Brief Report

Biological variation estimates for serum N-terminal pro-B-type natriuretic peptide from 8 healthy Turkish female individuals

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Abstract: N-terminal pro-B-type natriuretic peptide (NT-proBNP) measurements are widely used for diagnosing and assessing the severity of congestive heart failure (CHF). However, limited information is available about the within (CV_I) and between-subjects (CV_G) biological variation (BV). This study presents BV estimates for NT-proBNP in eight healthy Turkish women. Serum samples were collected weekly from 24 healthy subjects over 10 weeks and analyzed using the Siemens ADVIA Centaur® XP NT-proBNP assay. Outlier detection, variance homogeneity and trend analysis were performed followed by CV-ANOVA for BV and analytical variation coefficient (CV_A) estimation. The reference change values (RCV), the index of individuality (II), and the analytical performance specification (APS), were also calculated. NT-proBNP results from 11 males and three females were below the assay's limit of quantitation (LoQ). One female was identified as outlier between individuals. To fulfil criteria for variance homogeneity of the data from eight women, 7% of results were excluded. Ultimately, 104 analytical results from eight females were included in the analysis. NT-proBNP data for the female group were normally distributed and no trends were identified by regression analysis. The BV estimates were CV_I: 23.7% [95% confidence interval (CI): 19.0–30.4%] and CV_G: 8.22% (95% CI: 0.0–24.7%). The CV_A obtained from samples replicate results was 11.2% (95% CI: 9.4–13.9%), lower than the desirable APS for imprecision. The II indicated a low individuality of NT-proBNP. This study provides BV estimates for NT-proBNP derived from a protocol consistent with European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) recommendations. BV data on NT-proBNP represent an update of the presently available data, some of them obtained more than 20 years ago.

Keywords: Biological variation (BV); analytical performance specification (APS); N-terminal pro-B-type natriuretic peptide (NT-proBNP); reference change value (RCV); index of individuality (II)

Received: 29 August 2023; Accepted: 24 October 2023; Published online: 08 November 2023.
doi: 10.21037/jlpm-23-59
View this article at: https://dx.doi.org/10.21037/jlpm-23-59

Introduction

B-type natriuretic peptide (BNP) and its precursor N-terminal pro-BNP (NT-proBNP) are released primarily from cardiac ventricular myocytes in response to increased wall stress and stretch and play a crucial role in the diagnosis, prognosis, and management of various cardiovascular conditions (1,2). NT-proBNP measurements have become integral in the evaluation and monitoring of congestive heart failure (CHF), providing valuable insights into disease severity and response to treatment (3).

The considerable biological variation (BV) observed in BNP/NT-proBNP poses a potential constraint on their practicality for serial measurements in individual patients (4,5). Nevertheless, initial investigations demonstrated that employing BNP/NT-proBNP guidance for heart failure treatment led to a reduction in overall cardiovascular events and a delay in the occurrence of the first event compared to intensive clinically guided treatment (6).

BNP/NT-proBNP values' ability to aid in risk stratification, guide therapeutic decisions, and predict clinical outcomes has made these measurements an indispensable tool for clinicians. Therefore, it is crucial to understand and measure various sources of variation to accurately interpret changes in serial measurements within an individual. These sources include within-subject BV (CV_I), which represents random fluctuations around the individual's homeostatic set point (HSP), and between-subject BV (CV_G), which represents variation among different individuals' set points (7).

Since laboratory results are typically reported as single values, not considering BV can lead to misinterpretation and potentially unnecessary clinical interventions. Thus, having knowledge of the BV for a particular clinical test allows for proper interpretation of changes between serial measurements, and the statistically significant difference is reported as the reference change value (RCV) (8).

Knowledge of the BV of measurands serves multiple important purposes. It aids in defining analytical performance specifications (APS) (9,10), establishing personalized reference intervals (prRI) for individuals (11,12), evaluating the appropriateness of population-based reference intervals (popRI) through the index of individuality (II), and determining the number of samples required to estimate homeostatic set point (NHSP) within a certain percentage of the true value (13).

Data on BV of NT-proBNP have been published with markedly differing results [reviewed in the study by Wu (14)].

