Comparative Evaluation of Methods to Determine Intra-Individual Reference Ranges in Nutrition Support Team (NST)-Related Tests

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Abstract

Background The intra-individual reference range is generally narrower than the commonly used reference range. Consequently, close monitoring of changes in the laboratory test results of individuals based on the inter-individual reference range remains challenging.

Methods We examined the determination of individual reference ranges using four indicators of nutritional conditions: transferrin (TRF), albumin (ALB), retinol-binding protein (RBP), and transthyretin (TTR). The subjects comprised 20 healthy individuals and blood samples were collected and tested five times at 2-week intervals. We used the measurement results for the four indicators and examined individual reference ranges using four methods, including calculation methods based on the reference change value and Bayesian inference.

Results The resulting intra-individual reference ranges were narrower than the currently used inter-individual reference range for all measurements using four methods. Furthermore, the intra-individual coefficient of variation [CV (intra)] was smaller than the inter-individual coefficient of variation [CV (inter)] for TRF, RBP, and TTR for all subjects. The means CV (intra) for the four indicators were also lower than the corresponding CV (inter).

Conclusions The intra-individual reference range can be used to validate the standard deviation and coefficient of variation for currently used indicators. Moreover, Bayesian methods are speculated to be the most versatile.

Background

The interpretation of laboratory results generally uses the 95% confidence interval of the distribution of test results obtained from reference individuals selected under certain conditions. However, this reference range is interpreted as a combination of inter-individual and intra-individual variations; consequently, the reference range of an individual is often narrower than the combination reference range. In addition, close monitoring of variations in the clinical laboratory results of an individual is difficult and can lead to unnecessary secondary examinations if the values obtained exceed the general reference range [1–8]. For example, serum enzymes, such as γ-glutamyl transpeptidase and alkaline phosphatase, in addition to uric acid, total cholesterol, and albumin (ALB), have narrower intra-individual variations than inter-individual variations; thus, the reference range is consistent with inter-individual variations. Therefore, although there are significant changes in the values for an individual, such changes will not be detected as long as each is within the reference range, which is the reason for the low sensitivity of these reference ranges [4, 9].

Patients suffering from malnutrition tend to suffer for an extended period of time during which their condition typically worsens [10, 11]. Nutritional state management is a factor associated with metabolic disorders and slow healing of wounds, resulting in prolonged hospital stays [12]. Therefore, objective nutritional evaluation (objective data assessment: ODA) is essential for patients requiring close

monitoring of their nutritional state. This study examined the determination of individual reference ranges for four nutritional indicators: transferrin (TRF), ALB, retinol-binding protein (RBP), and transthyretin (TTR) [13, 14]. These indicators are also biomarkers with different half-lives. Each indicator can be determined using four methods. In Method (I), the standard deviations obtained from multiple measurements are considered as the standard deviation of the indicator, and the reference range is calculated as the mean ± 1.96 standard deviation measured for an individual. Method (II) uses the reference change value (RCV), which is calculated from the mean and the coefficient of variation (CV) of measurements obtained from an individual over time, i.e., the standard deviation (RCV) = RCV × mean × 1/100 is calculated and then used in the reference range = mean ± standard deviation (RCV). In Method (III), assuming that the individual reference range has a normal distribution, we define the range that includes 95% of the healthy measurement results as μ ± 2σ, estimated as mean ± 2S, and consider mean X − Cn (Cn = $t_{n-1}(0.025)\sqrt{(n+1)/n}$, $t_{n-1}(0.025)$: the top 2.5% of 5 distributions for freedom n − 1) < mean X < mean X + Cn as the reference range. Method (IV) is a reference range model in which variables are converted to present the measured values in a normal distribution. We first estimate inter-individual variations, intra-individual variations, and time effects in a mixed-effect model that uses measured values as the response variables, the individual as the random effects, and the point-in-time as fixed effects (or random effects). Next, the distributions of the measured values for an individual observed during medical examinations are estimated on the basis of Bayesian inference posterior distribution with inter-individual variations, intra-individual variations, and overall mean as prior distributions [15–18].

Methods

Devices and reagents

ALB was measured with a BM8060 automated biochemical analytical device (JEOL Ltd., Tokyo, Japan) using pure-auto S ALB reagent (KAINOS Laboratories, Inc., Tokyo Japan). TRF, RBP, and TTR were also measured with the same device using N-assay TIA Tf-H NITTOBO, N-assay LA RBP NITTOBO, and N-assay TIA prealbumin NITTOBO B-type R-1/R-2 (NITTOBO MEDICAL Co., Ltd., Tokyo, Japan) as reagents.

