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Effects of Biological and Analytical Variations on the Appropriate Use of "Reference Intervals" in Clinical Chemistry. Proposal of a Scheme for Longitudinal Assessment of Laboratory Values

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Summary: For twenty one different laboratory parameters the separate contributions of both biological (age, sex, hospitalisation) and analytical variations to the total variation have been examined. For each of the different laboratory parameters the reference interval and critical difference are given for the longitudinal evaluation of analysis results.

These critical differences are not only calculated on the basis of the analytical imprecision from day to day, as has been usual until now, but the mean individual deviations of the reference samples have also been included. The critical differences given in this paper are therefore essentially higher for some parameters compared with the critical differences on the basis of the analytical deviation, and are therefore much more realistic quantities to be used for longitudinal evaluation.

Finally for practical purposes a scheme is presented for use with longitudinal assessment of laboratory values, in order to enable the practising physician to consider these data in daily decision making.

Wirkungen biologischer und analytischer Varianzen auf den angemessenen Gebrauch von „Referenzintervallen“ in der Klinischen Chemie.

Vorschlag eines Schemas zur longitudinalen Bewertung von Laboratoriumsergebnissen.

Zusammenfassung: Der separate Beitrag von biologischer (Alter, Geschlecht, Krankenhausaufenthalt) und analytischer Varianz zur Gesamtvarianz wurde für 21 verschiedene Laboratoriumskenngrößen ermittelt. Für jede der verschiedenen Laboratoriumskenngrößen wird der Referenzbereich und die kritische Differenz für die longitudinale Bewertung der Analysenergebnisse angegeben.

Diese kritische Differenzen wurden nicht allein aufgrund der analytischen Impräzision von Tag zu Tag, wie bisher üblich, berechnet, sondern die mittleren individuellen Abweichungen der Referenzproben wurden einbezogen.

Die in diesem Beitrag angegebenen kritischen Differenzen sind deshalb, verglichen mit den kritischen Differenzen aufgrund der analytischen Abweichung, wesentlich größer; sie sind daher sehr viel realistischere Größen für die Durchführung einer longitudinalen Bewertung. Für praktische Zwecke wird ein Schema zur Anwendung bei longitudinaler Bewertung von Laboratoriumsergebnissen angegeben, um dem praktizierenden Arzt die Berücksichtigung dieser Daten im täglichen Entscheidungsprozeß zu ermöglichen.

Introduction

Results for the determination of concentrations of serum constituents are dependent on several analytical and biological factors. Recent developments in analytical laboratory techniques, such as introduction of automatic analysers, development of highly standardised techniques and highly sensitive detection reactions, etc., have considerably reduced the analytical bias. However, further study of biological factors influencing the laboratory data is necessary. Hitherto, results of laboratory investigations have usually been compared to reference intervals ("normal values") in order to determine normality or abnormality (transverse assessment), i.e. to discriminate between health and disease.

Reference values and inter-individual variations are determined from samples from an apparently healthy population, often a selected group of the total population. Means and standard deviations are calculated and reference intervals are generally given as $\bar{x} \pm 2S_T$. In this study the contribution of both biological (age, sex, hospitalisation) and analytical factors to the overall standard deviation is considered (tab. 1).

Intra-individual variations of laboratory values depend on several factors which have to be considered in long term studies (longitudinal assessment). Some of these can be standardised, e.g. time of venipuncture, posture of the patient, choice between venous or capillary blood for analysis, fasting or non-fasting states of the patient, etc. More or less constantly or slowly changing factors are age, pregnancy, overweight, seasonal influences, etc., whilst others such as sex, menopause, etc., can be regarded as constant. Of utmost importance in the clinical evaluation of laboratory data are significant changes in consecutive values observed in the same individual (longitudinal assessment).

Twenty one routine clinical chemical laboratory parameters in a large population have been studied in order to determine to what extent the result of an analysis must be different from a preceding result to indicate a significant change. This minimal required difference is called the critical difference d_k (1, 2).

