Being the first to recognize the intermediate filament (cytokeratin, Ck) nature of TPA (2), I wish to comment on the present use of the term TPA, now that monoclonal antibody (mAb)-based assays are being tried as substitutes for the assay with polyclonal antisera. The polyclonal antisera used to define TPA (3) was found to be directed against Cyk 8, 18, and 19 (4). When major portions of the primary structures of Cyk 8, 18, and 19 were reported (5, 6), most of the previous provisionally aligned partial sequences of TPA could be confirmed.

Mellerick et al. (7) found that mAbs to Cyks 8, 18, and 19 reacted differently with TPA prepared from cultured human carcinoma cells and the TPA in tumor patients' sera. This is not surprising, given that the Cyks must undergo various degrees of proteolysis before entering the circulation, a process that affects these proteins and their epitopes to different extents.

Sandström et al. (8) showed that mAbs to Cyks 8, 18, and 19 serve as well as anti-TPA as indicators in colon cancer, whereas a mAb to Cyk 18 is inferior in prostate and ovarian cancer. In another study, two different mAbs to Cyk 8 showed good correlation with each other in breast cancer but poor correlation for colon and lung cancer. These results confirm that serum TPA molecules from tumors at different locations contain different profiles of epitopes.

The recently introduced test kit for two selected epitopes in Cyk 19, Cyfra 21-1 (Boehringer Mannheim, Mannheim, Germany), has been proposed as a marker for non-small-cell lung cancer. Sensitivities of Cyfra 21-1 and TPA for some tumor locations are reported to be similar, according to results reported to the International Academy of Tumor Marker Oncology (9). A regression analysis of the IDaEL™ monoclonal TPA cyt IRMA (AB IDL, Borlänge, Sweden), relying on one epitope from Cyk 8 and one on Cyk 18, vs the polyclonal Prolifigen® TPA IRMA, using values in the latter test, <200 IU/L, for samples from 72 blood donors and cancer patients showed a correlation coefficient of only 0.56. In another study (10) comparing the polyclonal Prolifigen TPA IRMA with the monoclonal TPS™ IRMA from BEKI Diagnostics (Bromma, Sweden), which relies on an epitope M3 on Cyk 18, correlations between the two test kits were low, leading the author to conclude "that the vast historical knowledge collected for the Prolifigen® TPA kit can not be transferred using the TPS assay." This statement can be widened to cover the IDaEL assay as well (see Table 1).

In conclusion, therefore, assays intended to measure TPA that use mAbs recognizing one or more epitopes of Cyk must be supported by fresh clinical data. Such assays can be compared with but not supported by the clinical background of the TPA concept.

References

Björn Löning
Dept. of Organic Chem.
Stockholm University
S-10691 Stockholm, Sweden

Intensive Diabetes Management Requires Very Precise Testing of Glycohemoglobin

To the Editor:

The intraindividual variation of patients' test values can be used to estimate the maximum allowable analytical error (MAAE) (1). For analytes that can be controlled by the patient, e.g., glucose, the extent of control is the primary determinant of the MAAE. We had the opportunity to study the intraindividual variation of glycohemoglobin (GHB) in diabetics under extremely tight glucose control. Our estimates of MAAE contrast with those recently advanced by Phillipou and Phillips (2), who estimated intraindividual variation of GHB, as measured by affinity column, in two groups of patients with diabetes under stable clinical control.

These authors tested one group every 28 days over a 4-month period and the other every 84 days over a 15-month period. Their median intraindividual coefficients of variation (CV) were 4.2% and 7.1% for the 28- and 84-day groups, respectively. Using the equation relating analytical variation (CVa) to actual intraindividual variation (CV) first suggested by Cotlove et al. (3), CVa ≤ 0.5 CV, one may conclude that GHB methods should have a CVa of no more than 2.1% and 3.5% for the 28- and 84-day sampling protocols, respectively.