Subjects

Analysis of the individual reference ranges were conducted using data measured from 20 staff members of the SRL Diagnostics Pathology Laboratory (Tokyo, Japan). These volunteers [age: 45.2 ± 8.0 years (mean ± standard deviation)] presented no abnormal findings in in-house examinations, during interviews with an industrial physician, and had normal chest X-rays. The volunteers comprised 11 men (age: 46.4 ± 8.1 years), of which two were in their 30 s, five in their 40 s, and four in their 50 s, and nine women (age: 43.7 ± 8.0 years), of which three were in their 30 s, three in their 40 s, and three in their 50 s. These subjects were healthy adults and their dietary and exercise habits were not regulated [2]. All laboratory results were anonymous but linkable.

This study was conducted with strict adherence to the ethical policy on medical research involving humans, with approval from the SRL ethics review committee (approval no. 12 − 06).

Measurement Period

We followed instructions from each diagnostic kit company when measuring the corresponding test indicator. Fasting blood samples were obtained at 8:30 − 9:30 AM on five separate days at 2-week intervals (December 13 and 27, 2012; January 10 and 24, 2013; and February 4, 2013), and tests were performed on the centrifuged serum samples on the sampling day.

Statistical analysis

The four test indicators were measured five times for 20 subjects. We obtained the mean, standard deviation, inter-individual coefficient of variation [CV (inter)], and intra-individual coefficient of variation [CV (intra)] of the measurements from each individual over time. Using the mean of five measurements obtained for each subject, we calculated the mean and standard deviation for all 20 subjects and then examined the difference among the means of the subjects with the overall mean using the F-test and *t*-test.

Determining The Individual Reference Range

Method (I): This calculation method uses the mean ± 1.96 standard deviation based on multiple measurements and the standard deviation from each individual

We obtained the mean, standard deviation, and CV for each set of measurement results (measurements 1 and 2, 1–3, 1–4, and 1–5). We considered the standard deviation obtained for measurements 1 to 5 as the standard deviation of the test indicators, and calculated the reference range for each individual as the mean ± 1.96 standard deviation.

Method (II): This calculation method uses CV and RCV

We calculated the mean and CV of measurements from each individual obtained over time. RCV was calculated as follows: RCV = 2^(1/2)*Z*((CVa^2)+(CVi^2))^(1/2)) [19, 20], where Z = 1.96 (95%) or 2.58 (99%), Cva = measurement error, and Cvi = intra-individual variations. In this manner, we obtained the mean after the second measurement. We calculated the standard deviation (RCV) = RCV × mean × 1/100 and used the reference range = mean ± standard deviation (RCV).

Method (III): This calculation method uses measurement results of past normal time under the normal distributional assumption

Assuming that the individual reference range has a normal distribution, we defined the range in which 95% of the measurement results from healthy subjects fall within μ ± 2σ and estimate the error as the mean ± 2S. The smaller the number of measurements, the larger the estimation error. We determined the range in which the present measurement result X can be determined to be within or beyond the reference range based on previous measurements from healthy individuals (X1, X2, …, Xn). The present measurement results X are samples from the normal distribution population N (μ, σ^2) (μ and σ are unknown), which is equivalent to previous measurement results from healthy subjects (X1, X2, …, Xn). This is a test of the null hypothesis, and the same concept as the *t*-test for the difference between two groups can be applied.

The T-distribution becomes T = X-mean (n)/S^((1□(1^n)). If the top 2.5% of the t-distribution with freedom n − 1 t(n − 1)(0.0025) is used, when |T|>t(n-l) (0.025), the null hypothesis is rejected with a significance level of 5%, and it can be assumed that the physiological state changed due to a certain factor. In other words, we can consider the range in which the present data have a 5% false-positive rate, and the null hypothesis cannot be rejected (mean X−Cn < X < X mean X + Cn), as the reference range [21]. In this study, Cn = t(n□1) (0.025) ^((n + 1)/n). Using the value of 3.041 when n = 5 as the significance level α = 0.05 [21].

Method (IV): This calculation method uses Bayesian inference

We assumed that the individual reference ranges obtained using Methods (I)–(III) have a normal distribution of individual measurement results near the intra-individual reference range. However, the measurement results of an individual after an infinite number of measurements will be closer to the inter-individual mean than each individual measurement. As measurement values for an individual accumulate, the individual reference range calculated for each new measurement approaches the mean of measurement values for the individual. If we consider a value that falls outside the 95% confidence interval of the reference range obtained in this manner as abnormal, an abnormal finding for an individual can be detected earlier. A low or high baseline (the mean of measurements taken at each sampling) for an individual will not be considered abnormal, allowing false-positives to be eliminated. The individual reference range was thus obtained with the following procedures [15–18]:

(I)
Variables were converted so that the measured values had a normal distribution.
(II)
A mixed effects model that used measured values as the response variables, the individual as random effects, and the point-in-time as fixed effects (or random effects), allowed estimation of inter-individual variations, intra-individual variations, and time effects.
(III)
Using these inter-individual variations, intra-individual variations, and overall mean as prior distributions, we examined a reference range model that estimates the distribution of measured values for an individual by observation based on the posterior distribution of Bayesian inference.

