimeric CG isoforms. Given the previously described purity of the WHO reference reagents (3), the most likely explanation for the detection of nondimeric isoforms is that the specificities of the antibodies used by device manufacturers are incompletely characterized and may react with numerous CG isoforms. Unfortunately, details of the selectivity of these antibodies are proprietary information.

Unless a clinical advantage is identified, there is limited motivation to demonstrate that pregnancy screening tests detect isoforms other than intact CG. Detection of other forms of CG may be important for early detection of pregnancy. McChesney et al. (4) reported that in urine samples collected during early pregnancy, intact CG is sometimes transiently undetectable and sometimes present in lower concentrations than nondimeric isoforms. Similarly, Butler et al. demonstrated that hyperglycosylated CG is the predominant isoform in early pregnancy urine and advocated that assays be optimized to detect it (5). Investigating whether the different specificities (analytical selectivities) of qualitative urine tests affect diagnostic sensitivity is part of an ongoing study that includes further characterization of the concentrations of CG isoforms in early pregnancy urine.

References

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Novel Hemoglobin (Hb Grey Lynn) Substitution (α91Leu→Phe) Affects Heme Interactions and α₂β₂ Contacts

To the Editor:

Since the discovery of the causative mutation of sickle cell anemia, hemoglobinopathies have become the iconic molecular diseases, with some 900 mutations reported to date (http://globin.cse.psu.edu). Detection and characterization of novel mutations, facilitated by increasingly sensitive analytical techniques, continue to shed light on how molecular structure and function are preserved in the tetrameric molecule. Here we report the identification of a novel α chain substitution (α91Leu→Phe) in a patient who was also heterozygous for the αSEA 2-gene deletion.

A full blood count on this 40-year-old woman revealed a microcytic picture with a hemoglobin (Hb) concentration of 103 g/L, a mean cell volume of 63 fL, and a hematocrit of 0.33. These results prompted a hemoglobinopathy/thalassemia screen that revealed a normal cellulose acetate electrophoresis pattern, a marginally positive isopropanol stability test, and values within reference intervals for HbA₂ (3.0%), HbF (2%), and reticulocytes. Occasional HbH bodies were seen, and the Bio-Rad Variant cation exchange system for β-thalassemia revealed an aberrant component of 35.5% in the HbA₂ window.

Examination of whole lysate by electrospray ionization mass spectrometry on a VG Platform (1) indicated a new α-chain with a mass increase of 34 Da (Fig. 1A). Reversed-phase HPLC on a C-4 Jupiter column revealed a hydrophobic shoulder on the α-chain peak (not shown), and mass analysis indicated that this trailing edge was enriched in the +34-Da chain. Tryptic mass mapping (1) of these fractions within the range 300–1550 m/z proved uninformative, however.

The entire coding regions of both the hemoglobin, alpha 1 and hemoglobin, alpha 2 genes were individually amplified from genomic DNA using the primer pairs 5’-TGG AGG GTG GAG ACG TCC TG-3’ with 5’-CCA TGC TGG CAC GTT TCT GA-3’, and 5’-TGG AGG GTG GAG ACG TCC TG-3’ with 5’-CCA TTG TTG GCA CAT TCC GG-3’, respectively, and sequenced on an ABI 3130x/I genetic analyzer with Big Dye Terminator v.31 cycle sequencing chemistry according to manufacturer’s recommendations. This revealed apparent homozygosity for a CTT→TTT transition at codon 91 of the α1 gene. We have named the novel α91Leu→Phe substitution “Hb Grey Lynn” after the suburb of Auckland. This replacement of an alkyl leucine side chain by an aromatic phenylalanine is entirely consistent with the observed mass increase of 34 Da, and with both the increase in hydrophobicity and the normal electrophoretic pattern.

