More on Glycated Hemoglobin Measurement from Samples Dried on Filter Paper

To the Editor:

In response to the Letter by Eross et al. (1): we appreciate their comments on our work with glycated hemoglobin (gHb) from blood samples dried on filter paper (2, 3) but we believe that some important points in our paper were missed.

We, too, have had extensive experience with the thiobarbituric acid (TBA) method (4-7) and have used it with blood samples dried on glucose oxidase-pretreated filter paper. As described in our recent paper (3) measurement of gHb by affinity chromatography from blood dried on pretreated filter paper showed an increase in gHb during 14 days, with no further increase thereafter. For highly reproducible results we therefore standardized the day of elution. (Even with our TBA method we found a statistically significant increase from day 1 to day 7, with no change thereafter up to 21 days of testing.)

We chose to use affinity chromatography rather than TBA colorimetry for several important reasons. First, if the day of elution is standardized, reproducibility is excellent: our most recent data show inter-assay CVs of 4.1 and 3.8% for repeated spotting of frozen low- and high-gHb blood samples, respectively, over a six-month period. More importantly, the assay requires only 20 μL of blood (a sixth the quantity required for the TBA method) and is technically easier and faster than the TBA method. In addition, many clinicians find the reported units, % gHb, much easier to understand than nmol HMF. We see no clinical advantage in being able to store blood-spotted paper for three months; gHb reflects glycemic status for only the previous two to three months.

References

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Progestrone Receptors Should Be Measured in Postmenopausal Women Negative for Estrogen Receptor

To the Editor:

We disagree with some conclusions reached by Brocklehurst et al. in their Letter (Clin Chem 1986;32:2120), in which they discuss the incidence of estrogen receptor (ER) negative, progestosterone receptor (PR) positive tumors in pre- and postmenopausal women with breast cancer.

There is no disputing the fact that receptor-positive tumors are more likely to respond to hormone therapy than are their receptor-negative counterparts, but the association between receptor positivity in the primary tumor and an increased disease-free interval is much less clear cut. In a series of 508 patients with primary breast cancer analyzed after a maximum follow-up period of 91 months (median 36 months), we found no difference in likelihood of recurrence between the ER positive and ER negative patients (p >0.07). We obtained a similar result for 486 of these patients for whom we had also measured PR (p >0.7) (1). Raemaekers et al. (2) found that the ER status of the primary tumor does affect the likelihood of recurrence, but only in the first year after initial treatment. After prolonged observation the initial significant difference in recurrence rates was lost. In our paper these authors extensively reviewed the literature and refer to 27 papers in which an advantage was found for ER-positive tumors, but only after a short follow-up time, and often the effect was only seen in subsets of patients. They also refer to 13 papers in which follow-up times were longer and no advantage was found for ER positivity.

In a more recent analysis of our own data with respect to menopausal status when ER and PR are considered together, our results do not support the findings of Brocklehurst et al. that ER- PR+ tumors only occur in premenopausal women. We have studied a large number of patients and we have found an overall incidence of this particular phenotype of 5.8%, as compared with 2.8% reported in their Letter. When our patients are grouped according to menopausal status we found that 10.7% (36 of 336) pre-menopausal and 3.9% (33 of 854) post-menopausal patients had tumors that contained PR in the absence of ER.

Our results are similar to those found by McGuire (3) in a series of 1386 breast-cancer patients in which there was an ER- PR+ incidence of 9% and 3% for pre- and post-menopausal women, respectively. Brocklehurst et al. found that 13.6% (6 of 44) of their pre-menopausal but none of their postmenopausal patients (0 of 173) were ER- PR+. These data suggest that PR is being underestimated in their postmenopausal patients. Although ER- PR+ post-menopausal women form only a small part of the population of breast-cancer patients, they do respond to hormone treatment and therefore should receive this therapy.

We have recently shown that PR is of greatest value for prediction of response when measured in advanced primary tumors and when response is assessed on that primary. It is of less value when measured in either a primary tumor or in metastases and the response is assessed in metastatic deposits (4). However, if PR is measured before and after a short course of tamoxifen, tumors with an increase in PR have a 90% chance of response (5). PR predicts overall survival but not the disease-free interval, and it is a useful prognostic indicator after relapse. Because tamoxifen is a partial estrogen agonist, determination of its effect upon PR synthesis improves the predictive capacity of PR for response.