We assume that the mean μ0 and standard deviation σ0 of the test value X for data from a healthy subject have a normal distribution.

The test value of an individual i at time j, X_{ij}, is expressed with the following equation:

$$X_{ij} = \mu_i + t_{ij} + e_{ij}, (2)$$

where μi is the mean of the test result X for individual i through the time j = 1, n, where t_{ij} is the temporal variation of intra-individual test values and e_{ij} are measurement errors.

Let us assume that t_{ij} and e_{ij} have the same dispersions τ^2 and $\sigma\varepsilon^2$ regardless of the time and subject, and τ and ε are independent.

Then, the test value X_{ij} follows a normal distribution N (μi, $\tau^2 + \varepsilon^2$).

Both τ^2 and $\sigma\varepsilon^2$ are known and assumed to be $\sigma^2 = \tau^2 + \varepsilon^2$.

The population mean μ0 and standard deviation σ0 are predicted ahead of time, and with this prior distribution, the mean for N observations, and assuming a dispersion of σ2, follows a normal distribution of N (μn, σn) using Bayesian interference.

If

$$\mu_n = \frac{\sigma^2}{n\sigma_0{}^2 + \sigma^2}\mu_0 + \frac{\sigma_0^2}{n\sigma_0{}^2 + \sigma^2}\sum_{j=1}^{n} X_j \quad (3)$$

$$\frac{1}{\sigma_n^2} = \frac{1}{\sigma_0^2} + \frac{n}{\sigma^2} \quad (4) \quad \text{or} \quad \sigma_n^2 = \frac{\sigma^2\sigma_0^2}{\sigma^2 + n\sigma_0^2}$$

then individual reference values are assumed to have a distribution of μn estimate errors, temporal variations, and measurement errors of intra-individual examinations around the individual mean μn. Assuming that these errors have a normal distribution and are independent of each other, the lower and upper limits of individual reference values with n measurements are expressed as follows:

$$Lower\ limit = \mu_n - 1.96 \times \left(\frac{\sigma^2\sigma_0^2}{\sigma^2 + n\sigma_0^2} + \sigma^2\right)$$

$$Upper\ limit = \mu_n + 1.96 \times \left(\frac{\sigma^2\sigma_0^2}{\sigma^2 + n\sigma_0^2} + \sigma^2\right)$$

If there is no observation, the reference value for the test is the individual reference value.

Analytical Accuracy

We obtained acceptable accuracy for the four target indicators with the mean X-Rs-R method with two kinds of reference sera during the measurement period (December 2012, January 2013, and February 2013). The reference sera were L-Consela 1EX (Lot No. 079207) and L-Consela 2EX (Lot. No. 142207) for ALB and TRF, and Immunoquest L-1 (Lot No. A243A) and Immunoquest L-II (Lot No. K251A) for RBP and TTR. We obtained the mean total variations for the total variations in each reference serum in monthly sets collected over a 2 month period.

Results

Analytical accuracy

The mean total variations during the measurement period were TRF = 2.87, ALB = 0.12, RBP = 0.05, and TTR = 0.54, whereas the CVs were TRF = 1.07%, ALB = 2.88%, RBP = 1.38%, and TTR = 1.83% (Table 1).

Table 1
Total variation of 4 items during measurement period

Item abbreviation (Unit)	Control Sample	Total Variation	
TRF (mg/dL)	L-Consera1EX/2EX	SD	2.87
		CV(%)	1.07
ALB (g/dL)	L-Consera1EX/2EX	SD	0.12
		CV(%)	2.88
RBP (mg/dL)	Immuno-Quest L-Ⅰ/Ⅱ	SD	0.05
		CV(%)	1.38
TTR (mg/dL)	Immuno-Quest L-Ⅰ/Ⅱ	SD	0.54
		CV(%)	1.83

Determining The Reference Range By Each Statistical Analysis Method

We obtained the maximum, minimum, mean, and standard deviation for the four indicators measured in 20 subjects based on five measurements per subject. Figure 1 compares the results for each test indicator for the 20 subjects and the results of the five measurements for each subject. The data were statistically analyzed using the four methods described above and the following results were obtained: Method (I), mean ± 1.96 standard deviation; Method (II), mean ± standard deviation (RCV); and Method (III), mean ± 3.041 standard deviation (mean ± 2S) (Table 2). The RCV obtained from the results of five measurements for each test indicator were as follows: TRF = 11.64%, ALB = 12.68%, RBP = 20.54%, and TTR = 15.89% (Table 2). We also performed a statistical analysis with Bayesian inference [14–17]

[Method (IV)] (Table 3). Reference ranges for each test indicator shown in Table 3 were as follows: TRF = 190–340 mg/dL (men: 190–300 mg/dL, women: 200–340 mg/dL) [22], ALB = 3.8–5.2 g/dL [25], RBP = 2.7–6.0 mg/dL (men: 2.7–6.0 mg/dL, women 1.9–4.6 mg/dL) [24], and TTR = 22.0–44.0 mg/dL [21].

