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Biological variations of seven tumor markers

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ABSTRACT

Background: We sought to identify biological variations in the following tumor markers: pepsinogen I (PGI), pepsinogen II (PGII), carbohydrate antigen 724 (CA724), neuron-specific enolase (NSE), pro-gastrin-releasing peptide (ProGRP), carcinoembryonic antigen (CEA), and carbohydrate antigen 199 (CA199).
Methods: Serum samples were collected from 20 healthy Chinese individuals over 5 days. Samples were then screened for the presence of the seven aforementioned tumor markers. Within-individual coefficient of variation (CV_I), between-individual coefficient of variation (CV_G), confidence interval (CI) of biological variations, index of individuality (II), and the reference change value (RCV) of the seven tumor markers were calculated.
Results: Of the 7 tumor markers, index of individuality was all <1.0. ProGRP showed the lowest CV_I and CV_G, at 4.75% (CI: 3.96%–5.94%) and 16.42% (CI: 12.32%-24.61%), respectively. The 95% and 99% RCVs for ProGRP were 14.68 and 19.32, respectively, and were the lowest of the markers. In contrast, the CV_I and CV_G for CA724 were the highest, at 16.06% (CI: 13.83%–19.17%) and 96.95% (CI: 73.73%–141.59%), respectively. The 95% and 99% RCVs for CA724 were the highest, at 45.89 and 60.41, respectively.
Conclusion: Our findings provide additional information regarding the biological variation of tumor markers, and could be applied in a clinical setting.

1. Introduction

To accurately estimate longitudinal changes in individuals, it is important to take into account the biological variation of measurements. The two components of biological variation are the between-individual variability, which is the variation due to heterogeneity of physiological influences among subjects, and the within-individual variability, which is the biological variation in the same individual over time. According to Fraser et al. [1], changes in serial results for an individual could be due to deterioration or amelioration of the patient's condition, pre-analytical sources of variation, random analytical error, and within-individual biological variation around the homeostatic set point.

Numerous clinical guidelines for evidence-based medicine in oncology recommend the clinical application of serum tumor markers [2]. In many malignancies, tumor markers play an important role in patient management [3]. Tumor markers are useful in determining the progression of cancer in patients that are undergoing chemotherapy [4,5]. However, changes in tumor marker values over time are clinically relevant and can be difficult to assess [6]. The European Group on Tumor Markers recently proposed that an estimate of the inherent within-individual biological variation could be used to evaluate various cancer therapies [7].

An increasing number of tumor markers is used to monitor cancer patients and may sometimes aid diagnosis of cancer patients. These include pepsinogen I (PGI), pepsinogen II (PGII), carbohydrate antigen 724 (CA724), neuron-specific enolase (NSE), pro-gastrin-releasing peptide (ProGRP), carcinoembryonic antigen (CEA), and carbohydrate antigen 199 (CA199). PGI and PGII are being investigated as possible markers for gastric cancer, while CA724 may aid the therapeutic monitoring of stomach carcinomas. NSE is used to monitor therapy and progress in patients with small cell bronchial carcinoma and neuroblastoma. ProGRP is associated with neuroendocrine tumors, including small cell lung cancer. Elevated CEA serum concentrations have been demonstrated in a large variety of malignancies. The CA199 marker is mainly associated with pancreatic and gallbladder cancers.

There are no published studies regarding the biological variations of PGI, PGII, CA724, NSE, and ProGRP in the 2014 update of the Biologic Variation Database [8]. This study analyzed biological variations of 5 tumor markers (PGI, PGII, CA724, NSE, and ProGRP) and compared these variations in terms of CEA and CA199 with those in the database.

Abbreviations: CV_I, within-individual coefficient of variation; CV_G, between-individual coefficient of variation; CV_A, analytical coefficient of variation; CI, confidence interval; RCV, reference change value; II, index of individuality.

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Table 1
CV_A, CV_I, CV_G, and the corresponding CI for 7 tumor markers.

Tumor marker	Range of results	Upper limit of reference interval	CV_A (%)	CV_I (%)	CV_G (%)
PGI (ng/ml)	24.4–79.2	NA	2.17 (1.91–2.53)	8.21 (7.09–9.75)	23.01 (17.5–33.61)
PGII (ng/ml)	3.2–26.8	NA	3.39 (2.99–3.92)	12.21 (10.63–14.36)	37.32 (28.38–54.5)
CA724 (U/ml)	0.8–9.6	9.8	4.03 (3.53–4.71)	16.06 (13.83–19.17)	96.95 (73.73–141.59)
NSE (ng/ml)	5.3–16.3	16.3	2.12 (1.86–2.46)	13.2 (11.44–15.62)	16.84 (12.81–24.59)
ProGRP (pg/ml)	12.4–46.5	50.0	2.34 (1.99–2.81)	4.75 (3.96–5.94)	16.42 (12.32–24.61)
CEA (ng/ml)	0.61–2.73	5.0	2.15 (1.88–2.51)	10.95 (9.44–13.05)	46.74 (35.54–68.26)
CA199 (U/ml)	2.86–20.01	37.0	3.23 (2.83–3.77)	6.76 (5.82–8.05)	49.56 (37.69–72.38)

NA, no available reference interval; CV_I, within-individual coefficient of variation; CV_G, between-individual coefficient of variation; CV_A, analytical coefficient of variation; CI, confidence interval.