For the present investigation d_k has been calculated from literature data and has been compared to the actual differences found between classified data in this study. In this way an attempt was made to confirm the clinical relevance of these differences, i.e. the influence of factors such as age, sex, hospitalisation, etc., on laboratory parameters.

Tab. 1. Symbols used in this study acc. to Stamm (1, 2).

S_T^2 = variance in reference values

$$S_T^2 = S_B^2 + S_A^2 + S_{\text{other}}^2 \rightarrow S_T^2 = S_P^2 + S_I^2 + S_A^2 + S_{\text{other}}^2$$

$S_{T..}^2$ = total intra-individual variance when $S_I^2 = 0$

$$S_{T..}^2 = S_P^2 + S_A^2 + S_{\text{other}}^2$$

S_B^2 = biological variance

$$S_B^2 = S_P^2 + S_I^2 \quad | \quad S_P^2 = \text{intra-individual variance}$$

S_I^2 = inter-individual variance

S_A^2 = analytical variance

$$S_A^2 = S_S^2 + S_L^2 \quad | \quad S_S^2 = \text{variance in the run}$$

S_L^2 = long-term variance

S_{other}^2 = other variance, e.g. specimen collection

d_k = critical difference

$$d_k = 2\sqrt{2(S_P^2 + S_A^2)}$$

Materials and Methods

Subjects

The influence of sex, age and hospitalisation was studied by investigating laboratory data retrospectively from a population of 12000 patients. In order to eliminate the possible influence of disease all data from an individual were rejected if one of the data was outside the reference values. Different sub-populations were used for the study of the influence of hospitalisation, sex or age.

Although these procedures do not absolutely exclude all unhealthy patients, the influence of disease can be considered as negligible. The influence of hospitalisation was studied by dividing the subjects into hospitalised and out-patient groups.

Samples

Blood samples were taken between 7.30 a.m. and 9.00 a.m. and reached the laboratory within one hour.

The clinical patients had been fasting for twelve hours before the blood was taken; no limitations on diet were prescribed for out-patients.

Analytical procedures

There was a high degree of conformity in analytical procedures between the different participating laboratories, which used identical control sera, standards and methods. Moreover, the data from a hospital (where continuous flow analysis, Technicon, SMAC, was used) in a different area were compared with the data from the hospitals in the study area.

Analytical precision

The analyses concerned are listed in table 2 together with the coefficients of variation. The calculated critical differences are also given in table 2.

As expected, the analytical variation coefficient is larger in the data group obtained by continuous flow analysis. For every analysis, however, the relevant variation coefficient was used in the calculation of the critical difference d_k .

Tab. 2. Laboratory parameters with coefficient of variation (CV %) and critical differences (d_k).

Coefficient of variation and critical differences were calculated from data within the transverse reference interval.

	CV _P (%)	CV _A (%)			d _k		
		SMAC	Other methods		SMAC	Other methods	
		One hospital	One hospital	Area	One hospital	One hospital	Area
<i>Enzymes</i>							
Creatine kinase	71.6	14.9	2.9	14.0	207	203	206
Alanine aminotransferase	42.2	21.4	2.4	6.5	134	120	121
Aspartate aminotransferase	18.4	18.8	2.8	4.5	74	53	54
Lactate dehydrogenase	7.6	7.4	3.4	5.5	30	24	27
γ-Glutamyltransferase	34.7	10.1	2.9	11.8	102	99	104
Alkaline phosphatase	6.6	5.9	2.3	7.3	25	20	28
α-Amylase	—	—	3.8	3.9	—	—	—
<i>Electrolytes</i>							
Sodium	0.8	0.7	0.8	1.1	3.0	3.2	3.9
Potassium	4.6	2.3	1.7	2.3	15	14	15
Chloride	1.8	1.7	1.4	1.5	7.0	6.5	6.6
Calcium	1.7	2.1	1.5	2.6	7.6	6.4	8.8
Phosphate	7.4	3.5	2.9	2.9	23	23	23
<i>Intermediary metabolites</i>							
Bilirubin	24.5	13.9	2.7	3.4	80	70	70
Glucose	6.2	4.8	2.5	5.0	22	19	23
Cholesterol	5.6	2.4	3.0	6.2	17	18	24
Triglycerides	30.3	3.6	4.6	13.7	86	87	94
Total protein	2.7	2.2	1.6	2.1	10	8.9	9.7
Urea	13.4	5.1	3.2	5.9	41	39	41
Creatinine	4.0	5.8	3.1	5.5	20	14	19
Uric acid	8.7	5.0	3.8	3.6	28	27	27
<i>Iron status</i>							
Iron	26.6	5.6	5.3	9.4	77	77	80

