Article

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Least Significant Change (LSC) for Serum Concentrations of 25-hydroxyvitamin D

Running title: LSC for 25(OH)D

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Abstract: Background: The Least Significant Change (LSC) should be introduced and considered as a proper method to define the smallest clinically important difference between two consecutive measurements. **Methods:** The LSC was calculated based on 150 patients with a total 25-hydroxyvitamin D [25(OH)D] IDS-iSYS assay performed in triplicate. The LSC was determined by multiplying the calculated precision error by a factor of 2,77. The study group was additionally divided into subgroups according to gender, age, serum 25(OH)D concentration, and date of assays. **Results:** The LSC was 4,0 ng/ml (13,2%) for the entire group (n=150; 450 assays) and was not dependent on gender, age of patients or the date of assays (p>0.05). The LSC value depended only on the 25(OH)D concentration value. In the subgroup with vitamin D deficiency (<20 ng/ml), the obtained LSC value was 2.2 ng/ml (14.7%) and was lower compared to all other groups (p<0.05 for insufficiency, and p<0.0001 for optimal concentration value). In the subgroup with 25(OH)D concentration >50 ng/ml (n=4; 12 assays), the calculated LSC was 11.8 ng/ml (16.9%) and differed statistically only from the subgroup with vitamin D deficiency (p<0.005). **Conclusion:** An absolute LSC of 4.0 ng/ml was calculated for the IDS-iSYS assay used in our study, and should be considered when two (or more) assay results of 25(OH)D performed for a single patient are compared.

Keywords: 25-hydroxyvitamin D; least significant change; chemiluminescence; vitamin D; IDS-iSYS

1. Introduction

Modern medicine often uses statistical calculations; therefore, it seems reasonable to look for ways to make the best use in practice of the obtained numerical data [1–5].

The first definition used to assess the treatment process was the minimal clinically important difference (MCID), which is generally based on the assessment of the patient's health condition and treatment progress [6]. The purpose of determining the MCID was to answer the question of how large the change should be to be clinically significant. Thus, the minimal clinically important difference was assumed to be the smallest change in treatment outcome that the patient considers subjectively perceptible. However, at the stages of medical procedure where there are only numerical data to evaluate (e.g. measurement of body composition or laboratory diagnostic results), it was necessary to apply a concept that considered only the obtained data.

The main parameter used to assess the received numerical results is precision, presented by means of standard deviation (SD) and coefficient of variation (CV). Precision is defined as the degree of similarity of the results of multiple measurements using a single sample and a specific method,

hence it is a measure of repeatability [1–5]. Accuracy, in turn, is the degree to which a measurement matches the true value [1–5]. The objective of an analytical laboratory is to produce the best possible results in terms of both accuracy and precision. The quality of the results of a given laboratory is confirmed by appropriate validation procedures [3,5].

The main goal of evaluating a single test result is to confirm its consistency with the actual state, i.e. the real concentration of a given substance in circulation. Modern quality control systems are used for that purpose. The reference point is the concentration value measured by a reference method or calculated, e.g. as an average of all measurements.

The clinician making the diagnosis has a considerable number of laboratory and imaging tests at his disposal, as well as data from the medical interview, inter alia. All the information must be comprehensively analyzed based on the physician's knowledge and experience. When comparing two or more results of the same biochemical parameter, e.g. blood vitamin D marker concentrations from different periods, the person interpreting results may come across some doubts [7,8]. For example, whether two similar, "on the face of it", 25(OH)D total concentration results obtained several months apart indicate that the patient is not supplementing cholecalciferol appropriately [7,8]. Or whether the correctness of the supplementation contributes to some laboratory error. Similar doubts may arise in any medical process where a result in a numerical form is interpreted.

Thus, it proved important to find and develop a suitable statistical concept allowing for a reliable assessment of whether the interpreted numerical results reflected the actual change that occurred in the patient.

Such a practical tool could be the least significant change (LSC). That value is defined as the smallest difference between consecutive measurements that can be considered a real and non-random change. LSC is determined based on the previously calculated precision error (root mean square - standard deviation) [9–12].

