Ectopic Production of Creatine Kinase MB: Updated Evaluation by Mass Assays

To the Editor:

There have been several reports of apparently high concentrations of creatine kinase (CK)-MB in the sera of cancer patients [1–4]. In several of these cases, the high activity resulted from the presence of macro CK types 1 and 2 [5]. False-positive results for CK-MB can occur if nonspecific assays such as immunoinhibition are used [6]. These atypical isoenzymes are not inhibited by anti-CK-B antibodies, and high apparent CK-MB activities are produced. Macro CK type 1 (CK-BB bound to IgG) migrates between CK-MM and -MB, whereas type 2 (polymeric mitochondrial CK) migrates cathodic to CK-MM and can be readily differentiated from CK-MB by electrophoresis [7]. However, macro CK-BB linked to IgA can comigrate with CK-MB, and false-positive detection of MB is possible even with electrophoresis [8].

Annesley and McKenna [9] published the one clear case of ectopic CK-MB production by a tumor, by demonstrating high concentrations of CK-MB in homogenized tumor tissue and ruling out the presence of macro CK forms by heat stability tests. We describe a second case in which persistently high concentrations of CK-MB were detected in a patient with metastatic cancer. CK isoforms, myoglobin, lactate dehydrogenase isoenzymes, and cardiac troponins T and I were used to rule out the presence of acute myocardial injury.

A 71-year-old white man with a past medical history of coronary artery disease, acute myocardial infarction (AMI), hypertension, congestive heart failure, cerebrovascular accident, carotid stenosis, and atrial fibrillation presented to the Hospital of St. Raphael with a 4-day history of bright red blood per rectum, right lower quadrant pain, and hypotension. He was admitted to the Intensive Care Unit. An upper endoscopy revealed a gastric ulcer that appeared benign on gross inspection. No biopsies were performed. The gastrointestinal hemorrhage subsided with conservative therapy. Although his electrocardiogram was normal, his past history of coronary artery disease prompted testing for cardiac markers. Total CK and CK-MB were persistently increased over the course of this hospitalization with a relative index ranging between 21% and 30% (reference value <2.5%). Total lactate dehydrogenase was also increased, with an isoenzyme pattern containing a predominance of lactate dehydrogenase 5. A diagnosis of AMI was ruled out by the cardiologist. An abdominal CAT scan was obtained to further elucidate the source of his right lower quadrant tenderness. The liver showed marked hepatomegaly with diffuse hypodense nodules consistent with metastatic carcinoma of unknown primary. The patient was discharged to a hospice were he died several days later. Permission for an autopsy was denied.

Total CK and CK-MB (mass assay) were measured at the Hospital of St. Raphael with BM/Hitachi 747® (Boehringer Mannheim) and AxSYM® (Abbott Laboratories) analyzers, respectively. A subset of serum samples were sent to Hartford Hospital for further analysis. CK-MB was assayed on Opus Plus® (Behring Diagnostics), Access® (Sanofi Pasteur Diagnostics), and the ACS:180® (Ciba Corning), and CK isoenzymes and isoforms were assayed with the CardioRep® (Helena Labs.) [10]. Myoglobin and cardiac troponin I were assayed on the Opus [11], and cardiac troponin T (cTnT) was assayed on the ES300® (Boehringer Mannheim) [12]. To determine if heterophile antibodies were present, 20 µL of mouse IgG (Sigma) was added to a 200-µL aliquot of serum and incubated for 4 h at room temperature [13]. The mixture was assayed for CK-MB on the Opus.

Table 1 lists the results of the tests performed on several samples tested to further characterize the enzymes and proteins present in these serum samples. Each of the CK-MB mass assays showed increased concentrations exceeding the reference range by at least 10-fold. Because human anti-mouse antibodies can interfere with immunoassays that make use of murine monoclonal antibodies, mouse IgG was added to determine if this would remove the potential interfering agent. Table 1 shows that although a substantially lower residual result was present than was expected after dilution (45.1 µg/L), the high residual activity suggested that human anti-mouse antibodies could not be responsible for the majority of the apparent CK-MB present. The ratio of cardiac isoforms (MB2/MB1) showed results that were below the reference limit. This ratio may have been falsely low because isoforms were not originally ordered; therefore, the samples had not been properly preserved with EDTA to prevent in vitro isoform conversion before analysis [14]. The measurements were made to verify the presence of two CK-MB isoform bands, which ruled out the presence of a macro CK-BB:IgA complex comigrating with CK-MB (as one band would have been expected). Myoglobin concentration was normal, indicating no active heart, skeletal muscle, or renal disease. Results for both cardiac troponin T and I were below the cutoff concentration, demonstrating the absence of true cardiac injury.