Statistics

Data obtained from 12000 healthy persons were arranged in consecutive classes and for each class the means ($\bar{x}$), standard deviations (S_T) and the coefficients of variation (CV) were calculated. In order to determine the presence of absence of significant differences between means of two populations, the standard deviations of the difference between class means were calculated as follows:

$$SE_{\text{difference}} = \sqrt{\frac{S_{T1}^2}{n_1} + \frac{S_{T2}^2}{n_2}}$$

The difference between class means was considered to be significant ($p < 0.05$) when it exceeded $2 \cdot SE_{\text{difference}}$ (3, 4).

Significant differences are often found in large populations. With this method a significant difference e.g. between chloride content of serum in males vs. females was found ($p < 0.001$, table 4).

It is evident that this difference between values from males and females of 1 mmol/l had not hitherto been considered clinically relevant. Another procedure was therefore tried, which is suitable for use in daily clinical practice, i.e. the method presented by the group of Young (5) and confirmed by Stamm (1, 2). With the formula $d_k = 2 \sqrt{2(S_p^2 + S_A^2)}$ the minimal value of the significant difference between two consecutive determinations of a same constituent in the same individual was calculated, and designated by Stamm as the "critical difference", d_k .

Many investigators (5–11) determined values for the intra-individual variance S_p^2 . Means of these data from the literature (table 3, last column) were used in the calculation of our results.

Values for S_A were obtained from daily quality control analyses. Values for CV_P and CV_A and the critical differences are given in table 2.

As stated before we consider the difference between two values significant if its value exceeds the critical difference d_k .

Results and Discussion

As stated in the introduction, external influences on the clinical relevance of changing laboratory data were studied. In this introductory study the transverse reference values were assessed in different groups distinguished by age, sex and hospitalisation, and differences between group means were determined (tab. 4–7).

From a number of literature data (tab. 3) the so called critical differences, as used in the longitudinal assessment of patient laboratory data, were calculated and compared with the differences between the class means found in this study.

Tab. 3. Published intra-individual variation (coefficient of variation CV_P) data. CV_P values and literature references are given with the calculated $\overline{CV}_P$.

Literature references	(5)	(6)	(7)	(8)	(9)	(10)	(11)	$\overline{CV}_P$
<i>Enzymes</i>								
Creatine kinase	82.76	—	—	—	—	60.3	—	71.6
Alanine aminotransferase	57.89	—	—	26.4	—	—	—	42.2
Aspartate aminotransferase	14.79	—	15.1	24.2	—	19.3	—	18.4
Lactate dehydrogenase	6.19	9.0	7.3	—	—	8.0	—	7.6
γ -Glutamyltransferase	—	—	—	—	—	34.7	—	34.7
Alkaline phosphatase	6.33	—	5.8	4.8	6.4	8.5	7.9	6.6
α -Amylase	—	—	—	—	—	—	—	—
<i>Electrolytes</i>								
Sodium	0.93	—	1.4	0.7	0.5	—	0.5	0.8
Potassium	4.43	5.0	4.6	4.3	6.2	—	3.2	4.6
Chloride	1.52	1.4	2.1	2.1	—	—	—	1.8
Calcium	1.93	1.7	1.6	1.7	1.6	2.1	1.1	1.7
Phosphate	7.40	7.5	9.6	5.8	6.8	8.2	6.2	7.4
<i>Intermediary metabolites</i>								
Bilirubin	25.59	—	—	22.0	26.0	—	—	24.5
Glucose	—	5.6	6.5	—	—	6.5	—	6.2
Cholesterol	4.39	6.4	4.2	5.3	—	7.9	—	5.6
Triglycerides	33.33	—	—	—	—	27.3	—	30.3
Total protein	2.51	2.8	2.3	2.9	3.0	3.3	2.0	2.7
Urea	14.29	11.9	13.6	12.3	11.1	16.4	14.2	13.4
Creatinine	3.83	—	4.4	4.3	—	3.1	4.2	4.0
Uric acid	8.98	10.1	8.5	7.3	—	8.8	—	8.7
<i>Iron status</i>								
Iron	—	—	—	26.6	—	—	—	26.6

