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Biological variation data for kidney function related parameter: serum beta trace protein, creatinine and cystatin C from 22 apparently healthy Turkish subjects

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Abstract

Objectives: Biological variation is defined as the variation in analytical concentration between and within individuals, and being aware of this biological variation is important for understanding disease dynamics. The aim of our study is to calculate the within-subject (CV_I) and between-subject (CV_G) biological variations of serum creatinine, cystatin C and Beta trace protein (BTP), as well as the reference change value (RCV) and individuality indexes (II), which are used to calculate the glomerular filtration rate while evaluating kidney damage.

Methods: Blood samples were collected from 22 healthy volunteers for 10 consecutive weeks and stored at −80 °C until the day of analysis. While the analysis for serum creatinine was performed colorimetrically with the kinetic jaffe method, the nephelometric method was employed for cystatin C and BTP measurements. All analyses were carried out in a single session for each test.

Results: Analytical coefficient of variation (CV_A) for serum creatinine, cystatin C and beta trace protein was 5.56, 3.48 and 5.37%, respectively. CV_I and CV_G: for serum creatinine: 3.31, 14.50%, respectively, for cystatin C: 3.15, 12.24%, respectively, for BTP: 9.91, 14.36%, respectively. RCV and II were calculated as 17.94%, 0.23 for serum creatinine, 13.01%, 0.26 for cystatin C, 31.24%, 0.69 for BTP, respectively.

Conclusions: According to the data obtained in our study, serum creatinine and cystatin C show high individuality, therefore we think that the use of RCV instead of reference ranges would be appropriate. Although II is found to be low for BTP, more studies are needed to support this finding.

Keywords: beta trace protein; biological variation; glomerular filtration rate; reference change values.

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Introduction

Intra-individual biological variation is defined as the random fluctuations of an analyte around the homeostatic set for each individual. It is also referred to as within-subject biological variation (CV_I) [1]. Between-subject biological variation (CV_G), also known as inter-individual biological variation, is known as the difference between the homeostatic set points of different individuals [2]. CV_I is important for personalized reference intervals, while CV_G is important for population-based and cross-sectional reference values [3]. It is stated in the literature that the use of personalized reference intervals calculated using CV_I can be very valuable for the diagnosis of patients as well as for follow-up and treatment [4]. Each laboratory test has its unique biological variability. Being aware of this variation is important for understanding disease dynamics [5]. In everyday laboratory practice, biological variation data can be used at any stage of the total testing process. In the preanalytical phase, biological variation data can be used for test selection or appropriate sample selection, while in the analytical phase, each laboratory can set analytical performance targets such as analytical variation (CV_A), bias, total acceptable error (TE_A) based on biological variation data. Critical difference (CD) or reference change value (RCV) calculated using the biological variation data is employed to assess whether there is clinical significance between two consecutive results during a patient follow-up [6]. The increased awareness for both RCV and biological

variation has led to more research for the biological variation of several laboratory tests [7, 8].

Glomerular filtration rate (GFR), which is known to reflect kidney functions, is usually estimated based on serum creatinine (SCr) levels. However, SCr levels are affected by many factors such as changes in body composition due to gender, race, age, exercise or muscle diseases, and it is often incompetent to detect mild to moderate renal damage. Cystatin C, another biomarker used in GFR estimation, is a nonglycolyzed low molecular weight protein containing 122 amino acids, weighing 13 kDa, and is a member of the cystatin superfamily, one of the cysteine proteinase inhibitors [9]. Unlike SCr, serum cystatin C level is independent of gender, age or muscle mass [10]. β trace protein (BTP), which is reported as a relatively new GFR marker, is an enzyme from the prostaglandin D synthase class and is synthesized at a constant rate by glial cells of central nervous system [11]. In literature, it has been suggested that BTP's performance as a GFR marker is similar to cystatin C in adult patients [12–14].

SCr, cystatin C, and BTP test for estimating GFR have some advantages and/or disadvantages compared to each other in terms of molecular structure, cost-effectiveness, or biological properties. Although there is some information regarding biological variation for SCr and cystatin C [15], the data is not fully trustworthy because some of those studies do not follow the requirements in the guideline published by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Working Group (BV-WG) and most of them are not up-to-date [16]. Besides, there is no information on the biological variability of BTP neither in the literature nor in the biological variation database yet [15, 17].