References

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Choriongonadotropin and Pregnancy Testing: Prozone Phenomenon Defined

To the Editor:

In a recent Scientific Note, Gelletlie and Nielsen (1) evaluated several commercial pregnancy tests based on monoclonal antibodies to human choriongonadotropin (hCG). They addressed the problem of the prozone effect, which they described as a false-negative result caused by an excess of antigen (hCG), which inhibits the antigen/antibody reaction. This definition of the prozone phenomenon is at variance with that given in several authoritative sources in immunology, as well as a standard textbook of clinical pathology, and the standard manual for blood banking (2-4). These sources state that the prozone effect occurs when an excess of antibody saturates the antigenic determinants, preventing crosslinking and lattice formation. Conversely, the postzone phenomenon results when antigen is in excess and saturates the antibody binding sites, also inhibiting crosslinking and lattice formation. Between these two extremes is the zone of equivalence where the optimal concentration of antibody and antigen is found, leading to the desired antigen/antibody reaction. While these definitions derive from early observations of agglutination and precipitation reactions, they are applicable to the variety of different immunology-based techniques used in modern clinical-chemical methods.

It should be noted that in a recently published, comprehensive textbook of clinical chemistry (5), the antibody and antigen excess situations are thoroughly discussed, as well as the zone of equivalence, but the antigen excess case is called the prozone area. One suspects that this is an error in the first edition.

Both the prozone and postzone effects may result in a false-negative test result, so in a practical sense the distinction between the two phenomena may seem a moot point. Yet, as more immunology-based techniques and assays proliferate in clinical chemistry, it is useful to understand the molecular basis of antigen/antibody interactions, the problems that can result from a surplus of one or the other, and the corrective action that can be taken, such as dilution of the patient’s specimen to obtain the proper concentration of antigen. The terms “prozone” and “postzone” accurately connote which component of an immunological reaction is out of balance. Why not adhere in the clinical chemistry literature to the definitions already in use in immunology and related fields and avoid confusion?

References


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Is Paraoxon Hydrolitic Activity in Serum Predictive of Myocardial Infarction?

To the Editor:

McElveen et al. reported (1) that paraoxonase (arylesterase; EC 3.1.1.2) activity in human serum was bimodally distributed, both in a control group and in a group of patients with myocardial infarction (MI). They did not know whether the significantly lower activity of paraoxonase in the MI group indicated increased risk of MI or was a direct result of the infarct.

We compared the activity of paraoxonase in two groups of Hungarian children to elucidate this question. The 24 children in the first group were healthy, but one of their parents had had an MI before age 40. The control group consisted of 176 children; they and their parents were healthy.

We measured paraoxonase activity by a method modified from the method of Kirsch (2) and Eckerson et al. (3). Our assay system consisted of 10 μL of serum added to 0.8 mL of a mixture of 1.0 μmol of paraoxon (Serva, Heidelberg, F.R.G.) and 1.0 μmol of CaCl_2 in glycine buffer (50 mmol/L, pH 10.5) in the presence and absence of NaCl. Liberation of p-nitrophenol on enzymatic hydrolysis of paraoxon was monitored at 412 nm with a dual-beam recording spectrophotometer (Specord M-40; Carl Zeiss, Jena, G.D.R.) maintained at 25 °C. "Basal" paraoxonase activity is that measured without NaCl. Salt-stimulated paraoxonase activity was then measured with NaCl (final concentration, 1 mol/L) in both the enzymatic and reference cuvettes. The percentage of stimulation by NaCl was expressed as: [(activity with NaCl present - basal activity)/basal activity] × 100%.

Results for both study populations showed a clearly bimodal distribution of paraoxonase activity in the presence of NaCl. The distribution was not bimodal in the absence of NaCl; this is consistent with the results of others (3-4).

Pareaoxonase activity in both populations showed a similar, bimodal distribution. χ² for the goodness-of-fit test was 1.40%; the difference is not statistically significant. For the control population, the mean percentage stimulation of the activity by NaCl was 145.6% (SD 80.6%) of the basal activity. For the population of children whose parents had suffered MI this value was 114.0% (SD 82.5%), a significant difference (t = 1.71, p <0.1) by Student’s t-test.