RESEARCH ARTICLE

Biological variation of serum neuron-specific enolase and carbohydrate antigen 724 tumor markers

Shuo Wang | Min Zhao | Runqing Mu | Xin Zhang | Ke Yun | Hong Shang

Department of Laboratory Medicine, The First Hospital of China Medical University, Shenyang, China

Correspondence

Hong Shang, Department of Laboratory Medicine, The First Hospital of China Medical University, Shenyang, China.
Email: hongshang100@hotmail.com

Funding information

National Key Technologies R&D Program of China, Grant/Award Number: 2012BAI37B01

Background: The aim of our study was to assess two tumor markers, carbohydrate antigen 724 (CA724) and neuron-specific enolase (NSE), in 20 healthy individuals. Then, we determined their average intra- and inter-individual biological variations (CV_I and CV_G).

Methods: Blood samples were taken at weekly intervals for five weeks from 20 healthy subjects through a detailed questionnaire and physical examination. These samples were stored at -80°C and analyzed in a single run in duplicate. We calculated the biological variations, the reference change value (RCV), and the index of individuality (II) for the two tumor markers.

Results: The intra-individual and inter-individual biological variations were 50.3% and 103.4% for CA724, and 10.1% and 12.0% for NSE, respectively. The II values for CA724 and NSE were 0.48 and 0.85, respectively.

Conclusion: We determined the biological variations and indices of individuality for CA724 and NSE among 20 healthy subjects. These results will improve clinical applications of the markers.

KEYWORDS

biological variation, CA724, NSE

1 | INTRODUCTION

Tumor markers have an important role in early diagnosis, treatment, and follow-up monitoring of malignancies. They provide useful information that can be obtained during a non-invasive examination. The primary use of tumor marker measurements is to obtain reliable information about developmental trends of malignant cells and tissues before metastatic changes can be identified by other and convenient methods.¹ Tumor marker measurements sometimes provide more practical information than pathological diagnoses. Carbohydrate antigen 724 (CA724) is the most sensitive and specific indicator for gastric cancer.² CA724 is strongly associated with gastric cancer, particular among Chinese.³ Neuron-specific enolase (NSE) is another useful

Tumor marker, which is used for the diagnosis, treatment, and follow-up monitoring of patients with small-cell bronchial carcinoma.^{4,5} Serum NSE levels are significantly increased in patients with small-cell lung cancer. NSE also is the best indicator to predict the patient response to chemotherapy.

The question of whether a temporal change in a tumor marker signals increased disease activity requires a clinical decision by the patient's doctors. Changes in tumor marker concentrations for a particular individual can be due to deterioration or improvement of the disease, preanalytical variations, experimental variations during analysis, and biological variations among the population.⁶ A change in tumor biomarker status only truly reflects the patient's condition when preanalytical, analytical, and biological variations are excluded.

Biological variations of analytes are important to understand for clinical and laboratory applications. These variations can be used to calculate the reference change value (RCV), which can be used to evaluate the clinical significance of changes within a series of results. The

Abbreviations: NSE, neuron-specific enolase; CA724, carbohydrate antigen 724; RCV, reference change value; II, Index of Individuality; CV_I , Intra-individual biological variation; CV_G , inter-individual biological variation; CV_A , analytical imprecision; Z, the number of standard deviations required for a confidence interval (ie. 1.96% for 95%).

RCV value also be used to calculate the individual index (II), which can be used to assess the utility of population-based reference ranges.^{7,8} Biological variations of tumor markers provide objective criteria for accurate interpretations of clinical test results.⁹

The biological variations of some tumor markers have been determined and listed in the Westgard Database.¹⁰ However, few of these studies are available for NSE¹¹ and CA724,¹² and few studies have been reported in the literature. For patients with gastric or lung lesions, CA724 or NSE levels are determined on a weekly or monthly basis during follow-up examinations. The aim of our study was to determine estimates for the medium-term biological variations of CA724 and NSE in a group of 20 healthy Chinese individuals.

2 | MATERIALS AND METHODS

2.1 | Ethics approval

This study was approved by the ethics committee of the First Hospital of China Medical University.

2.2 | Participants

We recruited 20 apparently healthy subjects (9 men and 11 women) from laboratory staff in our hospital using a detailed questionnaire and physical examination. The median age was 29 years old (range 22–49 years) and the mean body mass index was 21.3 kg/m² (range 18.4–25.2 kg/m²). All volunteer subjects gave informed consent to participate in the study, according to the ethical standards of the China Medical University. Subjects self-reported as healthy, none of the subjects were taking any prescription medication (oral contraceptives excluded) or had any illness during the study, and none of the subjects had thyroid diseases or family history of thyroid diseases. Women were not pregnant or lactating, and had regular menstrual cycles. The following exclusion criteria were used: major medical illness, smoking, and heavy alcohol use. The subjects' weights were stable during the previous 2 months. The volunteer subjects did not exhibit any acute or chronic disease during our study, and they maintained their usual lifestyle.

