results between these 2 methods is not clinically relevant. Irrespective of the assay used for future NHANES monitoring, population reference ranges will change to higher values; however, MA-determined cutoff values for deficiency (10) could be directly applied to the LC-MS/MS because of its excellent agreement with the MA. Some advantages of the LC-MS/MS compared with the MA are that it provides information on the different folate species in addition to TFOL and it is less prone to interferences such as antibiotics. BR underrecovery of 5CH3THF, the main circulating form of folate, is likely the major reason for its lower results. A model will be required to convert results from the old or the new NHANES method for time trend analysis. Our model provides an excellent fit over a wide concentration range. This information may also be useful to the international community in that national data generated with the MA can now potentially be compared with US reference ranges.

We thank Joseph Jacobson (Battelle, Columbus, OH) and Irene Williams (Centers for Disease Control and Prevention, Atlanta, GA) for technical assistance with the LC-MS/MS and BR methods.

References


International Comparison of C-Peptide Measurements, Hsiao-Mei Wiedmeyer,1 Kenneth S. Polonsky,2 Gary L. Myers,3 Randie R. Little,1 Carla J. Greenbaum,4 David E. Goldstein,1 Jerry P. Palmer,5 (1 Departments of Pathology & Anatomical Sciences and Child Health, University of Missouri-Columbia School of Medicine, Columbia, MO; 2 Department of Medicine, Washington University School of Medicine, St. Louis, MO; 3 Centers for Disease Control and Prevention, Division of Environmental Health Laboratory Sciences, Centers for Environmental Health (F25), Chamblee, GA; 4 Benaroya Research Institute, Seattle, WA; 5 University of Washington and VA Medical Center, Seattle, WA; * address correspondence to this author at: Diabetes Diagnostic Laboratory, M767, Departments of Pathology & Anatomical Sciences and Child Health, University of Missouri School of Medicine, 1 Hospital Dr. Columbia, MO 65212; fax 573-884-8823, e-mail: LittleR@health.missouri.edu)

Background: C-peptide measurement has been widely used as a marker of insulin secretion in patients with diabetes. We assessed the comparability of C-peptide results obtained with different methods and by different laboratories and determined whether C-peptide results could be harmonized by normalization with a WHO reference reagent or with plasma.

Methods: We sent 16 different heparin plasma samples to 15 laboratories in 7 countries. The samples were analyzed with 10 different assay methods. A WHO C-peptide standard was also sent to each laboratory and used to determine the feasibility of normalizing results. To assess the impact of calibrator matrix on the comparability of results, we also used the mean results of all laboratories for 4 of the samples to normalize the remaining sample results.

Results: Between-laboratory variability increased with increasing C-peptide concentrations. Normalization of results with WHO reference reagents did not improve comparability, but normalization with samples significantly improved comparability among laboratories and methods. The 95% confidence interval estimate for the SD for the lab/method effect (0.0–0.061) using sample-normalized values did not overlap with the 95% CI estimate with the raw data (0.090–0.225).

Conclusions: C-peptide results generated by different methods and different laboratories do not always agree, especially at higher concentrations of C-peptide. These data support the concept of using a single laboratory for multisite studies and support efforts to harmonize C-peptide measurements by use of calibrators prepared in the sample matrix.

© 2007 American Association for Clinical Chemistry

Human C-peptide provides an accurate assessment of residual beta-cell function and thus has been widely used as a marker of insulin secretion in patients with diabetes (1, 2). Some studies have also suggested that C-peptide is
biologically active (3) and may play a role in preventing and possibly reversing some chronic complications of type 1 diabetes (4, 5). C-peptide is also important in the diagnosis of insulinoma/endogenous hyperinsulinemia (6).

Despite the fact that measurement of C-peptide has become increasingly important, the accuracy and between-laboratory comparability of C-peptide results have not been thoroughly evaluated. In 2002, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) organized a C-peptide standardization committee and funded an international comparison study of C-peptide assays to assess the comparability of C-peptide results within and among laboratories involved in diabetes-related long-term research studies. The goal of the study was to assess the degree of comparability of C-peptide results among methods and laboratories and to determine whether C-peptide results could be normalized to make comparing and combining data from different laboratories and studies more feasible. The comparison study was coordinated by a central laboratory at the University of Missouri-Columbia and was overseen by the C-peptide Standardization Committee. The members of the Committee and the participating laboratories are listed in Appendix A.

The study was conducted by the Diabetes Diagnostic Laboratory (DDL) at the University of Missouri-Columbia. The protocols were approved by the University of Missouri Health Sciences Institutional Review Board and all participants gave written informed consent. Three laboratories used more than one assay for C-peptide analysis, which resulted in a total of 20 laboratory methods being evaluated. Five laboratories used the Linco RIA; 1 additional laboratory used Linco C-peptide antibody with in-house isotope and precipitating reagents. Four laboratories used the Diagnostic Product Corp. (DPC) Immulite chemiluminescence immunoassay system, and 3 laboratories used the DPC RIA kit. Two laboratories used Tosoh Bioscience AIA-600II, an automated immunoassay system. The other methods—Liaison Immunoluminometric assay (ILMA) (DiaSorin), Biochem Pharma RIA, Shionogi RIA, Dako ELISA, and PerkinElmer Time-resolved fluoroenzymoimmunoassay (FIA)—were each used by 1 laboratory.