The thalassemic blood picture together with the occasional HbH bodies seemed more likely to be attributable to deletional α-thalassemia than homozygosity for the novel α gene mutation. Further screening for the α gene locus for the common α(+) , α(-2), α(+) , α(-2), α(+) , α(-3.2), α(-4.2), αSEA , FIL , MED , D, and α(+) deletions (2) did in fact show that this patient
was heterozygous for the αSEA deletion, which removes both the α1 and α2 genes from 1 allele, leaving only 2 functional α genes. This finding explains the comparatively high expression level (40%) of the variant α chain. In an alignment of 449 α globin sequences from different species, α91 is absolutely conserved as leucine, and this extraordinary level of conservation suggests an important functional role. X-ray structural analyses show that the alkyl side chain heads internally toward the heme plate, where it comes to within 2.4 Å of the Fe²⁺-bound proximal histidine (α87) and within 3.6 Å of the heme (Fig. 1B). Its main chain carbonyl is located on the surface and forms an H-bond with the guanidine group of Arg40 in the adjacent β2 subunit. On oxygenation, movements of the heme Fe²⁺ are transmitted through α87His and α91Leu onto the adjacent β2 subunit to regulate cooperatively and produce the characteristic sigmoidal O₂ binding curve (3). As this model correctly predicts, mutation of β40 Arg also impacts O₂ binding; its mutation to Ser in Hb Austin alters both affinity and cooperativity (4).

There are 2 predictable consequences of the novel α91Leu→Phe mutation, altered O₂ affinity and decreased molecular stability. We were unable to measure O₂ affinity, but we were able to demonstrate decreased molecular stability. A small amount of precipitate was collected after extending a standard isopropyl alcohol test to 40-min incubation. The precipitate was then dissolved in 1% formic acid and analyzed directly by mass spectrometry (Fig. 1A). This showed selective precipitation of the variant Hb, which was enriched from 40% in lysate to 56% in the precipitate. Only one mutation has been previously reported at this position, α91Leu→Pro. In this case the less conservative mutation was associated with mild anemia and a more marked instability (5).

This case highlights the importance of investigating abnormal Hbs at biochemical, hematological, and genetic levels to understand the pathological implications of the mutation and the risks it poses to carriers.

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References

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A Multiplex Quantitative Fluorescent PCR Test for Prenatal Diagnosis of Hb Barts Hydrops Fetalis

To the Editor:
I read with interest the paper by Ho et al. (1), which described a rapid prenatal diagnostic test for simultaneous detection of Hb Barts hydrops fetalis and exclusion of maternal contamination. The main background to their study is that PCR-based techniques are highly sensitive and are also prone to false-positive results in prenatal diagnosis due to amplifica-
tion of contaminating maternal DNA that may be present in the fetal samples. Current established PCR-based diagnostic tests require a separate test to exclude maternal contamination. Hence, the authors developed a novel quantitative fluorescent PCR (QF-PCR) assay that allowed diagnosis and exclusion of maternal contamination in one step. Their approach seemed excellent, with a sensitivity of 100% and specificity of 100%. However, I believe that the presented data did not justify use of the strategy in routine clinical practice.

First, the authors might add details about the incidence of misdiagnosis due to maternal contamination in fetal sampling. They cited a misdiagnosis rate of 3.8% attributable to maternal DNA contamination (2). Actually, this is a high rate of misdiagnosis, unacceptable for an experienced center. We acknowledge that maternal contamination is unavoidable in prenatal sampling, but measures can be taken to make contamination less likely. This problem is usually due to the inexperience of the operator at the time of sampling or of the laboratory technologist engaged in analyzing and separating the fetal material obtained. In our experience, the risk of maternal contamination in chorionic villous samples is remote with careful microscopic dissection to remove contaminating maternal decidua. For amniocentesis, samples contaminated heavily by blood can be cultured for 10–14 days before analysis. Culture of the cells reduces risk of maternal contamination. Although availability of a technique for differentiating tests is important, a low incidence of maternal contamination is vital. A QF-PCR test can easily detect the contamination, but it is no help to diagnosis. When maternal contamination is confirmed, a repeat sampling is usually required, which would add to the risk of contamination (i.e., 3.8%). In our experience in fetal sampling, the misdiagnosis rate due to maternal contamination in all fetal samples is ≤1%.

Second, the QF-PCR approach might not decrease the cost for diagnosis. This technique usually requires both special materials, which increase adds to the cost of regular PCR, and instruments that are not available at most centers. In fact, clinical decision-making for pregnancies at risk for Hb Barts hydrops fetalis has been changing in regions with a high prevalence of α-thalassemia. Ultrasonography now plays a major role in early detection of Hb Barts hydrops fetalis as early as 12–13 weeks, and can effectively differentiate normal pregnancies from those requiring an invasive evaluation (3–5), thus limiting invasive procedures to the few pregnancies identified to be at high risk by ultrasonography. This means that in 3 of 4 at-risk pregnancies, invasive procedures, which carry a 1% chance of miscarriage, are avoided. Because DNA study is relatively expensive, the medical saving might be noticeable. For the few centers with established QF-PCR services for aneuploidy testing, adding this multiplex approach to αSEA diagnosis would be simple and likely to be cost-effective.