Our study aims to calculate the CV_I and CV_G values of GFR markers including SCr, cystatin C, and BTP, hence, the RCV and individuality indices of those markers.

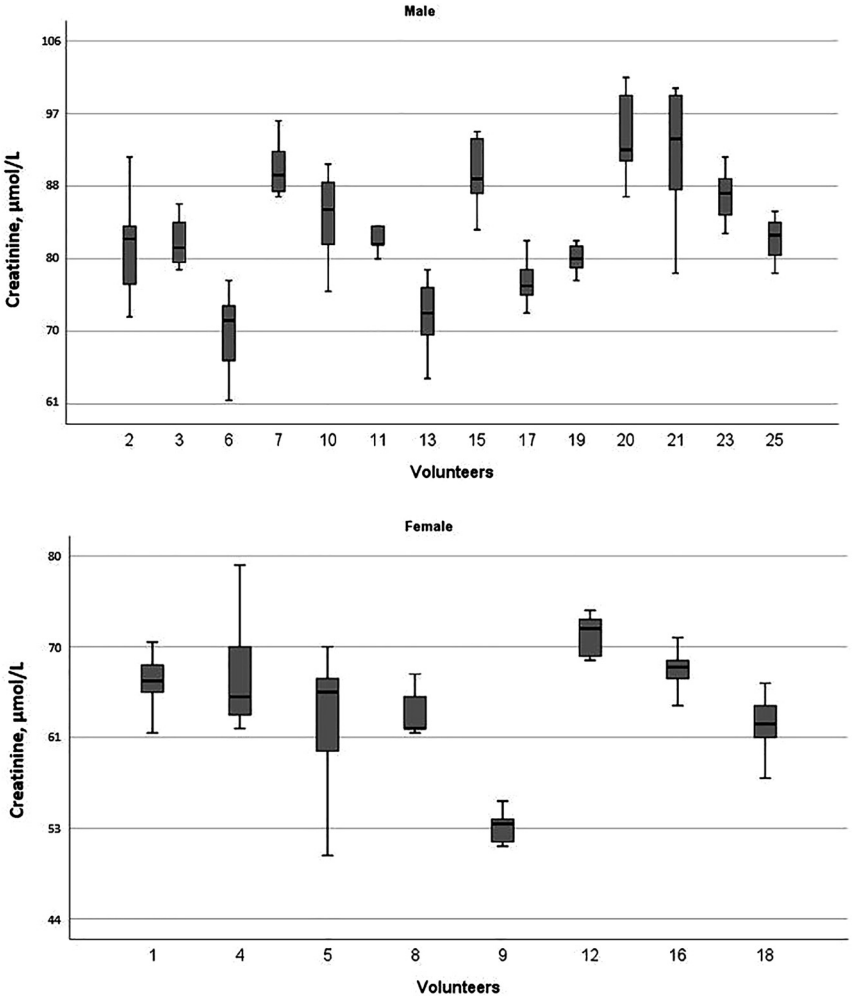

Figure 1: Distribution of creatinine results of volunteers by gender.