Influence of age

In table 4 data obtained for different age classes are given. Only in four cases did the difference between consecutive classes exceed d_k , i. e. for alkaline phosphatase, lactate dehydrogenase, potassium and phosphate in serum. For these constituents the age class 1–9 years invariably produced higher values than other age classes. A gradual increase with age was noted for glucose, urea, creatinine, α -amylase, cholesterol and uric acid. Values for glucose, creatinine, protein and uric acid content in the age class 1–9 years were significantly lower than in all classes over 20 years of age. The same was true for urea, α -amylase, bilirubin, cholesterol, protein, and uric acid in the 1–9 years class as compared to all other classes.

Small variations were noted for several consecutive class means of γ -glutamyltransferase, alanine aminotransferase, aspartate aminotransferase, creatine kinase and serum iron concentration.

The constancy of sodium, chloride and calcium concentrations was striking. This has also been reported

by Collove et al. (12) who suggested that it was due to endocrine regulation mechanisms.

The decreased levels of urea, creatinine, bilirubin and uric acid in the 30–39 years class must be noted as well as the low values for α -amylase activity in the 60–69 years class, and the decrease of creatine kinase activity after 69 years of age.

On account of the existing differences in blood creatinine levels between men and women, separate age classes for both sexes are given (tab. 5). From 10 years of age and onwards significant differences between age classes were found in females. In males significant differences were also seen between the 1–9 years and the 10–19 years age groups, and perhaps between 70–79 years and >80 years age groups.

Although a clinically relevant difference (15 $\mu\text{mol/l}$) existed overall between males vs. females this difference was not found in separate age classes. These findings are in good accordance with data from the literature (10, 13 to 22).

Tab. 4. Mean concentrations of serum constituents found in different age classes.

Age classes	1-9 years		10-19 years		20-29 years		30-39 years		40-49 years		50-59 years		60-69 years		70-79 years		>80 years			
	SI units	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	d _k
<i>Enzymes</i>																				
Creatine kinase	U/l	8	14	13	11	102	11	70	12	102	14	113	15	110	17	40	9	7	7	28
Alanine aminotransferase	U/l	18	6	82	8	60	8	96	8	47	8	89	6	89	6	61	7	15	5	9
Aspartate aminotransferase	U/l	18	9	104	10	56	7	109	9	68	8	87	7	100	7	62	7	19	8	6
Lactate dehydrogenase	U/l	6	173	29	129	50	134	87	135	30	128	59	143	81	132	40	141	13	137	41
γ-Glutamyltransferase	U/l	-	-	32	10	70	13	101	14	60	16	63	14	87	15	40	16	11	10	15
Alkaline phosphatase	U/l	12	109	23	48	58	39	94	39	68	41	87	43	78	46	50	45	29	45	11
α-Amylase	U/l	-	-	16	166	46	172	86	191	46	201	24	208	42	169	26	238	7	249	-
<i>Electrolytes</i>																				
Sodium	mmol/l	88	140	108	140	88	139	109	139	91	140	110	140	103	140	105	140	37	139	4.0
Potassium	mmol/l	86	4.7	111	4.1	116	4.1	107	4.2	96	4.2	149	4.2	120	4.2	144	4.3	144	4.3	0.6
Chloride	mmol/l	11	101	37	103	53	103	78	103	47	103	76	104	58	104	47	102	19	103	7
Calcium	mmol/l	25	2.4	33	2.4	38	2.4	97	2.4	61	2.4	84	2.4	80	2.3	56	2.4	28	2.4	0.2
Phosphate	mmol/l	10	1.8	26	1.2	50	1.2	61	1.1	56	1.2	93	1.1	83	1.1	60	1.1	18	1.1	0.3
<i>Intermediary metabolites</i>																				
Bilirubin	μmol/l	12	3	84	8	56	9	115	6	60	7	87	7	96	7	55	7	16	6	6
Glucose	mmol/l	16	3.5	51	4.4	58	4.9	90	5.2	48	5.3	83	5.1	83	5.2	48	5.5	16	5.3	1.2
Cholesterol	mmol/l	8	4.1	11	4.4	34	4.6	66	5.3	30	5.7	67	5.8	54	5.8	38	5.8	14	5.3	0.9
Triglycerides	mmol/l	8	1.2	41	1.2	80	1.3	85	1.4	100	1.4	100	1.4	100	1.4	100	1.4	45	1.3	1.2
Total protein	g/l	34	60	109	66	57	68	114	69	76	68	100	68	106	68	62	68	21	68	7
Urea	mmol/l	22	3.5	103	4.2	104	4.4	101	3.8	73	4.9	122	5.4	88	5.6	61	5.7	21	5.9	2.0
Creatinine	μmol/l	29	52	71	59	72	79	111	77	69	87	131	89	108	90	66	92	25	97	17
Uric acid	mmol/l	7	0.18	50	0.22	49	0.31	59	0.27	64	0.30	70	0.33	67	0.33	32	0.32	10	0.32	0.08
<i>Iron status</i>																				
Iron	μmol	-	-	25	18	57	16	66	20	63	17	55	17	52	20	27	17	6	13	14