From these results, it is possible that this is another case of ectopic CK-MB production. We were able to better characterize this isoenzyme as being CK-MB because more specific assays are available today for measuring this isoenzyme than at the time of the Annesley report [9]. Moreover, use of new cardiac-specific markers (cardiac troponin T and I) allowed confident exclusion of myocardial injury as a source of increased serum CK-BB (Table 1). The presence of macro CK forms were ruled out because macro CK does not cross-react with either the Conan anti-CK-MB antibody [15] (used in most commercial mass CK-MB assays) or the anti-CK-MB antibody used in the ACS:180 assay (which was developed separately from the Conan antibody). We also selected different mass assays for CK-MB to...
Table 1. Additional cardiac marker studies conducted.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Results</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB, Opus</td>
<td>66.2</td>
<td>0–5 μg/L</td>
</tr>
<tr>
<td>CK-MB, Opus + mouse IgG*</td>
<td>45.1</td>
<td>0–5 μg/L</td>
</tr>
<tr>
<td>CK-MB, Access</td>
<td>74.1</td>
<td>0–5 μg/L</td>
</tr>
<tr>
<td>CK-MB, ACS:180</td>
<td>51.1</td>
<td>0–5 μg/L</td>
</tr>
<tr>
<td>CK, isofrom, %MB</td>
<td>54.0%</td>
<td>0–4%</td>
</tr>
<tr>
<td>CK, isofrom, electrophoresis, MB/MBa</td>
<td>0.42</td>
<td>0.55–1.33</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>24.9</td>
<td>0–62 μg/L</td>
</tr>
<tr>
<td>cTnT, ES300</td>
<td>0.01</td>
<td>0–0.1 μg/L</td>
</tr>
<tr>
<td>cTnl, Opus</td>
<td>&lt;0.5</td>
<td>0–0.5 μg/L</td>
</tr>
</tbody>
</table>

* 200-μL sample plus 20 μL of mouse IgG (Sigma).

rule out the presence of an interfering agent that might have interacted with the assay’s detection method. Demonstration of CK-MB in tumor biopsy would have confirmed the presence of ectopic production. Several other possible explanations for these observations exist. As summarized by Chan et al. [16], CK-MB can be increased by release from nonmyocardial sources (e.g., trauma to skeletal muscles, grand mal seizures, inflammatory and non-inflammatory myopathies, etc.), cardiac injury other than AMI (e.g., cardiac contusions, myocarditis), and as a result of decreased clearance of serum CK-MB (in patients with hyperthyroidism and hypothyroidism). A high fraction of CK-MB relative to total CK can also be observed after extensive muscle damage or regeneration (such as in marathon runners or patients with Duchenne muscular dystrophy) [17, 18]. We have attempted to eliminate many of these causes of abnormal CK-MB through history review and laboratory testing. The patient did not have a history of extensive skeletal muscle turnover, seizures, skeletal muscle myopathies, or intramuscular injections. Cardiac injuries were ruled out by troponin. Moreover, myoglobin concentrations were within the reference interval, ruling out acute skeletal muscle injury, whereas thyroid function tests indicated no extent of thyroid disease. Nevertheless, because we were not able to obtain tumor tissue either before or after death, we cannot confirm the production of CK-MB by the tumor itself.

The clinical importance of ectopic production of CK-MB in a cancer patient is limited, as the incidence of this appears to be exceedingly rare. Such findings did not create much confusion for diagnosis of AMI because serial CK-MB measurements showed that the typical rise and fall pattern for total CK and CK-MB was absent. Testing with other cardiac markers, especially cardiac troponins, was helpful in further ruling out AMI. As more assays for cardiac troponins become commercially available, the use of CK-MB may decline over the ensuing years, further diminishing the importance of finding ectopic CK-MB patients. It will be interesting to see if case reports for ectopic production of cardiac troponin T or I are discovered, as the popularity of this testing increases.