The usefulness of the least significant change (LSC), a concept already known in theoretical statistics, was verified based on bone density and body composition measurements in a group of our pediatric hospital patients (at the age of 5–18) with the application of the dual-energy X-ray absorptiometry (DXA) method [10]. The study consisted of measuring bone mineral density (BMD) and body composition in a single DXA assay cycle with repositioning between measurements [10]. The primary objective of determining LSC based on DXA method in a pediatric patient population was to assess whether the change in the measured value observed during successive measurements was a real change and not a measurement error. Further, the same method was applied to calculation of LSC for peripheral quantitative computer tomography (pQCT) in our department [11,12]. A general rule of interpretation was adopted; if the difference in BMD or other variable assessed by DXA or pQCT in two consecutive measurements exceeded the established LSC value, it could be concluded with a 95% probability that the change was real [10–12].

As can be seen, the concept of the least significant change can be a useful tool for interpreting test results in a numerical value. Laboratory diagnostics is a discipline that mainly uses numerical data. To date, there have been no studies in scientific literature attempting to use LSC assessment to interpret 25-hydroxyvitamin D assays results. This justified the need to undertake this study, which presents a method of calculating LSC for practical application.

The least significant change, presented as an absolute and relative value, was developed based on a representative sample of 150 patients for whom routine 25(OH)D concentration assay was ordered. The Department of Clinical Biochemistry of the Children's Memorial Health Institute performs tests for "25-hydroxyvitamin D total", i.e. the sum of 25-hydroxyvitamin D2 from ergocalciferol (vitamin D2) and 25-hydroxyvitamin D3 from cholecalciferol (vitamin D3), both forms produced in the liver. Concentration of "25(OH)D total" is a marker of the body's vitamin D status [7,8,13]. On behalf of the Departments and Clinics in The Children's Memorial Health Institute, approximately 1,500 samples are usually tested monthly to determine 25(OH)D, using the chemiluminescent method. The large number of tests performed by the Department of Clinical Biochemistry [13,14] allowed for the selection of an appropriate number of serum samples for

practical determination of the LSC value. The test design was based on the similarity to the concept verified in the densitometric tests [9–12] and consisted of performing three rounds of the 25(OH)D total determination in one analyzer cycle.

2. Materials and Methods

2.1. Study Group

The study was conducted in parallel with the routine determination of 25(OH)D total at the Department of Clinical Biochemistry, the Children's Memorial Health Institute. 150 standard serum samples were collected from the outpatients, patients at the wards and those undergoing a commercial test. The age range of the study subjects (67 females and 83 males) was 1 month to 71 years old. The mean age in the study group was 11.8 years ±10.9, the majority of patients were in growth and maturation period of life.

The study group (n=150) was divided according to the following criteria: gender, age, 25(OH)D concentration and date of registration (blood draw) at The Children's Memorial Health Institute. According to age, the patients were divided into 5 subgroups: under 1 year of age (n=17), over 1 year of age to 3 years (n=15), over 3 to 12 years of age (n=43), over 12 to 18 years of age (n=64) and over 18 years of age (n=11). According to 25(OH)D concentration, the following 4 reference ranges were investigated: 25(OH)D <20 ng/ml (n=33), 25(OH)D above 20 ng/ml to 30 ng/ml (n=53), 25(OH)D above 30 ng/ml to 50 ng/ml (n=60) and 25(OH)D above 50 ng/ml to 100 ng/ml (n=4). Using the criterion of date of registration/date of blood collection, the study group (n=150) was divided into 5 equal subgroups of 30 study subjects each. The separate subgroups covered the following time periods: group I - 27.12.2023 to 14.02.2024 (Winter set), group II - 14.02.2024 to 11.04.2024 (late Winter-Spring set), group III - 11.04.2024 to 7.06.2024 (Spring set), group IV - 8.06.2024 to 14.08.2024 (late Spring-Summer set), and group V - 19.08.2024 to 4.10 2024 (late Summer-Fall set).

2.2. Methods

In the period from the end of December 2023 to early October 2024, the 25(OH)D concentration values for a total group of 150 patients were determined. Single blood draws from the patients were performed at the blood collection center by qualified nursing staff. 25(OH)D total concentration was determined routinely at the Department of Clinical Biochemistry using the IDS-iSYS diagnostic system and a method based on the chemiluminescence technology (IDS 25 VitD^s). The intra-assay repeatability declared by the IDS-iSYS manufacturer was 8.3% CV, on average, while the inter-assay repeatability was 12.1% CV. The practical measurement range (linearity) was from 4 ng/ml to 110 ng/ml, according to IDS-iSYS system document provided for test IDS 25 VitD^s as an information of the manufacturer.