Table 2

Comparison of current reference range and reference range calculated by three methods

Item abbreviation (Unit)	Results of statistical data analysis of five measurements of 20 subjects to be verified within this time range						RCV(%)	Method (I)		Method (II)			Method (III)		
	Max.	Min.	Between the maximum value and the minimum value difference	Average	standard deviation	CV (%)		Xbar±1.96SD (Results of this study)	The difference between the upper limit and the lower limit	Xbar±SD (RCV) (Results of this study)		The difference between the upper limit and the lower limit	Xbar±3.041SD (Results of this study)		The difference between the upper limit and the lower limit
TRF (mg/dL)	336	239	97	280	28.3	10.1	11.64	225	336	111	248	312	64	231	329	98
ALB (g/dL)	5.1	4.2	0.9	4.7	0.23	4.9	12.68	4.3	5.2	0.9	4.2	5.3	1.1	4.3	5.2	0.9

RBP (mg/dL)	5.3	1.5	3.8	3.5	1.08	30.9	20.54	1.4	5.6	4.2	2.7	4.3	1.6	3.1	4.0	0.9
TTR (mg/dL)	41.1	17.5	23.6	29.8	6.93	23.2	15.89	16.2	43.4	27.2	25.0	34.7	9.7	27.2	32.0	4.8

Table 3

Estimation of interindividual variability and intraindividual variation using mixed effects model

Item abbreviation (Unit)		Dispersion	Standard Deviation	Total	Reference Range used by current routine assay		
				standard deviation	SEX	lower limit	Upper limit
TRF(mg/dL)	Interindividual variation	770.96	27.77	30.61	Male	190	300
	Individual internal transition and measurement error variation	165.95	12.88		Female	200	340
ALB (g/dL)	Interindividual variation	0.05	0.22	0.28	Total	3.8	5.2
RBP (mg/dL)	Interindividual variation	1.13	1.06	1.11	Male	2.7	6.0
	Individual internal transition and measurement error variation	0.10	0.32		Female	1.9	4.6
TTR (mg/dL)	Interindividual variation	47.29	6.88	7.14	Total	22.0	40.0

Comparison Of Inter-individual Cv And Intra-individual Cv

Next, we compared CV (inter) and CV (intra) calculated using the five measurement results for the 20 subjects (Fig. 2). For TTF, RBP, and TTR, the CV (intra) was smaller than the CV (inter) for all 20 cases. For ALB, the CV (intra) was smaller than the CV (inter) in 18 of the 20 cases (Fig. 2). The mean CV (intra) of the 20 subjects was lower than CV (inter) for TRF, RBP, ALB, and TTR (Fig. 2).

Temporal Variations In Reference Range Estimates

Using the five measurements from the 20 subjects for the four test indicators (Supplemental Fig. 1), we examined variations in the four types of reference range estimates. The results for two of the 20 subjects (Sample No. 5 and Sample No. 17) are shown in Fig. 3. The first measurement was determined by comparing mean X−Cn < mean X < mean X + Cn and the reference ranges reported in previous studies [21–23]. For reference ranges estimated using Methods [(I)–(III)], the approximation tendency could be confirmed from the third measurement. In contrast, the reference range obtained with Method (IV) was much wider than those obtained with Methods (I) and (II), whereas it was narrower than that obtained with Method (III) (Fig. 3). Intra-individual reference ranges examined with the four methods in the present study were also narrower than inter-individual reference ranges currently being used after five measurements (Fig. 3).

Discussion

The typical procedure to determine a reference range is as follows [25–29]:

(1) Reference individuals are selected from healthy individuals. A population of reference individuals selected for each sex and age group comprises at least 120 individuals.

(2) Statistical analysis: mean ± 2 standard deviation (more accurately, 95% of the normal distribution is equivalent to mean ± 1.96 standard deviation, and mean ± 2 standard deviation is the range that includes 95.45% of the normal distribution).

(3) The above selection conditions for reference individuals, measurement conditions, and statistical analysis must be clearly stated.