Tab. 5. Mean serum creatinine concentrations in different age classes for both sexes.

Age class (years)		1-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	>80	total
Female	n	18	42	41	81	30	96	74	39	5	426
	$\bar{x}$	49	67	80	80	92	93	93	97	117	93
Male	n	11	24	35	40	33	30	39	26	19	257
	$\bar{x}$	42	55	78	76	83	86	85	89	93	78

Influence of sex

In table 6 the influence of sex on the level of serum constituents is given. Although significant ($p < 0.05$) differences between mean values in sex classes were found for urea, creatinine, bilirubin, γ -glutamyl-transferase, creatine kinase, cholesterol, uric acid, chloride, phosphate and iron, only in the case of creatinine did this difference approximate to the d_k value ($17 \mu\text{mol/l}$)

$$|x_m - x_f| > 2 \sqrt{2(S_p^2 + S_A^2)}$$

This difference therefore can be considered as clinically relevant which means that different reference

ranges must be used. This is not the case for the other serum constituents where statistically significant, but clinically non-relevant differences between sex classes were found.

Here the intra-individual variance exceeds the variance between sex classes. Literature data (10, 13, 19 to 22) for urea, creatinine, uric acid, cholesterol, creatine kinase, γ -glutamyltransferase and phosphate are in close agreement with our findings. Inconsistent values have been reported for α -amylase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and calcium.

Tab. 6. Mean concentrations and group variations of serum constituent values for both sexes. Significant differences between sex groups (SE_{diff}) and critical differences (d_k) are given.