Finally, not all prenatal testing requires a test for exclusion of maternal contamination suggested by the authors. For example, we use a regular multiplex Gap-PCR test to detect αSEA mutation of the α-globin gene in fetal samples. If the genotype of the fetus is revealed to be αSEA/αSEA or αα/αα, there is no need to do a differentiating test. If the fetus is revealed to have the same genotype as the mother (αSEA/αα), maternal contamination should be considered. However, due to the high sensitivity and specificity of ultrasonography in detection of Hb Barts hydrops fetalis, few cases require a repeated sampling.

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References

The authors of the article cited above respond:

Diagnosis of a fetal genetic condition requires fetal genetic material, which to date generally implies the need for invasive testing. Ultrasound for evidence of a hyperdynamic circulation can be used as a screening tool to identify at-risk pregnancies, but it is not diagnostic. In time perhaps the use of fetal DNA circulating in maternal plasma may offer more direct evidence of fetal thalassemia status. Many prenatal diagnostic centers now offer quantitative fluorescent PCR routinely for rapid aneuploidy detection. Our test can be readily implemented at only marginal cost. In fact, it eliminates the need for multiple primer sets in several double-deletion α-thalassemia syndromes.

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Rapid Differential Diagnosis between Subarachnoid Hemorrhage and Traumatic Lumbar Puncture by D-Dimer Assay

To the Editor:

Subarachnoid hemorrhage (SAH) and traumatic lumbar puncture (TLP) are both associated with blood-tinged cerebrospinal fluid (CSF). It is often difficult if not impossible to distinguish between SAH and TLP (1,2) by available CSF tests such as (a) measurement of xanthochromia of the supernatant after centrifugation, (b) counting of erythrocytes in the first and last tubes of collected CSF, or (c) searching of CSF microscopically for crenated erythrocytes (3–5).

We investigated the measurement of D-dimer (DD) in CSF and evaluated DD measurement as a means to distinguish between SAH and TLP.

We measured DD in CSF with the coagulation analyzer STA Compact® by the immunoturbidimetric assay, STA Liatest D-Di® (Roche Diagnostics). Erythrocyte counts were performed by optical microscopy with a Fuchs-Rosenthal chamber; CSF was considered to contain blood (CSF-H) when the count exceeded 500 erythrocytes/μL. CSF samples were obtained from the hospital emergency room and the intensive care unit. On arrival in the laboratory, the samples were inspected visually and an erythrocyte count was performed. Samples were then centrifuged and the supernatant collected and stored at −20 °C until groups of 10 were accumulated. Before processing, samples were thawed by incubation at 37 °C for 10 min, mixed by inversion, and allowed to settle for 15 min at room temperature before DD determination.

Between July 2005 and March 2006, we studied 146 CSF samples obtained from patients with ages ranging from <1 month to 82 years. The clinical histories of the patients were reviewed to determine the clinical diagnoses. Samples were classified as SAH samples, from patients with SAH clinically diagnosed by diagnostic computed tomography (CT) scan, or control samples, from patients with CSF-H due to TLP and from patients with nonhematic CSF.

Statistical analyses were performed with SPSS 12 for Windows. Because the Kolmogorow-Smirnov test showed that results did not follow a gaussian distribution, the non-parametric Mann–Whitney U-test was used to evaluate the difference between the 2 means. A P value <0.05 was considered statistically significant. The DD discrimination value that provided 100% sensitivity was established at 0.5 mg/L. Hence, we considered DD values ≥0.5 mg/L to be increased.

Of 146 samples collected during the study, 62 (42%) were hematic (CSF-H), of which more than half [35 (57%)] were from patients with an SAH diagnosis corroborated by diagnostic computed tomography CT scan. All SAH patients were adults and had CSF-H. The other cases of CSF-H (43%) were considered to be attributable to TLP, which represented 18% of the total CSF specimens.