In other words, the reference range of test values is expressed as a 95% confidence interval of inter-individual variations, including measurement errors. Medical examination data are repeatedly measured for each individual, and as new information is added to the data longitudinally (inter-individually), distributions can be analytically divided into inter-individual variations and other errors, i.e., intra-individual variations. The most natural interpretation of inter-individual variations is a variable model in which individuals have normal distribution around the inter-individual mean [15–18]. In contrast, a previous study on triglyceride determined the reference range for an individual using between 25 to 7,055 cases and found Cvi values ranging from 2.3–31.9% for the shortest measurement interval of several times a day to once every 2.5 months [30]. Earlier studies on high-density lipoprotein (HDL) cholesterol showed that a population ranging from 25 to 1,058 cases provided Cvi values of 4.8–10.0% [1, 2, 22–24, 31, 32]. The RCV for ALB was reported to be 14.5% [33], similar to the RCV value of 12.7% obtained in this study.

Three factors can cause variations in measured values: disease, physiology, and measurement technique [1, 2, 23, 24, 34]. Physiological variations may include the age, sex (including pregnancy and menstrual period), and dietary factors (such as meals, drinking, smoking, and stress) of an individual, inter-individual variations affected by genetic factors, and intra-individual variations such as the condition of

the individual prior to the examination (such as position, long- or short-term exercise), and conditions associated with blood sampling, such as the time of day [35]. In contrast, in terms of the limit of permissible errors for measurement methods, Tonks [36] divided ¼ of the reference range by the median of the reference range as the reference to evaluate the performance of the control survey for serological components; as a result, the maximum was set at 10%. Kitamura [9] and Cotlove et al. [37] focused on a component with intra-individual variations much narrower than the range of variations for the population by studying the physiological variations in an individual. They proposed the limit of permissible error (CV%) = ½ × (standard deviation for physiological intra-individual variations)/(mean of reference range) × 100. CV is expressed as CV% = standard deviation × 100/mean (%), which leads to total CV (CVt: total) = measured CV (CVa: analysis) + pre-measurement CV (CVp: pre-analysis) + inter-individual CV (Cvi: individual), which are indicators of intra-individual and inter-individual variations [38].

The concept of individual reference was proposed by Williams [39] in 1967, and a long-term evaluation of health conditions of individuals was considered to lead to the early discovery of chronic diseases. In many tests, variations caused by physiological factors were larger for inter-individual than for intra-individual assessments, which led to the acknowledgment of the importance of intra-individual variations [40]. In the current study, we examined individual reference ranges for Methods (I)–(III) and compared these with the commonly used reference range (inter-individual reference range). We found that the individual reference ranges calculated using the three methods were narrower, closely capturing physiological variations in each individual. Furthermore, we examined Method (IV) as a new model to calculate reference ranges. Method (IV) is a mixed model of inter-individual reference range and intra-individual reference range, which allows calculation of a reference range for each individual while using the inter-individual reference range routinely used in clinical settings. Consequently, Method (IV) would be easily accepted in routine clinical settings.

The present study examined 20 cases and we obtained good results in determining the individual reference range. Currently, the commonly used inter-individual reference range is the mean ± 1.96 standard deviation of the reference individual. With Method (III), initially proposed by Tango [21], we found that the range of upper and lower limits is wider for indicators where CV (inter) > CV (intra) and CV (inter) < CV (intra) of the 20 subjects are similar. The present study demonstrated that a small number of measurements leads to a high estimation error when setting the individual reference range, and that calculating standard deviation from the RCV using the method proposed by Fraser [41] [Method (II)] is useful. In contrast, using Fraser's method, the RCV must be obtained for each item ahead of time. The method proposed by Tango [21] is more versatile and the RCV converges after three measurements. Therefore, evaluating these methods with health examination and clinical data from actual subjects may provide information more useful in clinical settings. Nevertheless, these evaluations have taken the evaluation of the first measurement and the total fluctuation into consideration and thus, in terms of applicability, it is difficult to apply on new patients and experiment subjects.

On the other hand, since Bayesian inference can estimate individual referential area from the second measurement of new patients and subjects, its medical information is, all in all, more efficient and

appropriate than the ones from conventional ways. In other words, clinical physicians might judge within daily criteria (among individuals) by using the initial values and install Bayesian inference in the system for the second time onward. By using the initial values then the measured ones since the second time onward, an integrated individual criteria area can be estimated without choosing smaller items. This Bayesian inference is a mixed model of the collective criteria area among individuals (in the initial check) and individualized criteria area (the second time onward). In the initial check, no subject has previous values so the normal criteria area in daily check is used. Then, a shift to Bayesian inference from the second time can increase the applicability. Hence, if we could install and utilize LIS (Laboratory information System) in the medical check systems in hospitals, useful information could be attained without placing extra burden on clinical physicians. Furthermore, when the individualized referential area is narrower than the collective one, changes and development of the diseases of the patients or subjects might be spotted earlier for appropriate treatments. Moreover, when the individualized referential area is wider than the collective one, unnecessary treatments might be avoided.