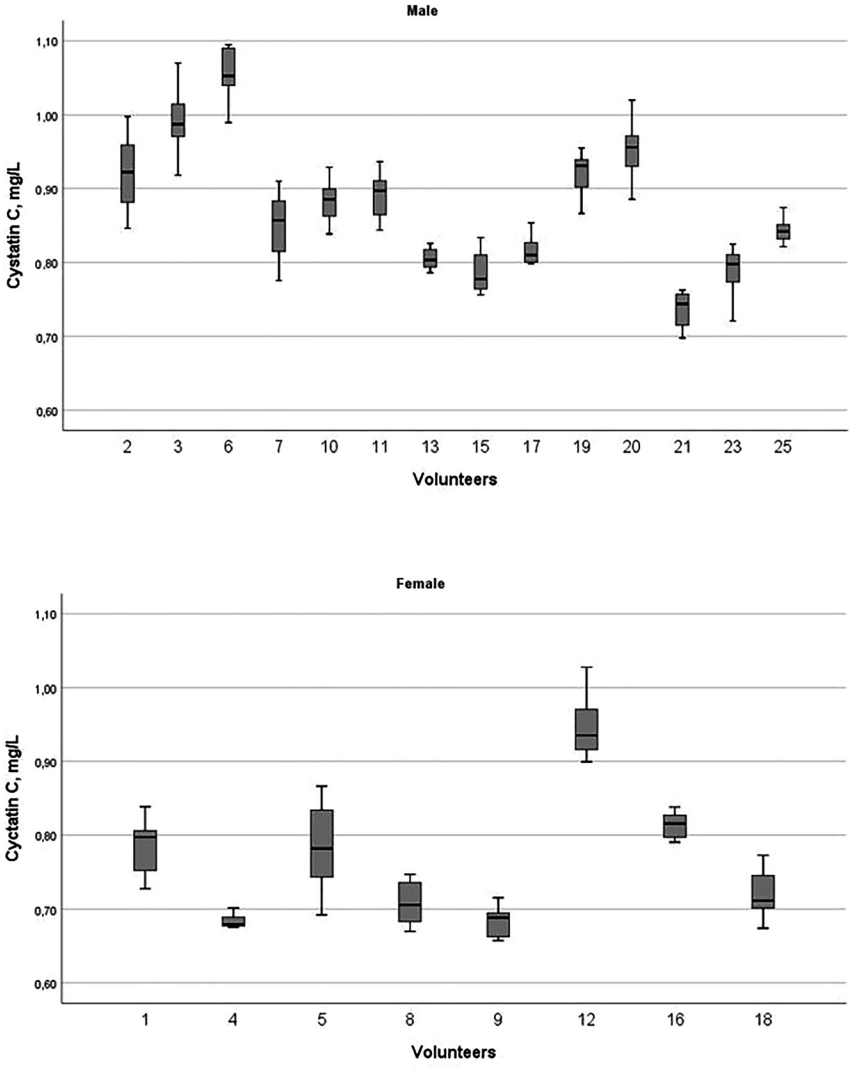

Figure 2: Distribution of cystatin C results of volunteers by gender.

Materials and methods

The study design and process were planned by considering the checklist for critical appraisal of studies of biological variation published by the BV-WG established by the EFLM [18]. The study was approved by the Bozyaka Training and Research Hospital, Izmir, Turkey clinical research Ethics Committee (Decision No. 4 dated 11.10.2017).

Study participants

Twenty-seven volunteers healthy in appearance who agreed to sign the informed consent form were included in the study in line with the Helsinki Declaration and Good Clinical Practice guidelines. Exclusion criteria determined by the EFLM BV-WG were implemented [19]: Those with a known diagnosis of diabetes, those with chronic kidney or liver disease, family history of thalassemia or other hemoglobinopathies, dyslipidemia, hepatitis B virus, hepatitis C virus, human immunodeficiency virus (HIV) carriers, patients with GFR <60 mL/min, and those with a history of hospitalization or serious illness in the last four weeks, or pregnant women. Thus, four volunteers were excluded from the study according to the exclusion criteria. The study was carried out with a total of 23 volunteers, 14 males, and nine females, between the range of 24–63 years. Since one subject could not sustain the sampling period for 10 weeks, the final number of volunteers was updated as 22 and all analyzes were performed on 22 volunteers' data.

Sample collection and preparation

Venous blood sampling performed by the same phlebotomist for 10 weeks on the same day at 8:00 and 10:00 am of each week. Venous blood samples were collected into gel-clot activator tubes (BD Vacutainer® SST II™ Advance, 5 mL, Catalog # 367,955, Plymouth, UK) following 8–12 h of fasting. Each blood tube was centrifuged at 1,500 *g* for 10 min, and each serum supernatant was divided into aliquots per analyte and stored at −80 °C until further analysis. On the day of analysis, serum samples were let to room temperature and centrifuged at 1,500 *g* for 2 min for homogenization, and then SCr, cystatin C, and BTP levels measured in duplicate.