A DD ≥0.5 mg/L was observed in 54 of 146 samples. All CSF-H with SAH were DD positive. Of the CSF-H without SAH final diagnosis, 70% (19) were DD negative (DD <0.5 mg/L). Of the nonhematic CSF, 11 (13%) had a positive DD value, 7 of which were pediatric. In adults, the mean (SD) DD concentration in CSF-H with SAH was significantly higher (P <0.0001) than that in CSF-H without SAH, 4.2 (2.9) mg/L and 0.5 (1.0) mg/L, respectively.

Distributions of DD concentrations in CSF in both groups (CSF-H with SAH and CSF-H without SAH) are shown in Fig. 1. The concentrations in SAH showed little overlap with concentrations in controls.

A potential limitation of the study was that some patients with SAH may have been negative on CT scan and thus included as controls, leading to overestimation of the sensitivity of the test. As we used only one analytical method, we cannot comment on the performance of other methods. In addition, the study was small. These considerations warrant caution in interpreting results and suggest a need for further studies to determine the diagnostic accuracy of DD testing of CSF.

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References


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Blood Lead Misclassification Due to Defective LeadCare® Blood Lead Testing Equipment

To the Editor:
In May, 2005, a partial recall of LeadCare® portable blood lead testing kits was initiated after proficiency testing revealed a negative error (mean, −25%; 95% CI, −15% to −35%) in blood lead concentrations (often called blood lead level in the US) analyzed with this device (1, 2). This bias exceeded previously recommended 95% error thresholds established with graphite furnace atomic absorption spectroscopy (3). The recall included 8 lots of defective sensors containing enough material for an estimated 500,000 patient tests distributed between September 2003 and May 2005.

The LeadCare device uses an anodic stripping voltammetry method to measure lead. Capillary sampling by fingerstick is the most common method of specimen collection. Assessments of the portable units by testing of venous samples from occupationally exposed workers have demonstrated that measurements obtained with these units show an insignificant positive error that decreases as blood lead concentrations increase (4).

A blood lead concentration ≥10 μg/dL (100 μg/L) has been established as the concentration at which children should receive clinical intervention (5).

We contacted 15 US laboratories identified as users of the analyzer by ESA Inc., the manufacturer of the testing unit. A total of 26,883 results were provided by 8 (53%) of 15 testing sites. Capillary samples were used to obtain all results reported in this study.

Data were recoded and classified according to age group (0–6, 7–15, or ≥16 years), test date (January 1–August 31, 2003, and September 1, 2003–June 30, 2005), recall status, and blood lead concentration category. Time periods for this study were determined by the market availability of defective sensors. Time period comparisons were limited to facilities with data from both time periods and with sufficient sample size (n = 15,024). Of 573 total test results, 12 included the lot number of the sensor. One facility provided both test and retest results (n = 95).

The median age of persons tested was 2 years (range 0–94 years), with 91% of all tests performed on children <6 years old, 7% on children 7–15 years old, and 3% on children and adults ≥16 years old.

Compared with mean blood lead concentrations collected before September 2003, the mean result of tests performed from September 2003 to May 2005 was 29% lower (P < 0.001) for children ≤6 years old and 22% lower for children 7–15 years old (P < 0.001). For children ≥6 years old, blood lead concentrations ≥10 μg/dL (100 μg/L) were found in 2.3% tested before September 2003 and in 1.7% tested after August 2003 (P = 0.01).

Children tested with the defective sensors were 4.5 times more likely to have blood lead concentrations ≥10 μg/dL (100 μg/L). When a defective sensor was used, the measured percentage of blood lead concentrations ≥10 μg/dL (100 μg/L) decreased in both the 0–6 year (P < 0.001) and 7–15 year (P = 0.01) age categories. Table 1 shows an outcome matrix comparing differences in the initial and retest results. An increase of 49.7% was observed in the prevalence of retest results that were above clinically important threshold values and thus sufficient to place the patient in a higher clinical category.

These analyses show a negative error among blood lead measurements obtained with defective sensors. These errors led to substantial misclassification, with nearly a 50% rate of underestimation of the prevalence of concentrations above clinically relevant thresholds, and may have resulted in misclassified patients receiving delayed or no treatment. The findings reported here have several limitations. Our analyses are based on the results from a limited number of testing sites. It is possible that the smaller time period before introduction of the defective sensors may influence results. In addition, our estimate of misclassification among those with retest results is based on a small sample of individuals from a single facility and solely on the percentage of persons whose retest result placed them in a higher clinically significant category. Nevertheless, this estimate is consistent with expected proportions of children with blood lead concentrations higher than selected thresholds, given a log normal distribution.