2.3 | Specimen collection

Blood samples were collected once per week for 5 weeks. All blood samples were collected at the same time of the day (between 7:20 and 8:10 am). The subjects were fasted overnight and seated for 20 minutes. Blood was collected using a standardized method to minimize sources of pre-analytical variation. We used 5-mL BD Vacutainer vacuum collection tubes containing polymer gel for serum collection (Becton Dickinson Biosciences, New York, USA). Whole blood was allowed to clot for 15 minutes, the serum was separated (3000× g, 10 minutes), and the samples were stored at –80°C. All samples were collected and processed before performing batch analyses.

2.4 | Analysis of tumor markers

Samples were thawed for 30 minutes at room temperature, mixed using a vortex-shaking apparatus, and analyzed in duplicate in the same batch in random order. Quality control assessment was performed with satisfactory results before the analyses. One analyst performed all tests using the same reagents (same lot numbers) to minimize analytical variations. The NSE and CA724 levels were measured using a Roche E170 immunology analyzer (Roche Inc., Mannheim, Germany). The reagents (Cancer Antigen 724, 00169337; Neuron-specific enolase, 00170027) and calibration standards (CA724, 00173758; NSE, 00175154) were from Roche. The controls were from Bio-Rad Tumor Marker Plus Control kit (54560).

2.5 | Statistical analysis

The Cochran maximum variance test and Dixon test were conducted to identify outliers. The Kolmogorov-Smirnov test was used to ensure normality for all data. The Shapiro-Wilk test was used to ensure data normality for each subject. Biological variation was analyzed using nested analysis of variance and Microsoft Excel. Intra-individual biological variation (CV_I) and inter-individual biological variation (CV_G) were calculated according to the method of Fraser and Harris.⁸ The confidence interval (95%CI) for the biological variation of these tumor makers was calculated according to the method of Burdick et al.¹³ Analytical variation (SD_A^2) was calculated as the difference between duplicate results for each specimen. Intra-individual biological variation was calculated as the total intra-individual variation minus one-half of the analytical variation. Inter-individual biological variation was calculated as the total variation minus the analytical variation and the intra-individual variation. Overall means were used to convert the components of biological variation into relevant CV. The index of individuality (II) was calculated using the following equation: $II = [(CV_A^2 + CV_I^2)^{1/2}] / CV_G$. The reference change value (RCV) was calculated using the following formula: $RCV = 2^{1/2} \times Z \times [(CV_A^2 + CV_I^2)^{1/2}]$, where the Z score was 1.96. An RCV with 95% probability was regarded as significant. The number of tests that needed to determine the homeostatic set point for the individual was calculated using the formula: $n = [Z * (CV_A^2 + CV_I^2)^{1/2} / D]$, where n was the number of specimens obtained, and D was the percentage closeness to the homeostatic set point.

3 | RESULT

3.1 | Subjects and calculations

No visible hemolysis was observed before determining NSE. Most of the results are consistent with normal distributions for the individuals (90% for NSE and 70% for CA724). The Cochran maximum variance test was used to identify outliers and detect precision for repeated measures results. We identified two outliers for NSE and one outlier for CA724. These outliers were removed from the analyses, but other data for the subjects were retained. The Cochran maximum variance test also was used to identify the outliers of the mean values for

TABLE 1 Mean, analytical, and biological variation and 95%CI for NSE and CA724

Tumor marker	Range of results	Mean	CV _A (%)	CV _I (%)	CV _G (%)
NSE (ng/mL)	6.2-13.7	9.8	1.9	10.1 (8.9-19.9)	12.0 (7.1-18.7)
CA724 (U/mL)	0.6-21.6	3.5	4.0	50.3 (46.1-102.8)	103.4 (70.4-155.5)

CV_A, analytical variation; CV_I, intra-individual biological variation; CV_G, inter-individual biological variation.

TABLE 2 II, RCV and n for NSE and CA724

	II	RCV(95% Probability)	RCV(99% Probability)	n (D = 10%)	n (D = 20%)
NSE (ng/mL)	0.85	28.3%	37.3%	4	1
CA724 (U/mL)	0.48	139.8%	184.0%	97	24

II, Index of Individuality; RCV, Reference change values; n, the number of specimens; D, percent closeness to the homeostatic set point.