References

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Acute Bacterial Prostatitis Induces Hematogenous Dissemination of Prostate Epithelial Cells

To the editor:  
Although not yet a part of clinical chemical practice, molecular techniques are sensitive tools to detect hematogenous spread of solid tumor cells in cancer and may become routine methods in the coming years.
(for review, see [1]). Provided that mRNA is expressed exclusively in tumor tissue and not by normal nucleated blood cells, its presence outside the organ identifies already disseminated cells. We have recently found hematogenous spread of prostate epithelial cells during radical prostatectomy [2] as well as during the course of transurethral resection of the prostate (unpublished data). The different mechanisms underlying hematogenous dissemination of cells from prostate gland remain largely unknown. Among the postulated factors are proteases, hypervascularization, growth factors, and impaired expression of adhesion factors (for review, see [3]). Among the diverse interactions during tumor proliferation, we believe inflammatory processes are likely to be among the key events that drive prostate cells from the acini to the blood vessels.

Between December 1995 and June 1996, we investigated 24 patients with acute bacterial prostatitis (ABP), using a prostate-specific membrane antigen (PSMA) nested reverse transcriptase (RT)-PCR assay [4] to detect prostate epithelial cell dissemination in peripheral blood. The clinical diagnosis of ABP was based on findings of dysuria, pollakiuria, marked malaise, high fever (>40 °C), chills, and prostate pain during digital rectal examination (DRE). The patients’ mean age was 37 years (range 24–45 years), and their mean serum PSA (Hybritech® assay) was 23.4 μg/L (range 8.9–63.2 μg/L). Escherichia coli was found in the urine of 17 patients; Enterobacter cloacae, Citrobacter freundii, and Proteus mirabilis were each found in 1 patient. Cytobacterial urine examinations remained negative for 4 patients. All patients were successfully treated with 400 mg of ofloxacin daily for 3 weeks.

Evidence of hematogenous dissemination of prostate epithelial cells was found in 41% of the ABP patients. PSMA RT-PCR positivity appears to be significantly more common in ABP patients (Fisher’s exact test, P <0.05), in that none of the 12 healthy volunteers tested for a non-specific effect of DRE were positive for this assay (Table 1). Because PSMA transcripts are not ordinarily found in polymorphonuclear cells under normal circumstances [4], the presence of an acute infection may increase the baseline of PSMA mRNA expression. To test this hypothesis, we used PSMA RT-PCR to test blood samples drawn from 10 female patients with bacterial sepsis (leukocytes >20 G/L) and found negative results in all cases.

ABP is associated with inflammation in part or all of the prostate gland [5]. Acute inflammation may promote hematogenous spread of prostate cells. Epithelial cells can migrate out of the prostate after (a) matrix destruction by polymorphonuclear leukocytes within neighboring acini and (b) hypervascularization because of the local inflammatory response. The occurrence of prostate epithelial cells in blood of noncancer patients such as ABP patients further emphasizes that nested RT-PCR protocols based on tissue- and not tumor-specific antigen mRNA detection should be interpreted with the utmost care. In the blood of prostate cancer patients, PSMA RT-PCR may be expected to detect prostate epithelial cells that are malignant [2, 4], but evidence that these cells have metastatic capabilities requires long-term follow-up.

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References

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Misleading Results with Opus® Magnum hLH Assay

To the Editor

We recently found unsatisfactory agreement of luteinizing hormone (hLH) results of the hLH Spec assay (Wallac) and Opus Magnum hLH assay (Behring Diagnostics) (Fig. 1A). Particularly disturbing were the occasional marked discrepancies.

According to the manufacturers, both assays are standardized against the WHO 2nd International Standard for pituitary hLH for immunoassay (code 80/552). Both assays are claimed to be highly specific, with cross-reactivities for hCG of <0.02%


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A spokesman from Behring replies:

To the Editor:

Glycoprotein hormones like LH occur as a complex mixture of circulating isoforms. Standardization and agreement among immunoassays is notoriously problematic, most probably attributable to the use of highly specific but different monoclonal antibodies in the various assays. Recent publications cited by Eskola-Williams above have demonstrated that certain LH tests underestimate or even completely fail to detect LH in some individuals who were shown by functional bioassays to have physiologically active molecules. Nucleotide mutations within codons 8 and 15 in the LH \( \beta \)-subunit gene have been described and appear to account for the majority of these immunologically unrecognizable LH isoforms. The frequency of this variant form of LH has been shown to be relatively high in the Finnish population, ~28%.