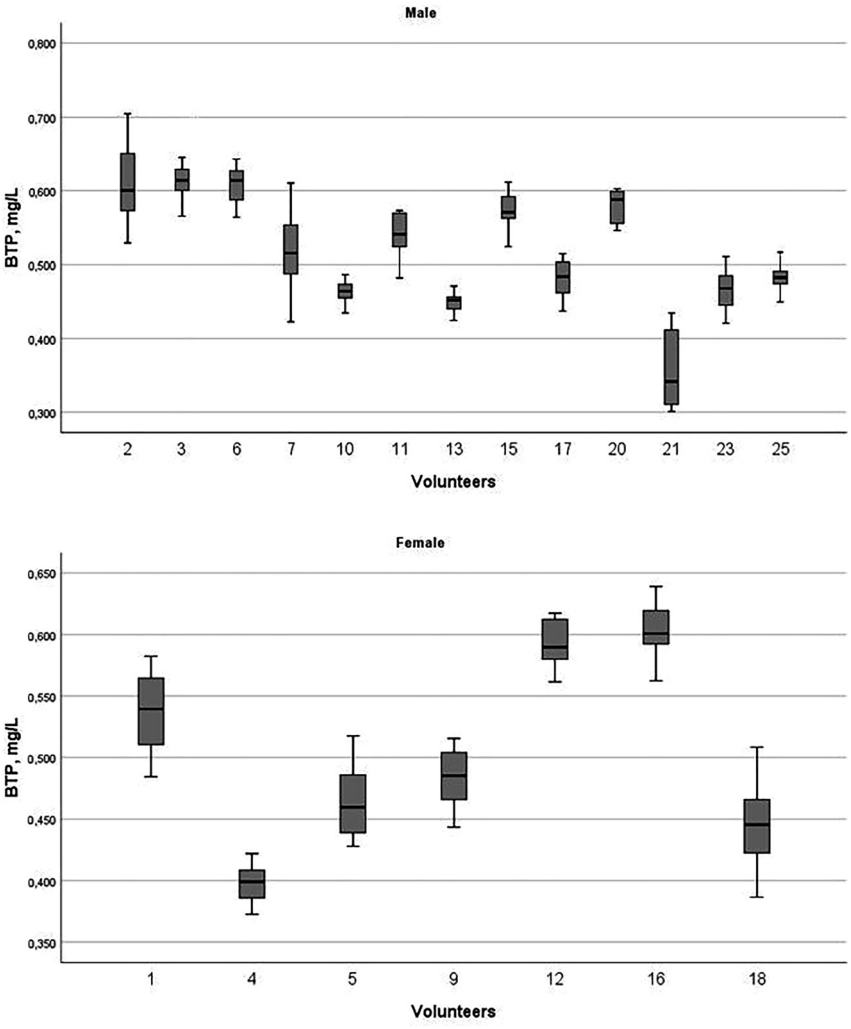

Figure 3: Distribution of BTP results of volunteers by gender.

Laboratory tests

Two levels of internal quality control material were used for Creatinine, Cystatin C and BTP. Within-run and total CV values for creatinine calculated using internal quality control material (Beckman Coulter control Serum; Beckman Coulter Inc., USA) were 3.44 and 3.97% for Level 1, 1.49 and 2.05% for Level 2, respectively. Within-run and total CV values for Cystatin C calculated using internal quality control material (N Latex Cystatin C, Siemens Healthcare Diagnostics Products, Marburg, Germany) were 1.62 and 1.91% for Level 1, 1.79 and 1.80% for Level 2, respectively. Within-run and total CV values for BTP calculated using internal quality control material (N Protein Control, Siemens Healthcare Diagnostics Products, Marburg, Germany) were 1.93 and 2.33% for Level 1, 1.22 and 1.32% for Level 2, respectively.

SCr levels were measured by colorimetrical method using the kinetic Jaffe method in a Beckman Coulter AU 5800 (Beckman Coulter Inc., Brea, CA, USA) analyzer. Serum creatinine kit (OSR 6178, Beckman Coulter Inc., Brea, CA, USA) was used for serum creatinine measurement. Serum cystatin C and BTP measurements were performed on Siemens Atellica NEPH 630 (Siemens Healthineers, Marburg, Germany) nephelometer. N Latex Cystatin C kit (Siemens Healthcare, Mississauga, Canada) was used for Cystatin C, while N Latex BTP kit (Siemens Healthcare Diagnostics Products, GmbH, Marlberg, Germany) was used for BTP. All analyses were carried out in a single run for each test. The estimated GFR (eGFR) was calculated using the CKD-EPI (Chronic kidney disease epidemiology collaboration) equation [20]. The CKD-EPI formula used is as follows:

- Female if SCr ≤0.7 mg/dL (≤62 μmol/L)
  $144 \times (SCr/0.7)^{-0.329} \times 0.993^{Age}$ [if black × 1.159]
- Female if SCr >0.7 mg/dL (>62 μmol/L)
  $144 \times (SCr/0.7)^{-1.209} \times 0.993^{Age}$ [if black × 1.159]
- Male if SCr ≤0.9 mg/dL (≤80 μmol/L)
  $141 \times (SCr/0.9)^{-0.411} \times 0.993^{Age}$ [if black × 1.159]
- Male if SCr >0.9 mg/dL (>80 μmol/L)
  $141 \times (SCr/0.9)^{-1.209} \times 0.993^{Age}$ [if black × 1.159]

Statistical analysis

Statistical Program for the Social Services (SPSS) (Chicago, IL, USA) 20.0 package and Microsoft Office Excel 2010 used for statistical analysis. CV_I, CV_G and analytical variation (CV_A), were calculated according to Fraser and Harris' method for data analysis [1, 3]. Briefly, outliers were detected and excluded before analysis. Outlier data in

within-subject analyses were determined with a Box-Plot and excluded from the analysis (Figures 1–3). The outliers in the between-subject results were detected according to Dixon-Reed criteria and also excluded from the analysis [21]. CV_A homogeneity was confirmed using the Barlett, and CV_I homogeneity by Cochran test [22]. The Shapiro-Wilk test was used to evaluate the distribution of within-subject and between-subject measurements. All measurements had normal distribution. After checking for variation homogeneity and normal distribution, CV_A, CV_I, and CV_G were calculated using nested ANOVA. The coefficients of variation (CV) were calculated as (SD/mean serum concentration) × 100 based on analytical, within-subject, and between-subject variations. RCV was determined using formula $2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}$ for 95 and 99% (z-score; 1.96 for 95% probability, 2.54 for 99%). Individuality index was calculated using the following formula: CV_I/CV_G

Results

The demographic data of the volunteers are presented in Table 1. For SCr, 16 out of 440 data analyzed were excluded from analysis: seventh volunteer's second and third-week measurements, eighth volunteer's eighth-week measurement, ninth volunteer eighth-week measurement, 11th volunteer's sixth-week measurement, 12th volunteer's sixth-week measurement, and 19th volunteer's second and third-week measurements.

For Cystatin C, six of the 440 data analyzed were excluded from the study: the second and third-week measurements of the second volunteer and the first-week measurement of the 20th volunteer.

For BTP, 42 of the 440 data analyzed were excluded from the study: all measurements of the 18 and 19th volunteers were excluded since the normal distribution of within-subject data was not observed, the outlier third-week measurement of the 21st volunteer was excluded as well.

Biological variation data for SCr, cystatin C, and BTP as well as RCV and individuality indices obtained from the data is summarized in Table 2.

Table 1: Characteristics of the study population.

Characteristic	Total	Male	Female
n	22	14	8
Age, year	40 (24–63)	42.5 (24–63)	38.5 (28–56)
Height, cm	178 (150–183)	179 (177–183)	161 (150–170)
Weight, kg	76 (51–88)	79.5 (63–88)	58.5 (51–62)
Body mass index, kg/m^2	23.8 (19.7–26.3)	24.6 (19.7–26.3)	21.8 (20.4–24.0)
Body surface area, m^2	1.94 (1.48–2.12)	1.99 (1.77–2.12)	1.63 (1.48–1.67)
Estimated GFR calculated using CKD-EPI equation, mL/min/1.73 m^2	100.6 (66.1–122.6)	106.75 (78.2–122.6)	95.6 (66.1–104.2)
Serum creatinine, µmol/L	74.3 (47.7–99.9)	83.1 (62.8–99.9)	66.3 (47.7–78.7)
Serum cystatin C, mg/L	0.81 (0.62–1.15)	0.85 (0.70–1.15)	0.74 (0.62–1.03)
Serum beta trace protein, mg/L	0.501 (0.281–0.712)	0.517 (0.281–0.712)	0.496 (0.356–0.639)

Corresponding values for continuous data are shown as median (interquartiles range). Laboratory median data (serum creatinine, serum cystatin C and serum Beta Trace Protein concentrations) represent the median of all values obtained during the 10 week study period.

Table 2: Summary of components of variation for creatinine, cystatin C and beta trace protein.