TABLE 3 Comparison of our results with previously published data on biological variations

	Our study			Federica Braga ¹¹			Zhihong Qi ¹²		
	CV _I	CV _G	II	CV _I	CV _G	II	CV _I	CV _G	II
NSE	10.1	12.0	0.85	13.6	11.5	1.44	13.2 (11.4-15.6)	16.8 (12.8-24.6)	0.78
CA724	50.3	103.4	0.48				16.1 (13.8-19.2)	97.0 (73.7-141.6)	0.17

CV_I, intra-individual biological variation; CV_G, inter-individual biological variation.

individuals, and to determine whether the discrete value of the test results for an individual was greater than the discrepancy of the entire population. The results indicated that NSE had one outlier, whereas CA724 had two outliers. We removed all data for these individuals from the analyses. Therefore, a total of 188 data points were used for the statistical analysis of serum NSE, and a total of 178 data points were used for the statistical analysis of serum CA724.

Serum CA724 levels of subject number 2 were high for all data collected during the study. We followed up this subject for 3 years with yearly physical examinations, but did not find any medical condition to explain the anomaly. Therefore, the results for this subject were removed from the analysis.

3.2 | Biological variations, indices of individuality, and reference change values for NSE and CA724

The overall means, analytical and biological variations, and II values for NSE and CA724 are presented in Table 1. The analytical variation was much smaller than the intra-individual biological variation. The II for CA724 was low (<0.6), whereas the II of NSE was larger (0.85). The RCVs for NSE and CA724 are presented in Table 2. The results obtained in our study were compared with those of two previous studies (Table 3). This comparison revealed that CV_I of CA724 was much larger in our study than in previous studies.

4 | DISCUSSION

Lung and stomach cancer are the most common cancers in China.¹⁴ NSE and CA724 tumor markers provide important information for

the treatment and follow-up of these patients. However, little was known about the biological variations of these markers. The valid estimates of the components of variation can be obtained from relatively small numbers of specimens collected from a small group of subjects over a reasonably short period of time.⁸ We based on this theory to design and implement the study of biological variation. Data regarding biological variation should be accompanied by name of manufacturer and methodology since methods change with time.¹⁵ Biological variation is an inherent variation that should be determined excluding preanalytical and analytical variation. In this study, we used standardized sample collection, processing, and analysis protocols to minimize pre-analytical and analytical variation. All analytical tests were conducted by the same technician using the same reagents. The analytical variations in our study were small (all <5%). This result achieved the general goal that analytical variation should be less than half of the intra-individual variation, and confirmed the accuracy of our data.

The intra- and inter-biological variations for NSE were 10.1% and 12.0%, respectively. These results were consistent with those reported previously by Braga et al¹¹ and Qi et al¹² Long-term and short-term biological variations were similar. We found only one published study¹² on the biological variation of CA724, which reported that the intra-individual biological variation was 16.1%, whereas the inter-individual biological variation was 97.0%. In our study, the intra-individual biological variation of CA724 was much larger than that in the previous report, 50.3%, whereas the inter-individual biological variation (103.4%) was similar with that in the previous report. The difference was due primarily to different sampling intervals. We collected samples every week for 5 weeks, whereas Qi et al¹² collected samples daily only for 5 days. We chose

to analyze weekly biological variation for a longer time because it might provide a more practical model for real-life clinical protocols analyzing tumor makers during follow-up examinations for patients with lung or stomach cancer.

The concept of RCV was introduced by Harris¹⁶ to determine whether a change in serial tests indicates a real change in a patient's condition; the difference between the two results should be larger than the combined variation for the two results. Our RCVs for NSE and CA724 were 28.3% and 139.8%, respectively. These results indicate that the follow-up test results for NSE and CA724 should change by 28.3% and 139.8% to be regarded as critically different; otherwise, the differences may be due primarily to other types of variation. The information obtained from RCV helps to improve the clinical application of tumor markers and improve patient treatment and monitoring. The disadvantage to this approach was discussed by Copper et al¹⁷ However, RCV is still considered as an objective tool for clinical and laboratory medicine.

Harris¹⁸ proposed that test results can be accurately evaluated with population-based reference intervals only when the index of individuality is greater than 1.4. An II value less than 0.6 indicates that population-based reference intervals are insensitive for the detection of unusual results for a particular individual. The II value of CA724 was 0.48, indicating that the range of values for a particular individual only span a small part of the population-based reference intervals. Reference intervals are insensitive for CA724, especially for determining whether a change was clinically significant. The overall II for NSE in our study was 0.85, which is higher than 0.6 but still less than 1.4. When we evaluate the result of serum NSE, both the reference interval and biological variation should be considered. Tumor markers are typically used for monitoring disease and not for cancer diagnosis. Therefore, serum CA724 and NSE with a low index are ideal for this purpose.