However, to the best of our knowledge, there are limited published studies (4,15-19) that examine the BV of NT-proBNP in healthy individuals, which do not meet the eligibility criteria outlined by the Biological Variation Data Critical Appraisal Checklist (BIVAC) for inclusion in meta-analyses, as established by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) (20).

In our current study, we investigated the BV of NT-proBNP in eight apparently healthy Turkish females, adhering to all the necessary preanalytical requirements outlined by the European Biological Variation Study (EuBIVAS) (21,22) in agreement to the BIVAC and the checklist provided by the working and task groups of BV of EFLM (20,23).

Methods

Study population and protocol

BV estimates for NT-proBNP were obtained from a Turkish population using serum samples collected from apparently healthy individuals, both males and females, enrolled following the methods previously described (24). The health status, inclusion/exclusion criteria, sample collection, processing, and storage protocol were based on the EuBIVAS, previously reported in detail (21,22). Briefly, exclusion criteria included the diagnosis of diabetes mellitus, dyslipidemia, chronic kidney and liver diseases, thalassemia and hemoglobinopathies, carrier status for hepatitis B, C, and human immunodeficiency virus (HIV), and female subjects who were pregnant or breastfeeding. In the first week, all participants underwent a series of laboratory tests to ensure they met the inclusion criteria. The subjects' use of medications and dietary supplements was recorded on a weekly basis (24).

The sample collection was carried out at Acibadem Labmed Clinical Laboratories and Acibadem Mehmet Ali Aydınlar University in Istanbul, Turkey. The study protocol received approval from the Institutional Ethical Review Board of Acibadem Mehmet Ali Aydınlar University in accordance with the World Medical Association Declaration of Helsinki.

The same phlebotomist collected serum samples on a weekly basis from 24 healthy subjects (12 male and 12 female) over a 10-week period, (February–April 2018), using Vacutainer Serum Separator Tubes (BD Gold, Franklin Lakes, NJ, USA). After centrifugation at 3,000 g, the serum was aliquoted and stored at –80 ℃. The samples were shipped in dry ice to Siemens Healthcare Diagnostics Inc., in Tarrytown, NY, USA, where the measurements were conducted. The samples remained stored at –80 ℃ until the analysis.

Determination of NT-proBNP was performed on the Siemens ADVIA Centaur® XP (Siemens Healthineers, Tarrytown, NY, USA) in December 2022, using the ADVIA Centaur® NT-proBNP (PBNP) assay, and the ADVIA Centaur® NT-proBNP calibrator.

The assay is designed to have a limit of blank (LoB) of

less than or equal to the limit of detection (LoD), LoD less than or equal to 20 pg/mL, and limit of quantitation (LoQ) of less than or equal to 35 pg/mL.

Samples from each subject were analyzed in duplicate within a single run, and ADVIA Centaur® NT-proBNP quality control (QC) at two different levels of concentration were used as internal controls of quality and were evaluated in duplicate for each run.

Data analysis

To obtain the BVs and analytical variation coefficient (CV_A) estimates with 95% confidence interval (CI), outlier detection, variance homogeneity analyses, and trend analysis followed by CV-ANOVA were conducted following the methodology described in previous EuBIVAS publications (25-27).

Initially, the measurement results lower than LoQ were excluded from the study. Outlier detection was performed using the Dixon-*Q* test to identify and exclude any outliers between subjects. Subsequently, the homogeneity of within-subject and analytical variability was assessed using the Bartlett and Cochran tests, respectively, on data transformed to coefficient of variation (CV). To determine if individuals were in a steady-state condition, a linear regression analysis between the mean concentrations of duplicate analyses from each blood drawing (representing the pooled mean group samples) and the corresponding blood drawing numbers (1, 2, …, 10) was performed. Subjects were considered to be in a steady state if the 95% CI of the slope included zero.

CV_I estimates were derived using the CV-ANOVA Røraas method (28). For estimating CV_G, ANOVA was performed on data that were natural log-transformed, and the normality of mean value data distributions was verified using the Kolmogorov-Smirnov test. The 95% CIs for BV estimates were calculated using the Burdick and Graybill method (29).