The study samples were selected randomly, the only logistic criterion being enough serum to perform three parallel determinations. Each patient's sample was distributed into 3 tubes, for all of which 25(OH)D concentration was measured in a single run of the IDS-iSYS device (intra-assay repeatability).

Specifically, 10 µL of patient sample was subjected to a pre-treatment step to denature the vitamin D binding protein (VDBP). The treated samples were neutralized in assay buffer and a specific anti-25(OH)D antibody labelled with biotin was added. Following an incubation step, acridinium labelled 25(OH)D was added. Following a further incubation step, the magnetic particles linked to streptavidin were added. After the final incubation step, the complex was captured using a magnet and a wash step performed to remove any unbound analyte. Trigger reagents were added and the resulting light emitted by the acridinium label was inversely proportional to the concentration of 25(OH)D in the original sample.

A statistical analysis was performed using Statistica software v.10 (StatSoft Inc., Tulsa, OK, USA). The normality of the distribution of variables was verified using the Shapiro–Wilk test. The Mann-Whitney test was used to determine the statistical significance of differences between the two subgroups. In the case of more than two subgroups, the non-parametric Kruskal-Wallis ANOVA test

was used. When the overall ANOVA coefficient was less than 0.05, post-tests for multiple comparisons (two-sided) were performed.

The basic principle for the interpretation of numerical results was the assessment of the difference in the concentration of a given parameter in two subsequent analyses. The precision errors (absolute and percentage, CV and CV%) firstly were calculated for each patient and presented as median (Q1-Q3).

The least significant change (LSC) as an absolute value was calculated, for groups, with a 95% confidence interval by multiplying the absolute root mean square precision error (CV) by a factor of 2.77 [7,8]. Least significant change as a percentage (LSC%) was calculated, for groups, with a 95% confidence interval by multiplying the percentage root mean square precision error (CV%) by a factor of 2.77 [7,8]. When the calculated LSC value is exceeded, it is assumed with a 95% probability that the change is significant, i.e. the change is greater than the repeatability error of the method and is clinically significant. Spearman correlation tests were performed to analyze the root mean square absolute and percentage precision errors (CV, CV%) in relation to 25(OH)D concentration values.

3. Results

3.1. General Characteristics

The average 25(OH)D concentration in the study group was 28.5 ng/ml ± 11.7. The median for 25(OH)D in studied patients was 28.3 ng/ml, with 4.2 ng/ml and 80.8 ng/ml as minimum and maximum concentrations. In studied group 22% of patients showed vitamin D deficiency, the 25(OH)D concentration values were lower than 20 ng/ml. Suboptimal (generally called insufficient) concentration, i.e. 25(OH)D >20-30 ng/ml, was reported in 36% of patients, while optimal 25(OH)D concentration value (>30-50 ng/ml) was noted in 38.3% of patients. The concentration considered high, 25(OH)D >50- 100 ng/ml, was recorded in four patients, representing 2.7% of the group under study.

3.2. Calculation of Precision Error (CV) and Least Significant Change (LSC)

The calculated root mean square precision error – absolute (CV) and percentage (CV%), and the least significant change – absolute (LSC) and percentage (LSC%), for the entire study group and separate subgroups are presented in Tables 1-5.

Table 1. Precision error (CV and CV%) and least significant change (LSC, LSC%) across the study group of 150 patients with triple assessments of 25(OH)D.

Variable	Number of patients	Number of samples	Median	lower quartile (Q1)	upper quartile (Q3)	Mean	SD
CV - absolute [ng/ml]	150	450	0.9	0.6	1.5	1.1	0.9
CV% - percentage [%]	150	450	3.7	2.2	5.7	4.1	2.5
LSC - absolute [ng/ml]	150	450			4.0		
LSC% - percentage [%]	150	450			13.2		

Table 2. Precision error (CV and CV%) and least significant change (LSC, LSC%) in relation to gender.

Variable	Gender	Number of patients	Number of samples	Median	lower quartile (Q1)	upper quartile (Q3)	Mean	SD
CV [ng/ml]	F	67	201	0.9	0.6	1.6	1.2	1.0
CV% [%]				3.7	2.1	5.2	4.0	2.4
LSC [ng/ml]						4.4		

LSC [%]						13.0		
CV [ng/ml]	M	83	249	1.0	0.5	1.4	1.1	0.8
CV% [%]				3.6	2.2	5.7	4.2	2.5
LSC [ng/ml]						3.8		
LSC% [%]						13.4		

Table 3. Precision error (CV and CV%) and least significant change (LSC, LSC%) in relation to age.