2. Materials and methods

2.1. Study subjects

We enrolled 20 healthy Chinese volunteers, comprising 11 males and 9 females (21–38 years, median age, 26 years) to this study. All enrolled individuals signed informed consent forms. Interviews and health questionnaires were conducted, along with routine blood and biochemical tests, to ensure that all individuals: led healthy lifestyles; had a relatively healthy status; and did not take any medications. Additionally, none of the enrolled women were pregnant or currently menstruating. During the study period, individuals maintained their normal lifestyles, which involved no excessive consumption of alcohol, tea, and tobacco products, and no strenuous exercise.

2.2. Specimen collection

Venous blood samples were drawn at 08:00, 12:00, and 16:00 on day 1, and at 08:00 on days 2–5 by the same phlebotomist. Blood samples from each individual were placed in serum tubes (BD Biosciences, San Jose, CA, USA) and centrifuged (3000 $\times g$, 10 min) once they had coagulated. Separated serum was collected from tubes and stored at −80 °C until required.

2.3. Analysis of tumor markers

Specimens were thawed at room temperature for 30 min, mixed thoroughly, and analyzed. All 7 markers (PGI, PGII, CA724, NSE, ProGRP, CEA, and CA199) were measured twice for each sample to ensure the accuracy of results. All specimens from an individual were analyzed in the same batch.

Internal quality control (IQC) samples were analyzed prior to the screening of specimens. Tumor markers PGI, PGII, and ProGRP were analyzed with an Architect i2000 immunoassay analyzer (Abbott Labs). Reagents (PGI, 43507LI00; PGII, 42454LI00; and ProGRP, 49171LP18), calibration standards (PGI, 30691LI00; PGII, 38225L100; and ProGRP, 44175LP26), and controls (PGI, 89607HN00 and 89608HN00; PGII, 89322HN00 and 89323HN00; and ProGRP, F5Y208-1 and F5Y208-2) were all from Architect Inc. Tumor markers CA724, NSE, CEA, and CA199 were assayed on a Cobas E601 immunology analyzer (Roche Inc.). Reagents (CA724, 18039901; NSE, 17462203; CEA, 18080102; CA199, 17769403), calibration standards (CA724, 173758-1; NSE, 175945-01; CEA, 179676-01; and CA199, 174979-01) and controls (multi-quality control, 173320 and 173339) were all from Roche Inc. The data on internal quality control were shown in Supplementary Table 1.

2.4. Statistical analysis

We conducted statistical analyses using SAS 9.3 (SAS Institute Inc.), with abnormal values excluded according to the method of Talwar et al. [9]. A normality test was performed using the Kolmogorov–Smirnov test, and logarithmic conversions were conducted on skewed indicators [10]. The within-individual coefficient of variation (CV_I), between-individual coefficient of variation (CV_G), and analytical coefficient of variation (CV_A) were calculated using a nested analysis of variance. CV_I and CV_G were evaluated according to the method of Fraser et al. [11]. The confidence interval (CI) of biological variation were analyzed according to the method of Burdick et al. [12]. The index of individuality (II) was calculated from CV_I and CV_G using the formula: $II = CV_I/CV_G$ [13]. The reference change value (RCV) was calculated using the formula: $RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$ [13]. Where Z values of 1.65, 1.96, and 2.58 represented probabilities of 90%, 95%, and 99%, respectively. For the RCV, probabilities of 95% ($P < 0.05$) and 99% ($P < 0.01$) indicated that differences were significant and highly significant, respectively. Comparison of intra-day and inter-day results for the markers was analyzed non-parametrically by the Friedman test, with results considered significantly different if the *P*-value was less than 0.05.

3. Results

3.1. CV_A, CV_I, CV_G, and CI for the 7 tumor markers

The CV_A values for all the tumor markers analyzed were below 5%. The CV_A, CV_I, CV_G, and CI for PGI, PGII, CA724, NSE, ProGRP, CEA, and CA199 are presented in Table 1. Of the 7 tumor markers, ProGRP exhibited the lowest CV_I and CV_G, at 4.75% (CI: 3.96%–5.94%) and 16.42% (CI: 12.32%–24.61%), respectively. CV_I and CV_G for CA724 were the highest, at 16.06% (CI: 13.83%–19.17%) and 96.95% (CI: 73.73%–141.59%), respectively.

3.2. Index of individuality and RCVs for tumor markers

The index of individuality for the tumor markers screened for was all <1.0 (Table 2). In addition, we calculated RCVs for the 7 tumor markers (Table 2). The 95% and 99% RCVs for ProGRP were 14.68 and 19.32, respectively, and were the lowest of the 7 markers. The highest 95% and 99% RCVs were observed for CA724, at 45.89 and 60.41, respectively.

Table 2
Index of individuality and RCV for the 7 tumor markers.

Tumor marker	II	RCV		
		90%	95%	99%
PGI	0.36	19.81	23.53	30.98
PGII	0.33	29.56	35.12	46.23
CA724	0.17	38.63	45.89	60.41
NSE	0.78	31.19	37.05	48.77
ProGRP	0.29	12.35	14.68	19.32
CEA	0.23	26.04	30.93	40.71
CA199	0.14	17.48	20.76	27.33

II, index of individuality; RCV, reference change value.