Components of variation	Creatinine	Cystatin C	Beta trace protein
n	424	398	434
Mean concentrations	76.9 (±11.5) µmol/L	0.84 (±0.11) mg/L	0.514 (±0.08) mg/L
CV_A	5.56 (5.08–6.13)	3.48 (3.18–3.84)	5.37 (4.90–5.96)
CV_I	3.31 (3.02–3.68)	3.15 (2.87–3.50)	9.91 (8.98–11.05)
CV_G	14.50 (11.15–20.71)	12.24 (9.42–17.49)	14.36 (10.92–20.97)
RCV	17.94%	13.01%	31.24%
II	0.23	0.26	0.69

Values are % (95% CI); CI, confidence interval; CV_A, analytical variation; CV_G, between-subject variation; CV_I, within-subject biological variation; RCV, reference change value; II, index of individuality.

Discussion

SCr, cystatin C, and BTP are parameters used to evaluate renal function. Biological variation data should be used by all laboratories to correctly manage the process of diagnosis, treatment, and follow-up of patients. As far as we know, our study is the first study in which the biological variation of these three parameters used in eGFR calculation is evaluated together and the biological variation for the BTP parameter is evaluated. In our study, within-subject biological variation data for SCr, cystatin C, and BTP are 3.31, 3.15, and 9.91%, respectively. RCV was calculated as 17.94, 13.01, and 31.24%, respectively; and the individuality index was found to be 0.23, 0.26, and 0.69, respectively.

Biological variation studies are challenging studies due to the need for strict time management, and resources to carry them out. Therefore, biological variation databases have been created to facilitate researchers to access available data. Referred articles in the biological variation databases have different results that can depend on several factors affecting CV_I such as the number, frequency, and duration of measurements, the total number of subjects, etc. Only 15 of the articles registered for SCr in the biological variation database created by EFLM were included in the meta-analysis [15, 16] and CV_I was given as 4.5% and CV_G as 14.3%. In the study by Gowans and Fraser [23], blood samples were taken from 15 healthy volunteers, seven male, and eight female, at four-week intervals for 40 weeks, and CV_I and CV_G values for SCr were found to be 4.1 and 14.1%, respectively. In the study published by M. Reinhard et al., 20 healthy volunteers were included for eight weeks, and CV_I for creatinine was found to be 4.7% and CV_G was 14.4% [24]. In our study, CV_I and CV_G values of SCr were 3.31 and 14.5%, respectively that the values were consistent with the literature. The minor differences among studies might be due to demographic differences such as an imbalance in male to female ratio. As is known, SCr concentration can be affected by many factors such as sex, nutrition, age, race, and muscle mass [25]. SCr measurement is one of the most requested tests in clinical laboratories as it offers an easy-to-follow, accessible, and internationally accepted method for GFR estimation to evaluate renal function [26, 27]. As seen in the study of Carobene et al. [28], although there were differences in the SCr measurement results between the enzymatic method and the Jaffe method, SCr measurement was performed using the Jaffe method in our study, since it was the widely used method. Investigating the question whether different methods create different biological variation values (measuring SCr with enzymatic vs. Jaffe methods), a multicenter study conducted in 2017 by Carobene et al. found CV_I and CV_G for the enzymatic method as 4.4 and 17.1%, respectively, while it was found to be 4.7 and 19.0% in the Jaffe method [29]. Both SCr methods produced similar biological variation profiles. However, the results were found to be similar between the methods, RCV values were also quite different. This difference in RCV is due to the different CV_A between the two methods. In the study of Carobene et al., the CV_A was 1.1% for the enzymatic method and 4.4% for the alkaline picrate method [29]. A similarly high CV_A was observed in our study, and this value did not meet the analytical performance target for precision.

Where patients' consecutive results are obtained from separate analytical systems, differences between the results may complicate the clinical interpretation, and these differences may also affect confidence intervals for eGFR estimation. In our study, the individuality index for SCr was calculated as 0.23. This value represents high individuality for SCr and in this case, the population-based reference ranges for the SCr parameter might be misleading for healthcare professionals. We reemphasize that it would be useful to implement the calculated RCV (17.94%) value instead of population-based reference ranges for monitoring patients with SCr.

In the case of cystatin C, only three of the 14 studies registered in the EFLM database were included in the meta-analysis, and as a result of those, CV_I and CV_G values were calculated as was 4.0 and 12.1%, respectively [15]. In a study by Carobene et al. [30], cystatin C measurements that were analyzed by turbidimetric method, CV_I and CV_G were determined as 3.9 and 12.0%, respectively. In another study that employing the nephelometric method, CV_I was found to be 4.5% and CV_G was found to be 13.0% [31], yet another study with a similar method reported a CV_I of 4.5% [32]. When our cystatin C results are compared to the results from the EFLM database, a general agreement can be observed. The minor differences among studies are thought to be due to reasons such as the participant numbers, or different analytical methods. However, the results obtained with turbidimetric and nephelometric methods are compatible as stated in the literature [33]. In our study, the individuality index for cystatin C was calculated as 0.26, and the RCV was found to be 13.01% and hence the use of individual-based reference intervals or RCV would be more reasonable than population-based reference intervals.