Variable	Age [years old]	Number of patients	Number of samples	Median	lower quartile (Q1)	upper quartile (Q3)	Mean	SD
CV [ng/ml]	<1	17	51	1.4	0.8	2.3	1.9	1.7
CV% [%]				4.33	1.8	5.7	4.3	2.4
LSC [ng/ml]						6.9		
LSC% [%]						13.5		
CV [ng/ml]	1-3	15	45	1.1	0.8	1.4	1.3	1.0
CV% [%]				3.5	1.8	5.7	4.4	3.5
LSC [ng/ml]						4.4		
LSC% [%]						15.4		
CV [ng/ml]	>3-12	43	129	0.9	0.5	1.3	1.0	0.6
CV% [%]				3.6	2.5	4.5	3.8	2.2
LSC [ng/ml]						3.2		
LSC% [%]						12.2		
CV [ng/ml]	>12-18	64	192	0.9	0.5	1.5	1.0	0.7
CV% [%]				4.1	2.1	5.8	4.2	2.1
LSC [ng/ml]						3.4		
LSC% [%]						12.9		
CV [ng/ml]	>18	11	33	0.7	0.4	1.8	1.2	1.0
CV% [%]				2.6	1.7	7.1	4.2	3.6
LSC [ng/ml]						4.1		
LSC% [%]						14.9		

Table 4. Precision error (CV and CV%) and least significant change (LSC, LSC%) in relation to the time period of a year.

Variable	Time period	Number of patients	Number of samples	Median	lower quartile (Q1)	upper quartile (Q3)	Mean	SD
CV [ng/ml]	Group I (Winter set)	30	90	0.8	0.5	1.3	1.0	0.6
CV% [%]				3.3	2.0	4.8	3.6	1.8
LSC [ng/ml]						3.1		
LSC% [%]						11.0		
CV [ng/ml]	Group II (late Winter-Spring set)	30	90	1.0	0.4	1.4	1.0	0.7
CV% [%]				3.7	2.1	5.9	4.1	2.5
LSC [ng/ml]						3.2		
LSC% [%]						13.2		
CV [ng/ml]	Group III (Spring set)	30	90	1.2	0.6	1.8	1.5	1.4
CV% [%]				5.4	2.5	7.1	5.1	2.7
LSC [ng/ml]						5.5		
LSC% [%]						16.0		
CV [ng/ml]	Group IV (late Spring-Summer set)	30	90	0.8	0.6	1.5	1.2	0.9
CV% [%]				3.4	1.8	4.3	3.9	2.7
LSC [ng/ml]						4.0		
LSC% [%]						13.1		
CV [ng/ml]		30	90	1.0	0.6	1.5	1.2	0.8
CV% [%]				3.4	2.2	5.0	3.9	2.2

LSC [ng/ml]	Group V (late Summer-Fall set)	3.9
LSC% [%]		12.3

Table 5. Precision error (CV and CV%) and least significant change (LSC, LSC%) in relation to 25(OH)D concentration.

Variable	25(OH)D concentration [ng/ml]	Number of patients	Number of samples	Median	lower quartile (Q1)	upper quartile (Q3)	Mean	SD
CV [ng/ml]	<20	33	99	0.6	0.3	0.9	0.7	0.4
CV% [%]				4.3	2.7	6.6	4.7	2.5
LSC [ng/ml]						2.2		
LSC% [%]						14.7		
CV [ng/ml]	>20-30	53	159	0.9	0.5	1.4	1.1	0.8
CV% [%]				3.5	2.2	5.7	4.3	2.9
LSC [ng/ml]						3.7		
LSC% [%]						14.2		
CV [ng/ml]	>30-50	60	180	1.3	0.7	1.6	1.3	0.7
CV% [%]				3.5	1.8	4.7	3.5	1.8
LSC [ng/ml]						4.1		
LSC% [%]						10.9		
CV [ng/ml]	>50-100	4	12	3.2	1.5	5.9	3.6	2.7
CV% [%]				4.4	2.4	7.9	5.1	3.8
LSC [ng/ml]						11.8		
LSC% [%]						16.9		

It was revealed that absolute median value of CV was 0.9 ng/ml and absolute LSC calculated in ng/ml, basing on triple assessments of 25(OH)D (n=450) was 4.0 ng/ml (Table 1). The root mean square percentage precision error (CV%) as well as percentage LSC (LSC%) appeared to markedly higher, 3.7 ng/ml and 13.2%, respectively.