On the basis of these findings, we recommend retesting of individuals whose blood lead concentrations were ≥6 μg/dL (60 μg/L) when measured with the LeadCare device between September 2003 and May 2005. We also recommend that LeadCare be not used for confirmatory testing of children with increased blood lead concentrations, because validation studies have evaluated the instrument only in the range of 1–42 μg lead/dL (10–420 μg/L) (4). This study underscores the value of proficiency testing as well as the importance of laboratory procedures that may identify unexpected results over time.

Table 1. Test/retest differences by clinically important blood lead thresholds (n = 95).

<table>
<thead>
<tr>
<th>Age Group</th>
<th>0–9 (retest)</th>
<th>10–14 (retest)</th>
<th>15–19 (retest)</th>
<th>20–44 (retest)</th>
<th>45–69 (retest)</th>
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<tbody>
<tr>
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<td>7</td>
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<td>45–69 (test)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers in bold type are the number of repeated tests above clinically important thresholds.

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Editor’s Note: Results are expressed in this Letter in conventional units first because these units are the ones familiar to many of the intended readers of this report.
Myocardial Infarction with Normal Coronary Arteries: A Role for MRI?

To the Editor:

We recently cared for several patients with chest discomfort characteristic of coronary disease, electrocardiogram (ECG) changes, and increasing troponin concentrations. Coronary angiography revealed mild or no coronary artery disease (CAD), so we evaluated these patients with contrast-enhanced cardiac MRI. Our data suggest that these patients had myocardial infarction (MI).

With Institutional Review Board permission, we reviewed our angiography database for patients with normal coronary arteries or mild coronary artery disease during the period from January 2005 to November 1, 2006, to augment those cases we found clinically. Inclusion criteria included presentation with acute coronary syndrome, increased serum troponin T concentration (>0.01 μg/L), or mild or absent CAD by angiography and a contrast-enhanced cardiac MRI (CE-CMR) considered diagnostic for infarction. Patients with prior infarction, known CAD, heart failure, pulmonary embolism, or suspected pericarditis/myocarditis were excluded. Eight patients met these criteria. All angiogram results were reviewed by one of the investigators (V.M.).

CE-CMR studies performed on a GE Signa CVi system (GE Medical Systems) were reviewed by J.F.B., who had no knowledge of other clinical data. Regional wall motion was characterized as normal, hypokinetic, or akinetic. Delayed enhancement was described as involving the endomyocardial border and transmural as involving the epicardial border alone without transmural extension or a midseptal myocardial stripe. Only solitary areas of subendocardial enhancement were considered diagnostic for infarction.

Six of the 8 identified patients (75%) were women. Risk factors for CAD were present in all patients except one. Hypertension, present in 75%, was the most common risk factor. Troponins increased had a rising and falling pattern, with a mean peak increase of 1.10 μg/L (range, 0.34–2.13 μg/L). Patterns of delayed hyperenhancement involved only the subendocardium (Fig. 1). Five of the patients had enhancement involving ≤50% of the myocardial wall; the remainder had at least 50% hyperenhancement. The inferior and lateral walls were involved in 6 patients (75%); the apical wall only was involved in 1 patient and anterior wall only in 1 patient. Mean ejection fraction, determined by MRI, was 60% (range, 40%–74%). Vasospasm was found in 2 of 3 patients who underwent coronary vasospasm study. All patients were treated for MI, and all were alive at a mean of 26 months (range, 6–59 months) later.

A subset of patients with clinical findings characteristic of acute MI have no or mild CAD. These patients meet the definition for acute MI, with increases in troponin combined with ischemic symptoms, pathologic Q waves, and/or ischemic ECG changes (1). In the absence of the finding of occlusive epicardial CAD, however, there is reluctance to diagnose infarction in these patients because troponin increases, although specific for cardiac damage, can occur for many reasons.