The Opus™ LH immunoassay (Behring Diagnostics) utilizes a polyclonal/monoclonal antibody pair, both specific to the \( \beta \)-subunit. The monoclonal antibody was selected for its high degree of specificity for LH. Method comparison studies
were performed with several commercially available assays, including DELFIA, and initial correlation coefficients were quite good (all $r > 0.9$, many > 0.95). Additionally, serial blood samples were obtained from 20 different women throughout the course of their menstrual cycles and clinically evaluated for LH, follicular (FSH), and estradiol on the Opus and other commercial systems. The Opus LH assay correctly identified the follicular, ovulatory, and luteal phase of the menstrual cycle in all cases, peaking on the same day as or one day earlier than the estradiol peak. In certain instances, however, the recovery of LH was lower that in some other commercial kits. The low bias was not attributed to calibration because recoveries were variable within the same method comparison. Discrepancies in recovery were believed to result from the differential recognition of various LH isoforms in individual patients. We did not, however, observe any samples in which the Opus assay totally failed to recognize LH.

Variable recoveries among LH assays utilizing monoclonal antibodies are common and, given that the clinical utility of the assay was demonstrated, the absolute value for LH seemed less important. As literature accounts became increasingly available describing the LH variant commonly seen in the Finnish population, however, more investigation into the specificity of the Opus reagents was undertaken. Results suggest that the antibody used in our current kit does not recognize the LH variant described in the Finnish study conducted by Eskola-Williams. Why we did not observe the degree of discordance described in the Finnish study is probably related to the relatively low frequency of the variant in the population we sampled, and similar specificity of the reagents used in several of the other assays. Indeed, good correlation was observed when the Opus assay was compared with the I3/A2 “intact” LH assay described in the Finnish study.

We agree that the value of a substantial number of LH samples will be underestimated in the Finnish population. The number of samples testing negative (homozygotes) for LH, however, will be relatively small. The references cited by Eskola-Williams describe the 28% population frequency as consisting of 24% heterozygous and 4% homozygous. According to our studies with menstrual cycle panels, accurate clinical information regarding cycle phase can still be obtained in most cases. We also agree that failure to recognize physiologically active LH, even in a small subset of patients, is undesirable, and test results not agreeing with clinical and other biochemical data should be interpreted with caution. A second-generation Opus LH assay has been developed that recognizes the LH variant described here while retaining excellent specificity. This new assay is currently available for research evaluation and will be commercially available beginning in the second quarter of 1997.

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Parameters Relating to Ultrasensitive Prostate-Specific Antigen Assays

To the Editor:
Several recent papers have dealt with the performance of prostate-specific antigen (PSA) assays at very low concentrations [1–3]. In these papers, no less than 5 different parameters have been discussed: the lower limit of detection (LLD), the biological detection limit (BDL), functional sensitivity (FS), the residual cancer detection limit (RCDL), and the minimal detectable concentration (MDC). One may wonder whether such a profusion of terms is meaningful or necessary. However, I believe that, although these parameters are to some extent presented or used as alternatives to each other, fundamental differences exist among them. Here, I aim at distinguishing two groups of parameters, each of which has a specific use.

Two types of questions may arise. First, given a certain measured concentration, does this measurement signify the presence of PSA, as opposed to assay noise? In other words, does the reading of the assay allow to state that the concentration is different from zero? The approach to this question involves statistical inference. First, a distribution of measurements should be obtained (with the same method and under the same conditions) in multiple specimens known to have zero concentration of the analyte. When the analytical signal falls far to the right on the distribution (or to the left for calibration curves with negative slopes), it is unlikely that a sample with actual zero concentration would yield a signal equal to or higher than the one measured. Stated differently, it is thus unlikely that the sample contains any analyte. So, ascertaining the presence of PSA in a clinical sample always and exclusively involves a measurement limit based on the distribution of results from a specimen devoid of analyte. Two parameters qualify as such: the LLD and the RCDL. These differ only in the nature of the blank specimens used: zero calibrators in the case of LLD, and actual clinical samples for the RCDL. In the latter, evidence for the absence of PSA is based on clinical information.