		Males			Females			SE _{diff}	P	d _k SI units			
		n _m	$\bar{x}_m \pm S_T$ SI units	n _f	$\bar{x}_f \pm S_T$ SI units								
<i>Enzymes</i>													
Creatine kinase	U/l	255	15 ± 9.0	289	12 ± 6.4	0.68	0.001	28					
Alanine aminotransferase	U/l	352	7 ± 3.2	214	7 ± 3.0	—	—	9					
Aspartate aminotransferase	U/l	369	8 ± 3.0	253	8 ± 3.0	—	—	6					
Lactate dehydrogenase	U/l	250	135 ± 18.6	146	137 ± 15.6	1.56	0.317	41					
γ-Glutamyltransferase	U/l	270	17 ± 13.1	205	12 ± 10.1	1.08	0.001	15					
Alkaline phosphatase	U/l	277	42 ± 10.1	190	43 ± 10.5	0.98	0.317	11					
α-Amylase	U/l	166	188 ± 54.6	144	188 ± 56.0	—	—	—					
<i>Electrolytes</i>													
Sodium	mmol/l	458	140 ± 2.4	356	140 ± 2.6	—	—	4.0					
Potassium	mmol/l	548	4.3 ± 0.4	436	4.3 ± 0.5	—	—	0.6					
Chloride	mmol/l	248	103 ± 3.1	179	104 ± 2.8	0.29	0.001	7					
Calcium	mmol/l	291	2.4 ± 0.08	214	2.4 ± 0.07	—	—	0.2					
Phosphate	mmol/l	270	1.1 ± 0.17	188	1.2 ± 0.15	0.02	0.001	0.3					
<i>Intermediary metabolites</i>													
Bilirubin	μmol/l	253	6.0 ± 3.4	354	8.0 ± 2.7	0.26	0.001	6					
Glucose	mmol/l	240	5.2 ± 0.6	237	5.2 ± 0.5	—	—	1.2					
Cholesterol	mmol/l	200	5.5 ± 1.0	119	5.6 ± 1.0	0.11	0.001	0.9					
Triglycerides	mmol/l	100	1.4 ± 0.4	100	1.4 ± 0.3	—	—	1.2					
Total protein	g/l	506	68 ± 8.0	270	68 ± 5.3	—	—	7					
Urea	mmol/l	378	5.0 ± 1.3	344	4.7 ± 1.9	0.12	0.046	2.0					
Creatinine	μmol/l	426	93 ± 15.5	257	78 ± 15.2	1.31	0.001	17.0					
Uric acid	mmol/l												
<i>Iron status</i>													
Iron	μmol/l	163	20.0 ± 4.7	163	16.0 ± 4.2	0.49	0.001	14					

Influence of hospitalisation

Differences between laboratory data from hospitalised and out-patients can be expected on account of different behaviour (bed rest, diet, etc.).

A number of analyses in blood are generally performed before admission to hospital in order to reduce the duration of hospitalisation. This means that longitudinal assessment of laboratory values is complicated by the differences due to hospitalisation found in intra-individual follow up. The clinical relevance of this item has been studied (tab. 7).

It appeared that the differences were clinically relevant only for serum glucose and serum protein, i.e. they are equal to or in excess of the critical difference (d_k).

The findings of *Young* (13), *Schwartz* (23) and *Kreutzer & Raymakers* (20) concerning the influences of bed rest on potassium, calcium and phosphate levels were not confirmed.

Other influences

Many other factors possibly influence the levels of laboratory data. Reports have been published on seasonal influences (12, 13, 20, 24, 25, 26), diurnal rhythm (13, 20, 23, 27, 28), smoking (29, 30), medication (20, 29 to 33), exercise (20, 23, 29, 34 to 39), pregnancy (20, 40, 41, 42), overweight (20, 23), blood transfusion (13, 29), intramuscular injection (23, 29), fasting (13, 20, 23, 29), and use of capillary or venous blood (13, 43, 44).

Clinical relevance

For the general practitioner and the clinical specialist it is impossible to constantly take all these factors into account. However, especially in longitudinal studies, this is in principle important. It must be considered that elimination of some factors can be obtained by strict standardisation of blood sampling, e.g. always between 8.00–9.00 a.m., always from resting patients, always in fasting conditions, etc.

Tab. 7. Mean concentrations of serum constituents from hospitalised patients and out-patients. Critical differences (d_k) are given for comparison.