Conclusion

In the present examination, TRF, RBP, and TTR had lower CV (intra) than CV (inter) in all 20 subjects, and the mean CV (intra) was lower than the mean CV (inter) for TRF, RBP, and TTR. These results indicate that the intra-individual reference value is useful for close monitoring of the variations in each individual caused by physiological factors and disease. In contrast, CV (intra) was higher than CV (inter) for ALB in two of the 20 cases although mean CV (intra) for ALB was lower than that of CV (inter), suggesting that there may be cases where the intra-individual reference value is not appropriately understood. Nevertheless, the preferred method for determining the individual reference range should allow close observation of temporal changes in test indicators with large inter-individual differences. Such methods will play an important role in the development of new biomarkers and in routine diagnosis. For nutrition support team (NST)-related test indicators in particular, the results obtained using the chosen method should closely reflect, for example, the postoperative nutritional state, allowing management of central venous nutrition and the reduction of complications (infections), thereby closely capturing the nutritional state of an individual [14, 15]. Furthermore, such methods could be widely applied to test indicators such as those related to pre- and post-dialysis tests and glucose tolerance tests.

Abbreviations

NST
Nutrition support team; CV:Coefficient of variation; RCV:Reference change value; TRF:Transferrin; ALB:Albumin; RBP:Retinol-binding protein; TTR:Transthyretin.

Declarations

Author’s contributions

YH and YT conceived the study design and performed the research. YH conducted all data analyses. YH and TS led the drafting of the manuscript, with significant contributions from YT, HO, and TK. All authors read and approved the final version of the submitted manuscript.

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Availability of data and materials

The dataset used and analyzed during the current study is available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was conducted with strict adherence to the ethical policy on medical research involving humans, with approval from the SRL ethics review committee (approval no. 12-06). All participants provided written informed consent prior to participating in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

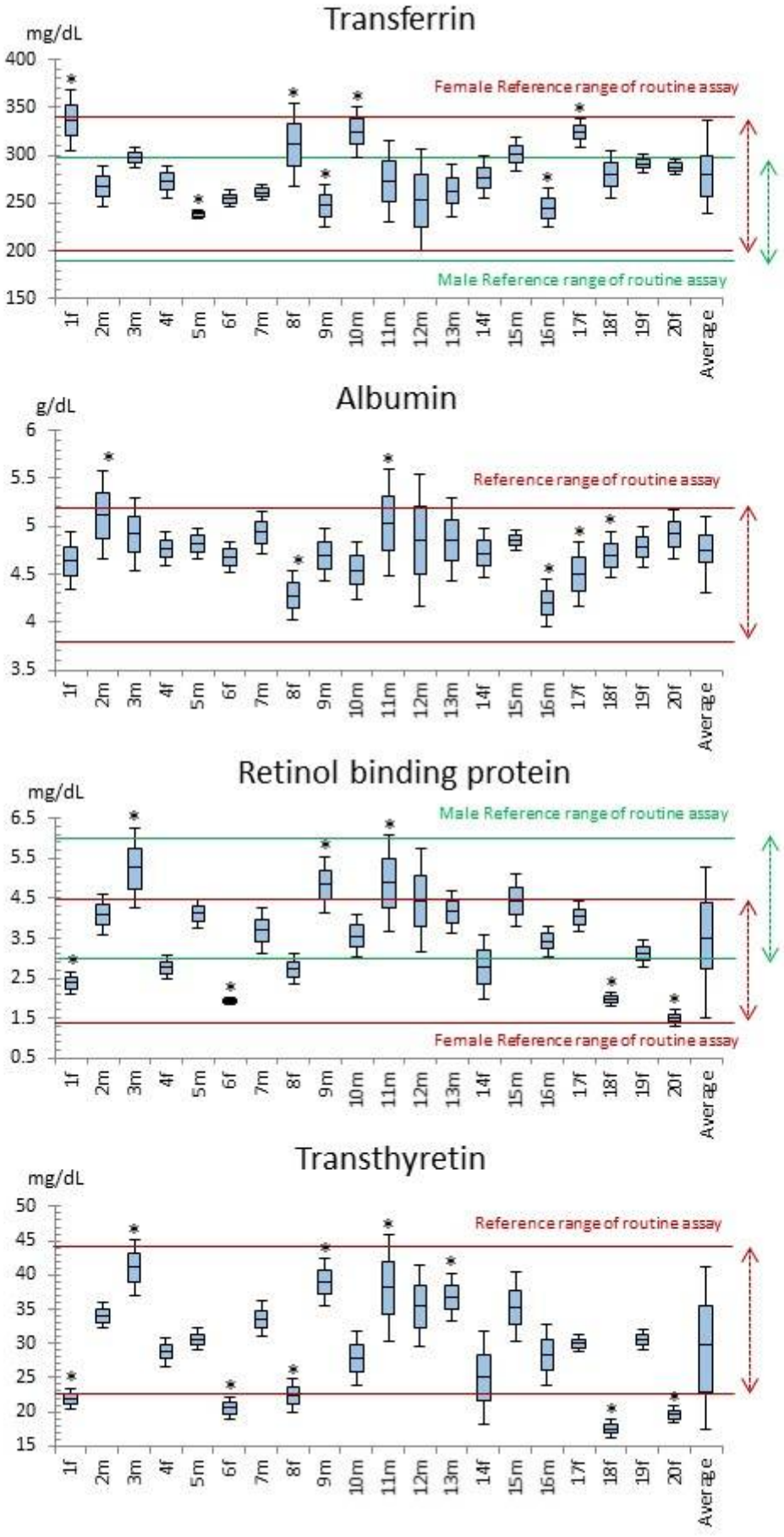
Transferrin
mg/dL
400
350
300
250
200
150
Female Reference range of routine assay
Male Reference range of routine assay
1f 2m 3m 4f 5m 6f 7m 8f 9m 10m 11m 12m 13m 14f 15m 16m 17f 18f 19f 20f Average
Albumin
g/dL
6
5.5
5
4.5
4
3.5
Reference range of routine assay
1f 2m 3m 4f 5m 6f 7m 8f 9m 10m 11m 12m 13m 14f 15m 16m 17f 18f 19f 20f Average
Retinol binding protein
mg/dL
6.5
5.5
4.5
3.5
2.5
1.5
0.5
Male Reference range of routine assay
Female Reference range of routine assay
1f 2m 3m 4f 5m 6f 7m 8f 9m 10m 11m 12m 13m 14f 15m 16m 17f 18f 19f 20f Average
Transthyretin
mg/dL
50
45
40
35
30
25
20
15
Reference range of routine assay
1f 2m 3m 4f 5m 6f 7m 8f 9m 10m 11m 12m 13m 14f 15m 16m 17f 18f 19f 20f Average