The following equations were utilized to calculate the desirable APS for analytical imprecision (CV_{APS}), analytical bias (B_{APS}), and the II:

$$CV_{APS} = 0.5 \times CV_I \quad [1]$$

$$B_{APS} = 0.25 \times \sqrt{CV_I^2 + CV_G^2} \quad [2]$$

$$II = \frac{CV_I}{CV_G} \quad [3]$$

The calculation for the asymmetrical RCV and for the NHSP, using the CV_A obtained from the duplicate measurements results of subjects' samples, were performed as previously described (24,25). NHSP were estimated for 15% and 20% deviation from the true HSPs. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics board of Acibadem Mehmet Ali Aydınlar University (No. ATADEK - 2023-07/251) and informed consent was obtained from all individual participants.

Results

NT-proBNP results from 11 males out of 12 and three females out of 12 were lower than the LoQ of the assay (<35 pg/mL). The only male with detected results was not included in the analysis. Additionally, one female was identified as an outlier between individuals and was not included in the analysis.

Consequently, the population used to derive the BV of NT-proBNP, consisted of 8 females, median ages of 23.2 years (range, 20–29 years). Physical activity, drug consumption, and smoking and alcohol intake of the study population are summarized in *Table 1*.

To fulfil criteria for variance homogeneity of the data, 7.1% of results were excluded (*Table 2*). In total, 104 analytical results were included in the analysis. NT-proBNP data were normally distributed and no trends were identified by regression analysis. The CV_I and CV_G estimates were 23.7% (95% CI: 19.0–30.4%) and 8.22% (95% CI: 0–24.7%) respectively (*Table 3*).

The APS for imprecision and bias, RCV, II and NHSP were calculated (*Table 4*). In *Figure 1*, median values and range of NT-proBNP concentrations for each individual are shown.

Discussion

This study exhibits a rigorous control over both the preanalytical and analytical phases. The study design and data processing were meticulously planned and implemented in accordance with the recommendations set forth by the EFLM (20,23) and adhered strictly to the EuBIVAS protocol (21,22).

The clinical relevance of NT-proBNP in the management of heart failure is well-acknowledged, with international guidelines emphasizing its significance in the diagnostic, prognostic, and therapeutic contexts. Specifically, it has become an indispensable tool for the evaluation of adult patients, primarily those over the age of

Table 1 Age, BMI, smoking habits, alcohol intake, drug consumption, and physical activity done by females enrolled in the study

Subjects	Females (n=8)
Age, years, median [range]	23.2 [20–29]
BMI, kg/m^2, median [range]	21.6 [17–26]
Physical activity, n	
No physical activity	5
>0 to <3 h/week	1
≥3 h/week	2
Smoking habits, n	
0 cigarette/day	6
>0 to <10 cigarettes/day	2
10–20 cigarettes/day	0
Drug consumption, n	
No drug	8
Type of drug	–
Alcohol intake[†], n	
0 U/day	6
>0 to <2 U/day	2
≥2 U/day	–

[†], one alcohol unit (U) correspond to 10 mL, equivalent to 8 grams, of pure alcohol (https://www.drinkaware.co.uk/alcohol-facts/alcoholic-drinks-units/what-is-an-alcohol-unit/). BMI, body max index.

Table 2 Number of results excluded due to outlier detection

Subjects	Females
Homogeneity (Bartlett and Cochran's tests), n	
Replicate (analytical homogeneity)	0
Samples (within homogeneity)	4
Subjects (within homogeneity)	0
Reed and Dixon, n	
Subjects (between)	1
Numbers of results used to estimate CV_I data, n	
Results	104
Subjects	8
% of outliers excluded to estimates CV_I data	7.1

CV_I, within-subject biological variation.

Table 3 CV_I and CV_G estimates for NT-proBNP with 95% CIs

Subjects	Females
Number of individuals	8
Total number of results	104
Mean number of samples/individual	6.63
Mean number of replicates/sample	1.93
Mean value, pg/mL (95% CI)	54.1 (50.7–57.6)
CV_A %[1] (95% CI)	11.2 (9.4–13.9)
CV_I % (95% CI)	23.7 (19.0–30.4)
CV_G % (95% CI)	8.22 (0–24.7)

[1], CV_A estimates were based on CV-ANOVA of duplicate analysis of female study samples. CV_I, within-subject biological variation; CV_G, between-subject biological variation; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CI, confidence interval; CV_A, analytical variation coefficient.