To date, there is no information for BTP in terms of biological variation in the database [15] and our biological variation determination might fill this gap. In the study, we calculated CV_I for BTP as 9.91% and CV_G as 14.36%. The

proximity of the values CV_I and CV_G, can be interpreted as the molecule not showing significant natural variation and not having distinct individual characteristics. Higher biological variation value of BTP compared to the other two biomarkers can be explained by its wide distribution in body fluids (such as cerebrospinal fluid, seminal plasma, plasma, and urine) as well as its synthesis from various tissues such as the brain, retina, melanocytes, heart, and kidneys [34]. Studies show that BTP is affected by several pathological conditions such as thyroid dysfunction, hepatic dysfunction, and infection. The short half-life indicates that this parameter is dynamic, suggesting that the observed high variation may be the cause.

In addition, deficiencies in standardization and cross-reactions due to the presence of different prostaglandin D synthase subtypes that catalyze the same reaction as BTP but have differences in amino acid sequences, evolutionary origins and cellular localization may create analytical variation for BTP [34]. More studies need to be performed for this parameter by detailing its physiological and analytical properties to verify our results. In this study, we found the RCV as 31.24% and the individuality index 0.69 for BTP. Although these findings represent that population-based reference ranges can be used in measurements with the current methodology and reagent to interpret the changes in BTP levels, RCV can be used in patient follow-up because the reference intervals used for BTP will be sensitive to abnormal changes in an individual.

It is already stated that it would be more appropriate to determine the CV_I used in the calculation of RCV in individuals with specific diseases to detect important changes beforehand [35]. In the study conducted by M. Reinhard et al., the CV_I value for SCr was 8.9% among 19 patients with mild to moderate renal dysfunction. Thus, this situation supports the need to derive biological variation data for specific diseases [24]. The biological variation of cystatin C has been studied much less in a population with kidney failure than in healthy individuals. In a study including 29 patients with type 1 diabetes with GFR levels ranging from normal to moderate, CV_I values of SCr and cystatin C were found to be similar (8.9% for cystatin C and 9.9% for SCr) [36]. In the study of Podracka et al. on 20 children after kidney and/or liver transplantation, the mean CV_I was found to be higher for Cystatin C compared to creatinine [37]. As a result of this study, cystatin C did not have any superiority to monitor renal function in patients with moderate renal impairment or healthy individuals compared to SCr. However, cystatin C is a superior parameter for monitoring renal function in situations with altered muscle mass (i.e.; patients with prolonged immobilization, muscular disease, or paralysis and children) [38]. However, the index of individuality lower than 0.6 we found for both SCr and cystatin C support the conclusion that both parameters show high individualities.

There are also possible limitations of the study. The fact that we obtained the results with the Jaffe method, which is widely available and used in our laboratory, instead of the enzymatic method recommended for creatinine in laboratory measurements, led to higher CV_A values when compared to analytical performance targets. The kit and the method we used for Cystatin C and BTP measurements may be why we could not achieve the analytical performance targets for CV_A based on biological variation. In addition, the unequal distribution of males and females in the study group can be considered as a possible limitation. Also, the fact that the biological variation estimates were not calculated separately according to the male and female subgroups is another limitation of the study.

As a conclusion, we derived biological variation data for SCr, cystatin C, and BTP following the criteria of the EFLM BV-WG. Laboratories can benefit from biological variation data at every stage of the total testing process and set analytical performance targets using this data. The indexes of individuality values for SCr and cystatin C present higher individualities, and the use of RCV values will be more reasonable than population-based reference intervals in a patient follow-up with SCr or cystatin C. We determined the CV_I and CV_G values for the BTP test and calculated its individuality index and RCV values for the first time in the literature, still, it is worth confirm these findings with further studies.

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Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: The Ethics Committee of the Bozyaka Training and Research Hospital approved this study (Resolution Number 4, dated October 11, 2017).

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