Our patient population consisted of 29 insulin-dependent diabetics who participated in the Diabetes Control and Complications Trial (DCCT) study (4) at the International Diabetes Center of Park Nicollet Medical Center. The study was conducted in accordance with our institutional review board requirements and in compliance with the regulations of the US Department of Health and Human Services. Each patient underwent an individualized regimen of intensive di-
abetes management (IDM). The regimen was designed to achieve blood glucose values as close to the normal range as possible with three or four daily insulin injections or treatment with an insulin pump. Patients adjusted their insulin doses according to food intake, exercise, and blood glucose tests performed four or more times per day. IDM included the use of insulin algorithms, frequent use of self-monitoring blood glucose meters, and the use of individualized meal plans. Patients were encouraged to closely follow IDM regimens, and were in frequent contact with dieticians and psychologists for review and support.

Intensive treatment of insulin-dependent diabetes mellitus resulted in a reduction of retinopathy by 54% to 76%, nephropathy by 54%, and neuropathy by 60%. Intensive therapy did not cause any worsening of neurobehavioral function or quality of life.

The patients who underwent IDM were followed for an average of 6.5 years. Serial hemoglobin A1c (HbA1c) data were measured at the University of Minnesota (5). Of the total monthly HbA1c observations for the patients, 91% were available. We grouped each patient’s monthly data from the most recent two complete years, 1991 and 1992, into successive 3-, 6-, 9-, 12-, 18-, and 24-month periods. For each period we calculated each patient’s total intra-individual CV (CVi), a combination of analytical variation (CVa) and intra-individual variation without any analytical component (CVi). Because of the non-gaussian CV distribution, we used the median CVi to indicate the central tendency of the data.

The CVi for each period (in months) was: 2.8% (3), 3.8% (6), 4.0% (9), 4.4% (12), 4.5% (18), and 4.8% (24). The CVa depends heavily on the number of HbA1c observations averaged and thus on the time over which these values are observed. The increase of CVa arises from the fact that, as more data are averaged, influences such as long-term patient compliance and seasonal variation are introduced. Unlike Phillipou and Phillips, we made no attempt to eliminate patients with trends in their HbG values.

Figure 1 shows the change over time in median with-in-year CVi, compared with the yearly grand mean of the HbA1c. Until 1990, there was a steady decrease in the average HbA1c and median CVi. The decrease and eventual stabilization of average HbA1c and median CVi can be attributed to improved treatment and the patients’ experience in dealing with modifications in diet, exercise, blood glucose monitoring, etc.

HPLC assays of HbA1c are capable of a precision of 0.1% (SD) at an average HbA1c of 7% (6) (CVa = 1.4%). We calculated the CVi from the CVa using the equation CVi = CVa2 - CVa. For the 3- and 12-month periods, we obtained CVa of 2.4% and 4.1%, respectively—a little less than one-half of the estimates of Phillipou and Phillips. Using CVa < 0.5 CVi, we determined therefore that the CVi should be no more than 1.2% and 2.1% for short-term and long-term monitoring, respectively. Thus, an HPLC assay with a CVa of 1.4% is acceptable for monitoring tightly controlled patients.

We believe that Phillipou and Phillips would obtain similar CVa if they monitored patients undergoing IDM. The CVa of 4.9% for the total HbG assay is not acceptable for monitoring such patients. The large analytical error of such imprecise assays can obscure significant trends in the well-controlled patient. Although IDM is not yet widely practiced (7), we believe that, as the message of the DCCT is disseminated, patients will more aggressively treat their diabetes and achieve more stable concentrations of GHB. It is thus attendant for clinical laboratories to produce the most clinically valid measurements of GHB possible. Although many laboratories may not be able to expend the effort to achieve such precision, each should assess its long-term CVa and improve or replace any systems with CVa exceeding 2-3%.

References

Nikheel S. Kolatkar
Park Nicollet Med. Center
George S. Cembrowski

Park Nicollet Med. Center
5000 W. 39th St.
Minneapolis, MN 55416
and Dept. of Pathol. and Lab. Med.
Univ. of Minnesota School of Med.
Minneapolis, MN

Patricia L. Callahan
DCCT Research Project
International Diabetes Center
Minneapolis, MN

Donnell D. Etzwiler
International Diabetes Center
and Dept. of Pediatr.
Univ. of Minnesota School of Med.
Minneapolis, MN

1 Author and address for correspondence.