55 years, who suffer from heart failure (1).

In the present study, we determined the BV of serum NT-proBNP in 8 healthy Turkish female individuals with a median age of 23.2 years (range, 20–29 years). It is crucial to recognize the potential limitations this might pose. While the demographic investigated provided valuable insights, it is not entirely representative of the typical heart failure patient, often being older adults of both genders. This discrepancy could influence the generalizability of our findings to the broader patient population. However, it is noteworthy that understanding the BV in a younger, healthy demographic can provide a foundation upon which future studies can build.

Results from male samples were below the LoQ of the assay (<35 pg/mL) for 11 out of the 12 samples and hence BV estimates could not be derived. This is in line with recent reports indicating sex-based differences in healthy populations (30). The lower circulating androgens and the potentiating effect of exogenous female hormone therapy contribute to the higher circulating NT-proBNP concentrations in females (31).

Specifically, females tend to exhibit higher NT-proBNP concentrations compared to males from late adolescence until middle age and this suggests that age- and sex-specific intervals may be necessary to more accurately assess the associated risk levels.

Interestingly, the previous studies did not provide

Table 4 APS for CV_{APS} and B_{APS}, RCV, II and the NHSP for NT-proBNP based on the BV estimates as reported in *Table 3*

Variable	Data
CV_{APS} %[a]	
Minimum	17.8
Desirable	11.8
Optimum	5.9
B_{APS} %[b]	
Minimum	17.8
Desirable	6.3
Optimum	5.9
RCV %[c] (decrease; increase)	−45.2; 82.4
II[d]	2.7
NHSP[e], n	
15%	12
20%	7

[a], CV_{APS} = 0.5 CV_I (desirable level). The factor for optimum and minimum performance specifications are arbitrarily set to 0.25 and 0.75 respectively. [b], B_{APS} = 0.25 $(CV_I^2 + CV_G^2)^{0.5}$ (desirable level). The factor for optimum and minimum performance specifications are 0.125 and 0.375, respectively. [c], RCV were calculated as described in the text delivering asymmetric values for rise and fall at the probability level of 95% for significant unidirectional change, applying CV_A estimates based on duplicate measurement of all study samples. [d], II = CV_I/CV_G. [e], NHSP $[Z^*(CV_A^2 + CV_I^2)^{1/2}/D]^2$ where D is the allowed percentage deviation from the true homeostatic set point, and Z is 1.96 (for a P value <0.05). NHSPs associated with 15%, and 20% deviations from the true homeostatic set points are calculated. APS, analytical performance specification; CV_{APS}, analytical performance specification for imprecision; B_{APS}, analytical performance specification for bias; RCV, reference change values; II, index of individuality; NHSP, number of samples required to estimate the homeostatic set point; NT-proBNP, N-terminal pro-B-type natriuretic peptide; BV, biological variation; CV_I, within-subject biological variation; CV_G, between-subject biological variation; CV_A, analytical variation coefficient.

differentiated BV estimates for males and females, despite reporting differences in concentration between the two sexes (17). Consequently, the CV_G estimates were incorrectly obtained by combining two heterogeneous groups, leading to a misleading overestimation of the CV_G value and consequently a misleading value of the B_{APS} and II.

For the measurands with high individuality, where the CV_I is small if compared to the CV_G (defined as II = CV_I/CV_G), for a correct interpretation of serial results, the use of the reference intervals (RIs) should be replaced by the RCV. In such situations, the individual is the best point of reference for assessing change, armed with the knowledge of the CV_I of the measurand. The II has been in fact proposed as a discriminator for the usefulness of RCV values. II values lower than 0.6 indicate marked individuality, while RIs are suitable for the assessment of measurands with an II >1.4 (7).

The II observed in this study, was found to be 2.7. This finding implies a diminished degree of individual variability in NT-proBNP.