3.3. Absolute and Percentage Precision Errors

When precision errors (CV, CV%) were analyzed in relation to gender, age, and time period of the year there were no statistical differences between girls and boys (Table 2), between different age groups (Table 3) or when a time period of year were compared (Table 4).

3.4. Least Significant Change (LSC) in Relation to Age, Gender, Time of Assays and 25(OH)D Concentrations

There were no statistically significant differences between the calculated LSC values in the studied subgroups according to gender (for absolute LSC, p=ns; for percentage LSC, p=ns; Table 2), and to age (p value for absolute LSC, p=ns; p value for percentage LSC, p=ns; Table 3).

When divided according to the time period (date of assay), there were no statistically significant differences between the calculated LSC values in the studied subgroups (p=ns for LSC absolute; p=ns for percentage LSC; Table 4).

When 25(OH)D concentration was controlled for, there were no statistically significant differences between the calculated LSC values for the root mean square percentage error (p=ns, Table 5), however, there were statistically significant differences between the calculated LSC values for the root mean square absolute error (p<0.0001) in the subgroups under study (Table 6).

Table 6. Absolute least significant change (LSC) in relation to 25(OH)D concentration. P value for multiple (two-sided) comparisons - Kruskal-Walli's test. Independent variable: 25(OH)D concentration.

25(OH)D concentration [ng/ml]	<20	20-30	>30-50	>50-100
<20	XXX	<0.05	<0.0001	<0.005

20-30	<0.05	XXX	ns	ns
>30-50	<0.0001	ns	XXX	ns
>50-100	<0.005	ns	ns	XXX

ns, not significant; XXX, not applicable for statistical analysis.

The calculated absolute LSC value in the subgroup with vitamin D deficiency, with concentrations of 25(OH)D<20 ng/ml, differed statistically significant from all other subgroups with higher 25(OH)D concentrations (Table 6). The subgroups that did not differ significantly from each other were those with suboptimal concentration (20-30 ng/ml) and optimal concentration (30-50 ng/ml). The subgroup with high 25(OH)D concentration (50-100 ng/ml) differed significantly only from the subgroup with its deficiency (<20 ng/ml) (Table 6).

Finally, Spearman correlation tests between 25(OH)D concentration values and absolute and percentage root mean square precision errors (CV, CV%) were performed showing the highly significant correlation with r=0.44 for absolute root mean square precision error (CV; p<0.0001) but not for the root mean square precision error expressed in percentages (CV%; r=-0.14; p=0.08).

4. Discussion

In clinical practice, a physician analyzing a single test result considers several aspects. The main issue is whether the measurement is reliable, defined as the high accuracy and precision of the test. These qualities should be confirmed by control procedures used by a laboratory.

The interpretation of numerical data appears to be more difficult when it is necessary to compare two test results obtained in a time interval. The correct direction of expected changes is one of the determinants of the patient's current health condition. A favorable change in the test result can take different directions. When the concentration of a marker of a disease entity is assessed, significant decrease or increase is expected. In the case of a substance being a component of a systemic homeostasis (e.g. vitamin D), an increase in the concentration over a certain period may be a confirmation of the correct supplementation.   It may also be desirable to maintain relatively constant blood concentration over a defined period, e.g. a drug administered intravenously. In all the above cases, being able to determine whether there has been a change or not is the basic question posed by the person assessing the test results. Another question is the extent of the change for it to be considered significant for the treatment process. Proving statistical variability calculated using a standard method does not necessarily have to translate into clinical significance. In the evaluation of laboratory results, a skilled physician often decides, based on own experience and intuition, whether a noticeable difference between two results should be considered. This situation is comparable to assessing a patient's health condition by determining the minimal clinically important difference (MCID) based on the physician's subjective judgement and the patient's well-being.