5 | CONCLUSION

We used standardized methods to estimate weekly biological variations of serum NSE and CA724 for 5 weeks in 20 healthy subjects. We also calculated RCV and II. Our results showed that II was low for both NSE and CA724, which suggests that we should pay attention to the RCV when assessing patient results.

ACKNOWLEDGMENTS

We thank all participants for their cooperation and sample contributions. This study was supported by the Ministry of Science and Technology of the People's Republic of China (National Key Technologies R&D Program of China, Grant number 2012BAI37B01). We thank for the BioMed Proofreading LLC for English language copyediting.

ORCID

Hong Shang <http://orcid.org/0000-0001-5333-8943>

REFERENCES

1. Tuxen MK, Soletormos G, Petersen PH, Schioler V, Dombernowsky P. Assessment of biological variation and analytical imprecision of CA 125, CEA, and TPA in relation to monitoring of ovarian cancer. *Gynecol Oncol*. 1999;74:12-22.
2. Gaspar MJ, Arribas I, Coca MC, Diez-Alonso M. Prognostic value of carcinoembryonic antigen, CA 19-9 and CA 72-4 in gastric carcinoma. *Tumour Biol*. 2001;22:318-322.
3. Chen XZ, Zhang WK, Yang K, et al. Correlation between serum CA724 and gastric cancer: multiple analyses based on Chinese population. *Mol Biol Rep*. 2012;39:9031-9039.
4. Shibayama T, Ueoka H, Nishii K, et al. Complementary roles of pro-gastrin-releasing peptide (ProGRP) and neuron specific enolase (NSE) in diagnosis and prognosis of small-cell lung cancer (SCLC). *Lung Cancer*. 2001;32:61-69.
5. Wojcik E, Kulpa JK, Sas-Korczynska B, Korzeniowski S, Jakubowicz J. ProGRP and NSE in therapy monitoring in patients with small cell lung cancer. *Anticancer Res*. 2008;28:3027-3033.
6. Fraser CG. Data on biological variation: essential prerequisites for introducing new procedures? *Clin Chem*. 1994;40:1671-1673.
7. Fraser CG. Desirable performance standards for clinical chemistry tests. *Adv Clin Chem*. 1983;23:299-339.
8. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci*. 1989;27:409-437.
9. Plebani M, Giacomini A, Beghi L, et al. Serum tumor markers in monitoring patients: interpretation of results using analytical and biological variation. *Anticancer Res*. 1996;16:2249-2252.
10. Biological variation database, and quality specifications for imprecision, bias and total error (desirable and minimum). The 2014 update. www.westgard.com/biodatabase1.htm. Accessed December 2, 2014.
11. Braga F, Ferraro S, Mozzi R, Dolci A, Panteghini M. Biological variation of neuroendocrine tumor markers chromogranin A and neuron-specific enolase. *Clin Biochem*. 2013;46:148-151.
12. Qi Z, Zhang L, Chen Y, et al. Biological variations of seven tumor markers. *Clin Chim Acta*. 2015;450:233-236.
13. Burdick RK, Graybill FA. *Confidence Intervals on Variance Components*. New York: Marcel Dekker Inc.; 1992.
14. Chen W, Zheng R, Zuo T, Zeng H, Zhang S, He J. National cancer incidence and mortality in China, 2012. *Chin J Cancer Res*. 2016;28:1-11.
15. Fraser CG, Lippi G, Plebani M. Reference change values may need some improvement but are invaluable tools in laboratory medicine. *Clin Chem Lab Med*. 2012;50:963-964.
16. Harris EK, Yasaka T. On the calculation of a "reference change" for comparing two consecutive measurements. *Clin Chem*. 1983;29:25-30.
17. Cooper G, DeJonge N, Ehrmeyer S, et al. Collective opinion paper on findings of the 2010 convocation of experts on laboratory quality. *Clin Chem Lab Med*. 2011;49:793-802.
18. Harris EK. Effects of intra- and interindividual variation on the appropriate use of normal ranges. *Clin Chem*. 1974;20:1535-1542.

How to cite this article: Wang S, Zhao M, Mu R, Zhang X, Yun K, Shang H. Biological variation of serum neuron-specific enolase and carbohydrate antigen 724 tumor markers. *J Clin Lab Anal*. 2018;32:e22327. <https://doi.org/10.1002/jcla.22327>