Therefore, employing a RI for females based on the population, appears suitable for interpreting successive NT-ProBNP measurements. However, the traditional perspective on the use of the RCV should be reassessed considering emerging evidence that underscores the significance of personalized RI. Carobene *et al.* using EuBIVAS data, demonstrated that when calculating CV_I in the single individual (referred to as CV_P), the resulting values exhibited a surprisingly broad range that was independent of the measurands concentration (12). This indicates that despite a reduced level of individuality within the population, there can still be advantages in utilizing a prRI based on the individual's own data. To simplify the concept, a glance at *Figure 1* depicting the data of individual subjects is sufficient to observe that the intra-individual variability of subject 7 (CV_P =17.4%) is considerably lower compared to subject 4 (CV_P =27.7%).

Utilizing the CV_A and CV_I estimates derived from the entire population described in this study, the generalized asymmetrical RCVs were determined to be −45.2% to 82.4% (decrease to increase), as shown in *Table 4*. Nevertheless by applying CV_A estimates based on duplicate measurements of all study samples and utilizing CV_Ps instead of CV_I, the RCVs obtained were −37.9% to 61.1% and −49.4% to 97.7% for subject 7 and subject 4, respectively.

It means that to positively impact the patient's report, it is essential to consider all relevant variables that characterize our patients (11). To note that this evaluation requires significant effort to be effective. In fact, key unanswered questions, including determining the reliability of homeostatic setting points and CV_P values based on the number of patient results, addressing uncertainty related to analytical components, updating prediction intervals with new results, and incorporating physiological drift

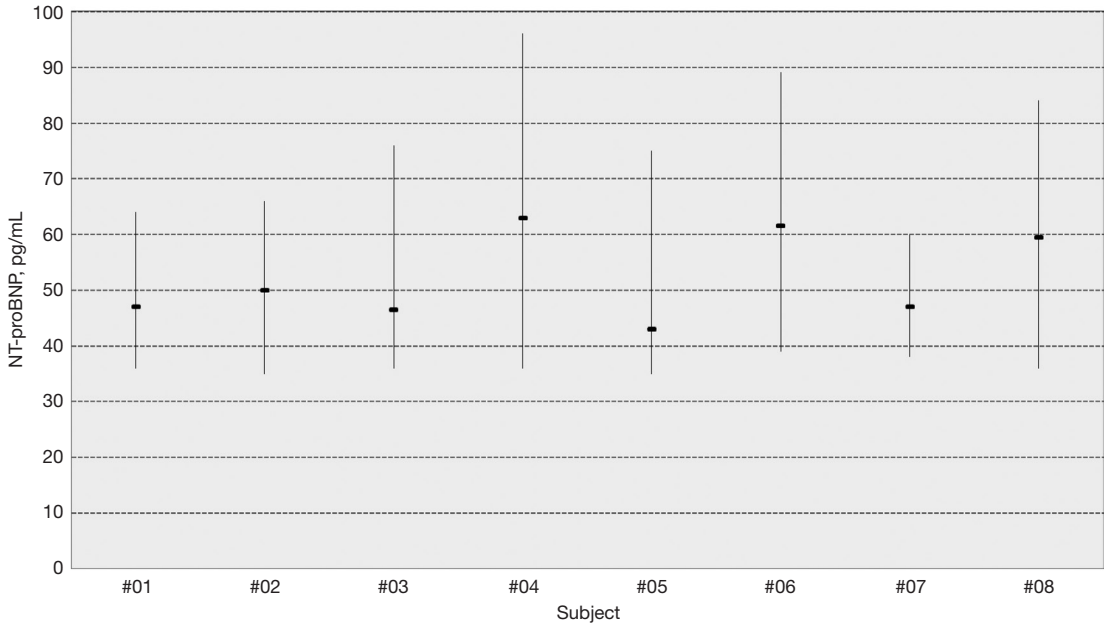

Figure 1 Median values (dots) and range of NT-proBNP concentrations (vertical error bars) for each individual included in the study after exclusion of outliers. NT-proBNP, N-terminal pro-B-type natriuretic peptide.

due to seasonal and age-related factors, still remains (32). Addressing these questions requires dedicated effort and ongoing studies to provide comprehensive solutions. It is through these continued efforts that we can enhance the patient's report and improve clinical practice.