This paper tested for the first time the practical applicability of least significant change (LSC) for the assessment and interpretation of laboratory results assessing vitamin D supply. To the best of our knowledge there are no data providing LSC both absolute or percentage for total 25(OH)D concentrations assessed in children nor for adults. The concept of the study assumed both intra-individual variability and that resulting from the approximately 10-month analysis period showing influence of period of the year, i.e. possible seasonal variations of the IDS-iSYS (IDS 25 Vit[s]) system [15–17] and most likely difference in concentrations between winter and summer months [18–20]. It can be assumed that the model of the research can be compared to the routine of daily laboratory work.

Our study was the first providing complex analysis of absolute precision, relative precision, absolute and relative least significant change (LSC, LSC%) for 25(OH)D assays performed in 450 samples from 150 patients, mainly in growth and maturation period, using IDS-iSYS system of measurement. Our main findings are that absolute and relative precision error values (CV, CV%) and in consequence least significant changes (LSC, LSC% - calculated as CV or CV% times 2,77), were not

statistically different when age, gender, and the time period of the assessments were controlled for. The potentially expected dependence of LSC on the patient's age - despite approximately 2-fold higher absolute LSC values in the group of patients below the age of 1 than in the other groups - was not statistically confirmed (p> 0.05). Further, based on the data obtained from the analysis of 25(OH)D concentration in the selected group of patients, the absolute LSC value was 4.0 ng/ml, whereas LSC expressed as % appeared as 13.2%, however, both were proposed by us as applicable for this specific IDS-iSYS assay system only. In general terms, our findings showed that two or more 25(OH)D concentration measurement results, when compared, will differ significantly from each other in a clinical and diagnostic context when the difference between them will be higher than 4.0 ng/ml. In consequence, our study pointed out that monitoring procedure with the use of LSC, at least in pediatric cases of our hospital, should be based on absolute root mean square precision error (CV) but not on relative/percentage root mean square precision error (CV%) that appeared as less sufficient.

In contrast, 25(OH)D concentration appeared to be the only variable that influenced the obtained LSC. In the subgroup with vitamin D deficiency (<20 ng/ml), the obtained LSC value was 2.2 ng/ml (14.7%), thus lower than in the other subgroups (Table 6) and was statistically significantly different. It appeared that in subgroups with a suboptimal (>20-30 ng/ml) and optimal (>30-50 ng/ml) 25(OH)D concentrations LSC values were not significantly different from each other; the calculated LSC were 3.7 ng/ml (14.2%) and 4.1 ng/ml (10.9%), respectively. The last subgroup, with high 25(OH)D concentration values (>50 ng/ml), differed statistically significantly in the LSC value only from the subgroup with vitamin D deficiency (<20 ng/ml). However, the comparison of that subgroup to the others was difficult due to its small size (n=4). It was also observed that the absolute root mean square CV value and that of absolute LSC when analyzed according to increasing 25(OH)D concentrations (Table 5) trended to increase both, what was not observed when comparing the relative/percentage values of these variables. But Spearman correlation tests between 25(OH)D concentration values and absolute and percentage root mean square precision errors (CV, CV%) provided answer – results showed significant correlation with absolute precision error (CV; p<0.0001) but not for precision error expressed in percentages (CV%). Further, the correlation between 25(OH)D and the root mean square CV% trended to be negative.

The practical conclusion of the study is the obtained absolute LSC value, which, for the purpose of result interpretation, can be assumed as approximately 4.0 ng/ml. Thus, a person comparing two or more 25(OH)D concentration measurement results of the same patient obtained in different periods of time can state with a probability of 95% that, when the values differ by more than 4.0 ng/ml, the difference is of diagnostic significance. Such diagnostic assumption can be made without reservations for suboptimal and optimal concentrations, i.e. 25(OH)D >20 ng/ml up to 50 ng/ml, the case for over 75% of the patients in the studied group. The situation appeared slightly different for low 25(OH)D concentration values (<20ng/ml), where the absolute LSC appeared to be 2.2 ng/ml. In turn, in the high concentration range (>50 ng/ml), the value of the calculated absolute LSC turned out to be several times greater, 11.8 ng/ml. However, the small size of that group (n=4) prevented an effective comparison with the others, as well as diagnostic use of this LSC.

The authors of the study believe that the LSC values presented above allow for their practical use in the interpretation and assessment of 25(OH)D concentration results, considering the discussed limitations. It is advisable to extend the study on a larger patient population. The expected study population should consist of a comparable number of participants with different 25(OH)D concentrations.

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