Fig. 1

Figure 1

Mean and standard deviation for measurements of four test indicators for each subject. The mean and standard deviation for five measurements of transferrin, albumin, retinol-binding protein, and transthyretin for each of the 20 subjects, along with the mean and standard deviation for all 20 cases. *p < 0.05 vs. average.

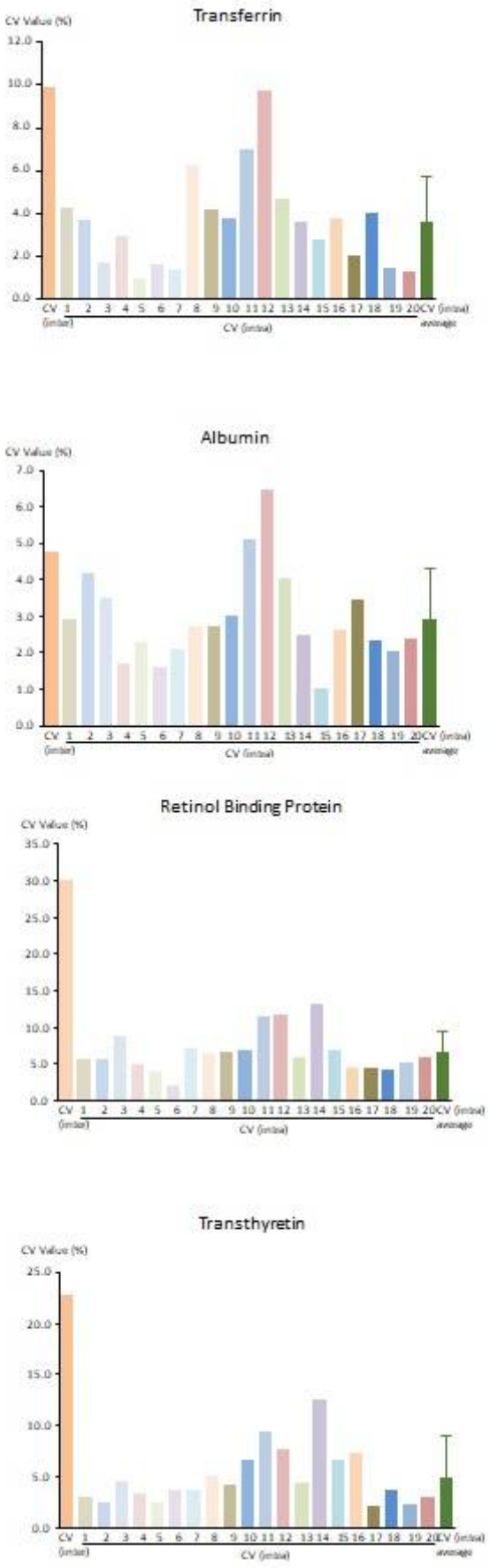
Transferrin
CV Value (%)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
CV (intra)
CV (inter)
Albumin
CV Value (%)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
CV (intra)
CV (inter)
Retinol Binding Protein
CV Value (%)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
CV (intra)
CV (inter)
Transthyretin
CV Value (%)
25.0
20.0
15.0
10.0
5.0
0.0
CV (intra)
CV (inter)

Figure 2

Comparison of inter-individual and intra-individual CVs. CV (inter) and CV (intra) calculated for each measured indicator using five measurements from the 20 cases, along with the mean and standard deviation of CV (intra) for the 20 cases.