In contrast, parameters such as FS, BDL, and MDC do not qualify as limiting values of a zero-sample distribution and therefore cannot be used to judge individual specimens. It is easy to see that FS does not take into account the distribution of null-specimens but only the variability in samples with very low concentrations. The BDL and the MDC do take into account the distribution of values in specimens devoid of analyte but also reflect variability in samples with very low concentrations.

A second type of question is, how does a given assay perform at very low concentrations? In this respect, limiting values such as LLD and RCDL reveal only part of the picture, because they relate only to blank
specimens. Clinically, it is more relevant to use parameters that in some way reflect precision for specimens with very low concentrations that are different from zero. These include FS, BDL, and MDC. Within this group, however, only MDC is based on firm statistical reasoning: It specifies the concentration that has a certain probability to yield an estimated value above that for blank specimens. In contradistinction, the BDL combines the LLD and variability at low concentrations in an arbitrary way. And by its very definition, FS (the lowest concentration that achieves an interassay CV of <20%) is arbitrary as well.

Therefore, although all these parameters reflect various elements of the performance of an assay, only the LLD and the RCDL are useful as guides to test interpretation.

References

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Imunoassay of Catecholamines and Metabolites

To the Editor:

Taran et al. [1] recently stated that “immunoassays of catecholamine remain the preferred method for routine measurement of these molecules in biological fluids.” A perusal of the relevant literature for pheochromocytoma or neuroblastoma diagnosis over the past 20 years will show this to be a dubious assertion. Rather, HPLC with electrochemical detection (HPLC-ED) appears to be in routine use by >90% of teaching hospital and major clinical laboratories worldwide. Although the 1991 review [2] cited by these authors to support their statement does refer to the putative use of immunoassay in the measurement of catecholamine metabolites, it is concluded that initial attempts in this direction suffer from marginal sensitivity and specificity [2]. More recent reviews on the diagnosis of pheochromocytoma make no mention of immunoassay as an analytical method in this area [3, 4].

The authors are clearly expert in the development of immunoassays for a wide range of clinically important analytes, but we do not believe some of the generalizations made in their paper were justified; it would have been preferable, moreover, if they had (e.g.) indicated the limitations of their methodology when applied to pheochromocytoma diagnosis. Additionally, most of the literature they cite is pre-1990 and does not reflect newer concepts in the diagnosis of catecholamine-producing tumors. The propitious production of a single monoclonal antibody to homovanillic acid (HVA) [1] with suitable specificity might be applicable in routine use as part of a screening procedure for neuroblastoma, but perhaps the authors should also have noted that testing for increased HVA alone is insufficient for biochemical diagnosis of all neuroblastomas [5].

References
methods, which are indeed well suited to the measurement of this type of molecule. If Smythe and Duncan have interpreted our article in this manner, it may well be that we were rather overenthusiastic in stating our case. We have no desire whatever to spark off a potential war of words between immunologists and those who champion chromatographic techniques in this field.

Last, we agree with the idea that the measurement of HVA alone is not sufficient to establish a biochemical diagnosis of all neuroblastomas, and this is not written in our paper.

References


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Difference in Hemoglobin-Binding Ability of Polymers Among Haptoglobin Phenotypes

To the Editor:

Langlois and Delanghe [1] described several functional differences between haptoglobin phenotypes in this journal. They reviewed the functional properties of haptoglobin phenotypes such as ability for hemoglobin (Hb) binding, antioxidative capacity, and inhibition of prostaglandin synthesis, which were strong in Hp-1, intermediate in Hp-2, and weak in Hp-2. However, we do not agree with their results about the ability for Hb binding.

Hp-2-1 and Hp-2-2 phenotypes form many polymers, and analysis of the functional properties of each of the isolated polymer proteins had been very difficult [2]. We designed new analytical methods such as crossed Hb electrophoresis [3] and two-dimensional affinity electrophoresis, using Hb [4] to identify the Hb-binding ability of each of the haptoglobin polymer proteins of Hp-2-1 and Hp-2-2 phenotypes. From the results of crossed Hb electrophoresis, we found that a haptoglobin molecule combined with equimolar amounts of Hb to form at least a hexamer. We confirmed by two-dimensional affinity electrophoresis that the larger polymer of the Hp-2-2 phenotype had a lower affinity to Hb, but a Hp-2-1 polymer of very high molecular mass had high affinity to Hb. The electrophoretic pattern of Hp-2-1 polymers changed in the serum of patients with hemolytic disease because of the different affinities to Hb and the different turnover rates of each polymer. From these phenomena, we concluded that the total affinity to Hb changes with the alteration of the Hp-2-1 polymer pattern and that the Hb binding ability in Hp-2-1 is not always intermediate.