Heparin plasma samples were collected from 8 nondiabetic volunteers after they had fasted overnight and 60 min after they had consumed a standard meal (Boost™, Novartis Medical Nutrition). The samples were shipped frozen on dry ice and kept at −70°C in the laboratories before analysis. Laboratories were instructed to analyze the specimens on specified days, in the same manner as they would analyze clinical specimens, and to provide a single result for each specimen.

Along with patient specimens, the laboratories received a WHO C-peptide reference reagent (standard) to assess its commutability and to determine the feasibility of normalizing results to this standard (7). This standard was prepared in the central laboratory with a vial of C-peptide stock solution into which was dissolved 1 ampoule of WHO IRR 84/510 standard (10 μg) in 25 mL of distilled water (8, 9). The resulting solution (400 ng/mL or 132.4 nmol/L) was shipped on dry ice along with the samples. Each laboratory serially diluted the standard solution with their own assay buffer to make 4 preparations that were then analyzed in each analytical run.

To assess the impact of the calibrator matrix on the comparability of results, 4 of the samples were used to normalize the results from the remaining 12 samples. The mean of all laboratories’ results for each sample was used as the assigned value for each of these sample calibrators. To compare the effect of normalization with WHO standards and normalization with samples, normalization was applied to the same 12 samples for both analyses. The 4 samples used as sample matrix calibrators were therefore not included in the normalization with WHO standards.

We performed all data analyses with Excel and SAS. Most laboratories in this study reported C-peptide results in nanograms per milliliter, but a few reported results in SI units (nmol/L). All C-peptide results were converted to SI units (nmol/L) before data analyses. Because scatter plots of the data indicated increasing variability in responses with increasing C-peptide, the model assumed constant CV. Weighted regression methods were used with weights inversely proportional to the variance. We performed iterative re-weighting because the variance had to be estimated from the modeled regression results.

For each laboratory/method, 2nd-degree equations were fitted by use of laboratory/methods values for the WHO standards on y and the “true” values for the standards on x; weights based on constant CV were used in the weighted regression analyses. In all but one case the coefficient for the squared term was not significantly different from zero, and in that case the fitted quadratic curve visually showed minimal curvature. Therefore, we performed normalization with simple linear relationships. The weighted regression analyses were run again using the normalized values and the same analyses were performed when we used samples as calibrators.

Visual inspection of the data showed that one laboratory’s results (using the DPC Immulite method) did not show consistent increases with increasing C-peptide concentrations and was therefore considered an outlier (Fig. 1a). This laboratory’s results were therefore excluded from all further evaluation.

The assay survey of participating laboratories showed that 6 of the C-peptide assay methods were already calibrated to WHO standard IRR lot 84/510 (DPC RIA, DPC Immulite, Linco RIA, Tosoh AIA, DiaSorin ILMA, and in-house RIA). Two of the laboratories reported that their methods were not calibrated to WHO standards (PerkinElmer FIA, Shionogi RIA), and no information was available for 2 of the methods (Dako ELISA, Biochem Pharma RIA).

C-peptide results before and after normalization with WHO standard (Fig. 1A and 1B) showed that the 95% confidence interval (CI) estimate for the SD for the lab/method effect (0.172–0.456) overlapped with the 95% CI.
estimated with the raw data (0.090–0.225). This result suggests that WHO normalization was ineffective in reducing the variability of C-peptide results within and among the lab/methods.

As with the WHO normalization, weighted linear regression analyses were used for each of the assay methods for normalization with the 4 patient samples. Results (Fig. 1C) showed that the 95% CI estimate for the SD for the lab/method effect (0.0–0.061) using normalized values did not overlap with the 95% CI estimated with the raw data (0.090–0.225). This result shows that normalization with sample calibrators was effective in reducing variability of C-peptide results.

The importance of measuring C-peptide has increased significantly in recent years with the evidence from the DCCT that higher C-peptide concentrations are associated with improved hemoglobin A1c concentrations, less hypoglycemia, and less retinopathy and nephropathy (10). Furthermore, stabilization of C-peptide concentrations is being used as a measurable endpoint for immunomodulatory trials in type 1 diabetes (11, 12). Our data show that a wide variety of C-peptide assay methods are being used by laboratories worldwide, and that the C-peptide results generated by the different methods, and even in some cases by different laboratories using the same method, are not always comparable. This finding has important implications for studies in which C-peptide results from different laboratories involved in multicenter studies are compared or combined for data analyses and for clinicians following patients over time if samples are tested at different laboratories.