		Hospitalised patients		Out-patients		
		n	$\bar{x}$	n	$\bar{x}$	d_k
	SI units		SI units		SI units	SI units
<i>Enzymes</i>						
Creatine kinase	U/l	232	102	208	78	180
Alanine aminotransferase	U/l	229	23	226	19	25
Aspartate aminotransferase	U/l	238	25	235	22	12
Lactate dehydrogenase	U/l	230	369	228	324	74
γ -Glutamyltransferase	U/l	220	24	230	21	21
Alkaline phosphatase	U/l	231	98	231	92	13
α -Amylase	U/l					
<i>Electrolytes</i>						
Sodium	mmol/l	239	140	249	144	5
Potassium	mmol/l	237	4.3	220	4.3	0.6
Chloride	mmol/l	236	100	179	102	7
Calcium	mmol/l	242	2.3	225	2.4	0.2
Phosphate	mmol/l	230	1.1	239	1.1	0.2
<i>Intermediary metabolites</i>						
Bilirubin	μ mol/l	232	9.9	234	7.9	6.0
Glucose	mmol/l	234	7.4	227	5.2	0.9
Cholesterol	mmol/l	250	6.1	236	6.4	1.1
Triglycerides	mmol/l	230	1.4	234	1.6	1.2
Total protein	g/l	250	61	250	69	6
Urea	mmol/l	236	5.9	230	5.6	2.2
Creatinine	μ mol/l	231	96	229	88	14
Uric acid	mmol/l	241	0.36	237	0.33	0.08
<i>Iron status</i>						
Iron	μ mol/l	174	8	231	14	11

It is impossible to standardise other factors such as age, sex, seasonal influences, pregnancy, blood transfusion, etc. In order to improve the benefit of using laboratory data a scheme was developed by which the influence of a number of factors on 21 common laboratory data can be shown (tab. 8). We have differentiated between factors influencing laboratory data without clinical relevance and those which may be relevant for clinical decision making,

i.e. those where the differences observed exceed the critical difference (d_k) value.

The use of this scheme enables the practising clinician to take into account any possible alteration in circumstances since the previous blood sample was taken. In this procedure the patient is used as his own reference in a longitudinal follow up, such as in the course and development of disease, and in the treatment phase.

Tab. 8. Several influences on clinical chemical serum parameters. Legends: ↑ clinically significant increase; ↓ clinically significant decrease; + increased; - decreased; O no influence; ♀ female; ♂ male. Seasons: 1 Spring; 2 Summer; 3 Autumn; 4 Winter.

	In- creas- ing age	Male/ Fe- male	Hos- pital- isa- tion	Cir- cadian rhythm	Seasonal	Capil- lary sam- ple	Preg- nancy	Intra- mus- cular injec- tion	Non- fast- ing pa- tient	Smok- ing	Stress	Over- weight
Creatine kinase	-	O	+	O	O		-	+	O	O	↑	
Alanine aminotransferase	O	O	+	+-	O	O	O	O	-	O	-	
Aspartate aminotransferase	O	O	+	+-	O	O	O	+	+	O	+	O
Lactate dehydrogenase	↓	O	+	+-	↑ ₂ + ₄	O	+	O	+	O	+	+
γ-Glutamyltransferase	O	O	+	+-	O		O	O	O	O	O	
Alkaline phosphatase	↓	+	+	↑↓	+ ₃		↑	O	+	O	O	↑ ♀
α-Amylase	+	O	O	O	O		+-	O	O	O	O	
Sodium	O	O	-	+-	+ ₂	+	-	O	O	O	+	
Potassium	↓	O	O	O	O	O	-	O	O	O	+	
Chloride	O	O	-	+-	+ ₂	+	O	O	O	O	O	
Calcium	O	O	O	+-	- ₁ + ₄	+	-	O	+	O	+	O
Phosphate	↓	-	O	+-	+ ₂ - ₄	-	+	O	+	O	↑	O
Bilirubin	+	-	+	+-	- ₁ + ₃	O	O	O	+	O	O	O
Glucose	+	O	↑	↑↓	+ ₂	O	+-	O	↑	+	+-	O
Cholesterol	+	-	-	+-	+ ₁ - ₂	+	+-	O	+	+	+	+ ♂
Triglycerides	O	O	+	+-	- ₄		+	O	↑	+	O	
Total protein	+	O	↓	↑↓	- ₂	+	-	O	+	O	+	+ ♀
Urea	+	+	O	+-	+ ₂	O	-	O	+	+	↑	O
Creatinine	+	↑	+	+-	O	+	O	O	+	O	↑	
Uric acid	+	+	+	+	+ ₂		-	O	+	-	O	↑
Iron	O	+	+	+-	O		-	O	O	O	-	

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