The CV_A estimate obtained in our study is below the desired. This would suggest that the analytical system employed in our study likely meets these requirements. However, the measurement uncertainty does not follow a linear pattern between the LoQ and the upper measurement limit. It increases near both the LoQ and the upper measurement limit. Therefore, if the measurement result of the analyte falls near the LoQ or the upper measurement limits, the standard deviation (SD) and, consequently, the total variation will be higher. Additionally, if the measurement result is below the LoQ, it cannot be accepted as reliable data, and these results should not be incorporated into further calculations.

These issues highlight the limitations of the existing methods. In cases where such limitations arise, the method should be refined to accurately measure the lower levels of the analyte, which correspond to the normal levels found in healthy individuals.

Moreover, it is important to consider the strict pre-analytical protocol employed in this study according to the EuBIVAS (21,22), and the fact that the CV_A estimates are based on duplicate analysis of all samples. In routine settings with long-term analysis, it is possible for the CV_A estimates to surpass the APS.

In this study, another noteworthy discovery was the identification of NHSPs for NT-proBNP, with values of seven and 12 accompanied by an approximate deviation of 20% and 15% respectively (*Table 4*). This suggests that when the NT-proBNP concentration is close to the action limit, clinical decision-making might require multiple measurements to ensure accurate interpretation.

Until now, to the best of our knowledge, six papers (4,15-19) have been published that examine the BV of NT-proBNP in healthy individuals. Two of which (18,19) reported BV data diurnal variation, therefore, according to the BIVAC they are not eligible to be included in the meta-analysis.

In the remaining four published papers, CV_I estimates range from 10% (16) up to 58% (15). Three of these papers, published prior to the EFLM recommendations, reported BV estimates without CI_S measures: this presents a challenge when attempting to make a direct comparison between these historical data and the new data presented in this study. Furthermore, these studies would have been categorized as BIVAC grade C. When assessed using the BIVAC (20) quality evaluation, certain BIVAC quality items (QIs), specifically QI 7 (steady state) and QI 10

(variance homogeneity), were not satisfied. It is likely that publications lacking or inadequately addressing essential details related to BIVAC QIs may result in less reliable CV_I estimates (33).

Limitation

It is essential to recognize certain limitations inherent to our study, which should be taken into account in the planning of future research projects. This study exhibits a limitation related to its small sample size while maintaining the rigorous control over both the preanalytical and analytical phases. We were in fact limited by the ability to measure below the LoQ and hence were not able to get results for males because, apart from a single exception, all the NT-proBNP males' results were lower than the LoQ.

Another limitation of this study is the age demographic of the sampled population. While this provides valuable insights into the BV of NT-proBNP in this specific age group, it may not be entirely representative of the broader population, especially those most clinically relevant to heart failure.

Moreover, the analyses were performed using only one manufacturer's reagents. However, estimates of BV that describe the natural fluctuations of the measured quantity should not be influenced by the specific reagent used, as demonstrated in previous EuBIVAS studies (34,35).

Nevertheless, our findings provide a foundational understanding of NT-proBNP BV in the studied group, which could serve as a stepping stone for subsequent research in more clinically relevant populations. It would be beneficial for future studies to expand on this work by including a more diverse age group, with a particular focus on the heart failure demographic.

Conclusions

In conclusion, the fully BIVAC-compliant BV data on NT-proBNP represent an update of the presently available data, some of them obtained more than 20 years ago. In our study, CV_I estimate was homogeneously distributed and lower than previously published estimates, which means that more stringent CV_{APS} were derived.

Acknowledgments

This study was supported by Siemens Healthineers.
Funding: None.

Footnote

Peer Review File: Available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-59/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jlpm.amegroups.org/article/view/10.21037/jlpm-23-59/coif) and report that this study was supported by Siemens Healthineers. C.D. is an employee of Siemens Healthineers. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics board of Acibadem Mehmet Ali Aydınlar University (No. ATADEK - 2023-07/251) and informed consent was obtained from all individual participants.

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doi: 10.21037/jlpm-23-59
Cite this article as: Carobene A, Abou-Diwan C, Locatelli M, Serteser M, Coskun A, Unsal I. Biological variation estimates for serum N-terminal pro-B-type natriuretic peptide from 8 healthy Turkish female individuals. J Lab Precis Med 2024;9:3.