Normalization using specimens achieves a modest improvement in comparability but the harmonization may be inadequate to fulfill the requirements of clinical trials. Our data reaffirm the difficulty in achieving standardization of C-peptide measurements because, like many polypeptides, C-peptide does not appear to behave the same way in pure calibrators as it does in patient samples, i.e., the commutability of pure standards is poor (13, 14).

Recently, isotope-dilution liquid chromatography-mass spectrometry has been proposed as a reference measurement procedure for serum C-peptide (14, 15) Further studies are needed to determine if this method may be useful in standardizing measurements of plasma/serum C-peptide.

It is important that comparability of C-peptide results between laboratories be addressed before the initiation of large-scale trials involving multiple laboratories performing C-peptide analyses. The data presented here support the concept of using a single core laboratory for such studies. Our data also illustrate the need for efforts to standardize C-peptide measurements by use of materials prepared in sample matrix.

Appendix

Members of the C-Peptide Standardization Committee and Participating Laboratories

NIDDK C-Peptide Standardization Committee. David Goldstein (University of Missouri), Gary Myers (Centers for Disease Control and Prevention), Jerry Palmer (University of Washington), Kenneth Polonsky (Washington University), Judith Fradkin (NIDDK), Lisa Spain (NIDDK), Randie Little (University of Missouri), Hsiao-Mei Wiedmeyer (University of Missouri).

Participating Laboratories. Anette Ziegler, Kerstin Koczvara, Diabetes Research Institute Munich (Germany); Paolo Pozzilli, University Campus Bio-Medico (Italy); Charlotte Becker, University Hospital Malmö (Sweden); Ezio Bonifacio, San Raffaele Hospital (Italy); Merete Frandsen, Thomas Mandrup-Poulsen, Steno Diabetes Center (Denmark); Anders Isaksson, Mona Landin-Olsson, Lund University (Sweden); Armando Mendez, Linda Jones, University of Miami (FL); Jean Bucksa, Vicky Makky, University of Minnesota Medical Center, Fairview (MN); Veronica Luzzi, Gene Sherrrow, Washington University (MO); Liz Rinehart, Linco Diagnostic Services.
Inc. (MO); Jon Nakamoto, Anne Caston-Balderrama, Quest Diagnostics Nichols Institute (CA); Vinod Gaur, Northwest Lipid Metabolism and Diabetes Research Laboratory, University of Washington (WA); Alethea Tennill, University of Missouri (MO); Akira Shimada, Taro Maruyama, Keio University (Japan); Spiros Fourlanos, Royal Melbourne Hospital (Australia).

Grant/funding support: This project was funded by the Centers for Disease Control and Prevention through a funding contract (No.200200409985), supported by the NIDDK Special Statutory Funding Program for Type 1 Diabetes Research.

Disclosures: None declared

Acknowledgments: We thank Judith Fradkin and Lisa Spain for their support. We also thank Alethea Tennill, Curt Rohlfling, and Donghua Huang of the Diabetes Diagnostic Laboratory, University of Missouri, for their technical assistance with this project, as well as Richard Madsen, University of Missouri, for statistical consultation.

References
8. WHO synthetic C-peptide reference material 84/510, National Institute for Biological Standards and Control (UK).
11. K-ras in stool has been proposed for the detection of pancreatic and colorectal cancer (CRC). Different analytical techniques have been developed, but studies of this biomarker in the general population are lacking. We investigated the prevalence and potential determinants of mutant K-ras in stool in a large sample of unselected older adults and assessed the association with colonicoscopy findings.

Methods: In stool samples from 875 older adults (age range 50–75 years) participating in a large-scale population-based cohort study, we used mutant-enriched PCR and allele-specific hybridization reaction to analyze mutations in codons 12 and 13 of the K-ras gene. We assessed the association between mutant K-ras in stool and risk factors for gastrointestinal cancer sites, exocrine pancreatic insufficiency determined by fecal pancreas elastase 1, and colonicoscopy findings.

Results: The overall prevalence of mutant K-ras in stool was 8% (95% confidence interval 6%–10%). There was a tentative association between increased fecal pancreas elastase 1 and mutant K-ras in stool (P = 0.09). Patients with advanced colorectal neoplasia diagnosed within 2 years after stool collection (24 with advanced adenomas, 7 with CRC) all tested negative.

Conclusion: The proposed assay identifies mutant K-ras in stool at a higher prevalence than has been reported for other analytical techniques. Our findings do not support the use of this assay for CRC screening, but its potential use for early detection of pancreatic cancer (in combination with other markers) requires further investigation.

© 2007 American Association for Clinical Chemistry

Given that somatic mutations of the K-ras gene are observed in ~40% of colorectal cancers (CRCs) (1–3) and in >80% of pancreatic cancers (4), mutant K-ras in stool specimens has been proposed as a potential component of marker combinations aimed at the early detection of these cancers (5–10). Conclusions drawn from existing studies must be viewed cautiously, however, because most of the study populations were highly selective and rather small (5–10), and different analytical techniques were used and