Ischemia and even myocyte injury can occur in the absence of overt epicardial CAD. It is possible that coronary occlusion resolved before angiography, but we also acknowledge that coronary angiography is...
not a perfect tool (2). Epicardial CAD or alterations in coronary vasomotion that changed the severity of epicardial coronary lesions could have been missed or underestimated. We believe that mechanisms such as these were the most likely etiologies for the troponin increases in our patients. Although troponin increases can occur in response to moderate to severe pulmonary embolism with acute right heart overload, heart failure, and myocarditis, these were not present clinically in our patients. CE-CMR is an accurate method for detecting MI and has high sensitivity and excellent spatial resolution (3). The delayed hyperenhancement seen in our patients is not specific for infarction, but we observed solitary subendocardial locations typical for MIs (4), and we were able in all cases to rule out other diseases that present this way. We cannot exclude myocarditis, but the changes seen on cardiac MRI were not typical for this disorder (5), which usually shows one or several foci of contrast enhancement, most often in the left epicardial ventricular free wall. We are unaware of documented cases of myocarditis involving a solitary area of the subendocardium. Our data are similar to a small series recently reported (2) in that most patients were women and most of the abnormalities were in the inferior wall.

In these patients presenting symptoms typical of ischemia accompanied by ECG changes and biomarkers classic for MI, the MRI images were also diagnostic. Perhaps MRI detected old insults, but our data suggest that when patients have a classic presentation, one needs to be cautious in relying solely on angiography for definitive information.

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References

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Correction

In the article entitled “Identification of a New Metabolite of Astilbin, 3'-O-Methylastilbin, and Its Immunosuppressive Activity against Contact Dermatitis”, by Jianming Guo, Feng Qian, Jianxin Li, Qiang Xu, and Ting Chen (Clin Chem 2007;53:465–471; DOI: 10.1373/clinchem.2006.077297), Table 2 was aligned incorrectly, and entries were lost in the bottom-most row. The correct table is provided below. The printer regrets this error.

Table 2. $^{13}$C NMR spectral data of astilbin and methylated astilbin.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Position</th>
<th>Astilbin $^{13}$C NMR</th>
<th>3'-O-Methylastilbin $^{13}$C NMR</th>
<th>$^{13}$C-$^1$H long-range COSY (C$^\rightarrow$H), 3'-O-Methylastilbin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-2</td>
<td>84.8</td>
<td>85.4</td>
<td></td>
</tr>
<tr>
<td>C-3</td>
<td>79.4</td>
<td>79.9</td>
<td></td>
</tr>
<tr>
<td>C-4</td>
<td>196.7</td>
<td>197.3</td>
<td>2, 3</td>
</tr>
<tr>
<td>C-5</td>
<td>166.3</td>
<td>166.9</td>
<td>6</td>
</tr>
<tr>
<td>C-6</td>
<td>98.2</td>
<td>97.8</td>
<td></td>
</tr>
<tr>
<td>C-7</td>
<td>169.7</td>
<td>170.2</td>
<td>6, 8</td>
</tr>
<tr>
<td>C-8</td>
<td>97.2</td>
<td>98.3</td>
<td></td>
</tr>
<tr>
<td>C-9</td>
<td>164.9</td>
<td>165.4</td>
<td></td>
</tr>
<tr>
<td>C-10</td>
<td>103.2</td>
<td>103.5</td>
<td>6, 8</td>
</tr>
<tr>
<td>C-1'</td>
<td>130.0</td>
<td>130.8</td>
<td>2, 5'</td>
</tr>
<tr>
<td>C-2'</td>
<td>117.1</td>
<td>113.4</td>
<td>2, 6'</td>
</tr>
<tr>
<td>C-3'</td>
<td>147.3</td>
<td>150.5</td>
<td>5', 3'-OCH$_3$</td>
</tr>
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<td>148.1</td>
<td>149.9</td>
<td>2', 6'</td>
</tr>
<tr>
<td>C-5'</td>
<td>116.3</td>
<td>117.6</td>
<td></td>
</tr>
<tr>
<td>C-6'</td>
<td>121.3</td>
<td>123.3</td>
<td>2, 2'</td>
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<tr>
<td>Rhamnose</td>
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<tr>
<td>C-1''</td>
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<td>103.8</td>
<td>3, 1''</td>
</tr>
<tr>
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<td>73.2</td>
<td></td>
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<tr>
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<td>72.9</td>
<td>73.6</td>
<td>4'', 1''</td>
</tr>
<tr>
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<td>19.2</td>
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<td>OCH$_3$</td>
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<td>57.9</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Recorded in CDOD$_3$ at 300 MHz; chemical shift (\delta) values in ppm.