It is well known that a Hb molecule adheres to the beta subunit of haptoglobin [5], but the kind of alpha subunits among haptoglobin phenotypes may affect the affinity of haptoglobin molecules to Hb [4, 6]. The heteroconjugate polymer of Hp-2-1 phenotype may have a characteristic formation with high affinity to Hb. One must therefore consider the functional differences of each of the polymers of high molecular mass in a discussion on the functional properties of each Hp phenotype.

References


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The authors of the article referred to respond:

To the Editor:

The data provided by Okazaki and Nagai regarding the affinity between hemoglobin (Hb) and haptoglobin (Hp) polymers could not have been incorporated in our review article [1] since their paper [2] had not yet appeared at the time our review was accepted for publication in this journal (June 1996).

In our review, we discussed the available literature about quantitative Hb binding as a function of Hp type and Hp concentration. In their letter above, Okazaki and Nagai confuse the term “affinity” with hemoglobin-binding capacity (HBC), the latter being an expression of the protective capacity of human serum Hp against hemolysis. Existing literature data about the stoichiometry of Hp–Hb binding are mainly based on older immunodiffusion methods [3, 4], which underestimate serum Hp 2–2 concentration because the diffusion of high-M, immune complexes is impaired in gels (typically in the analysis of polymeric Hp 2–2) [5]. However, the more recent immunonephelometric methods for determining Hp concentration are not phenotype-dependent. Making use
of fixed-time nephelometry (from Behringwerke AG), we found a good correlation between serum Hp concentration and HBC. After addition of Hb to human serum, the HBC was determined by gel permeation chromatography on a Protein PAK Glass 300 SW column (Waters Nihon Millipore), followed by photometric detection of the eluting Hp–Hb complexes at 418 nm. For Hp 1–1 (n = 16), Hp 2–1 (n = 48), and Hp 2–2 (n = 36), we determined the following ranges for HBC (defined as grams of Hb per liter of serum) by Hp phenotype: 1.70–6.76 for Hp 1–1, 1.30–5.41 for Hp 2–1, and 1.22–4.81 for Hp 2–2 (P < 0.05, ANOVA) (unpublished results). The observation of Okazaki and Nagai that the electrophoretic pattern of Hp 2–1 changes after transfusion has been used as an argument for the existence of different affinities for Hb between Hp polymers [2]. However, observations made after blood transfusions should always interpreted with caution in light of the complexation of albumin with the heme from the transfused erythrocytes [7], which gives rise to additional bands of methemalbumin on the gels. Banding patterns may indeed change in the hours after transfusion. In contrast to the fast removal of the Hp–Hb complexes by the liver [8], these methemalbumin complexes can be observed in plasma by 5 h after hemolysis and persist in the plasma for a few days.

These data are further confirmed by the biological effects of Hp phenotypes on the metabolism of vitamin C [6]. The Hb-catalyzed vitamin C oxidation is related to the serum Hp concentration. In Hp 2–2 plasma, vitamin C concentrations are lower than in the other types because of less-effective protection against the Hb/iron-driven peroxidation [6]. Consequently, the conclusions summarized in our review regarding quantitative Hb binding of Hp phenotypes remain valid.

We have also studied the in vitro effect of Hb addition to human Hp 2–1 serum on the distribution of Hp 2–1 polymers on starch electrophoresis. Addition of increasing concentrations of Hb (from 0.05 to 3 g/L) did not induce any change in the typical electrophoretic pattern of Hp 2–1 polymers (unpublished results). The observation of Okazaki and Nagai that the electrophoretic pattern of Hp 2–1 changes after transfusion has been used as an argument for the existence of different affinities for Hb between Hp polymers [2]. However, observations made after blood transfusions should always interpreted with caution in light of the complexation of albumin with the heme from the transfused erythrocytes [7], which gives rise to additional bands of methemalbumin on the gels. Banding patterns may indeed change in the hours after transfusion. In contrast to the fast removal of the Hp–Hb complexes by the liver [8], these methemalbumin complexes can be observed in plasma by 5 h after hemolysis and persist in the plasma for a few days.

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