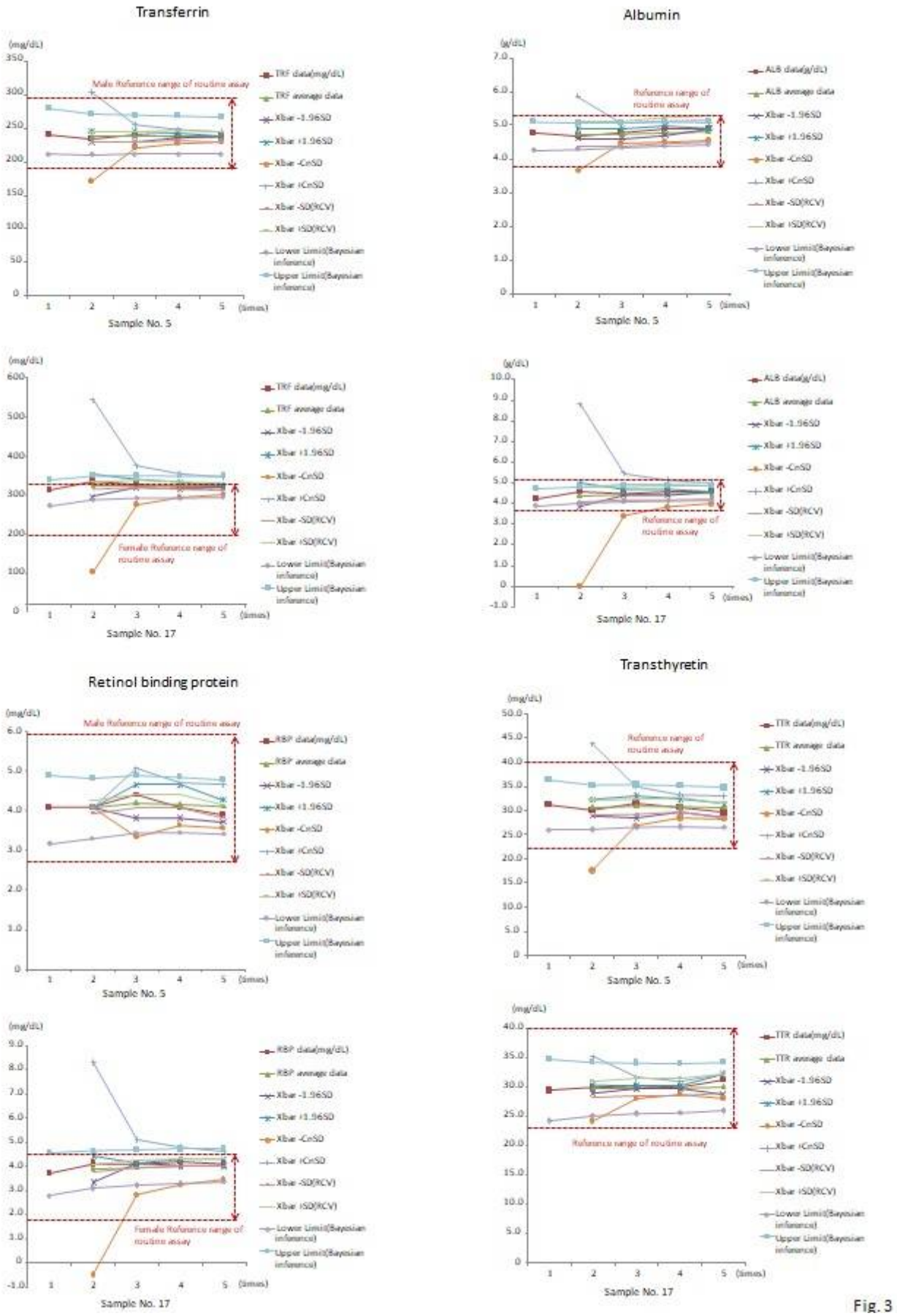

Fig. 3

Figure 3

Temporal changes in measured values for the four test indicators. Temporal changes in five measurements of transferrin, albumin, retinol-binding protein, and transthyretin for subjects No. 5 (man) and No. 17 (woman).