Biological a analytical variability

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Subject Clinical Biochemistry

- Proper and right indication of laboratory tests (test panels)

- Proper and right interpretation of laboratory results
- Znalost principů analytických metod k posouzení senzitivity, specificity a možných interferencí (např. lékových)

Evaluation of laboratory result

- Reference intervals
  according the age, sex (gender) ethnicity, index of individuality
- Clinical significance of difference between two following results of the same patient according delta check, CD (critical difference), RCV (reference change value)
- Decision limit (cut-off value)
- Biological halflife of the analyte

„Normal“ values

- Reference intervals – morning, fasting
- Population-based – interindivid. biol. var.
- Subject-based – intraindivid. biol.var.
- „healthy“ population, n=? (120 vs. 20)
- Analytical method dependent ref. intervals (biochemical OK, immunochemistry – big differences, comparable results: TSH, PSA, PCT)

Reference intervals (RI)

- 2.5 – 97.5 %, event. 99. percentil (hs-cTnI)
- 5 % of healthy persons are out of RI !!

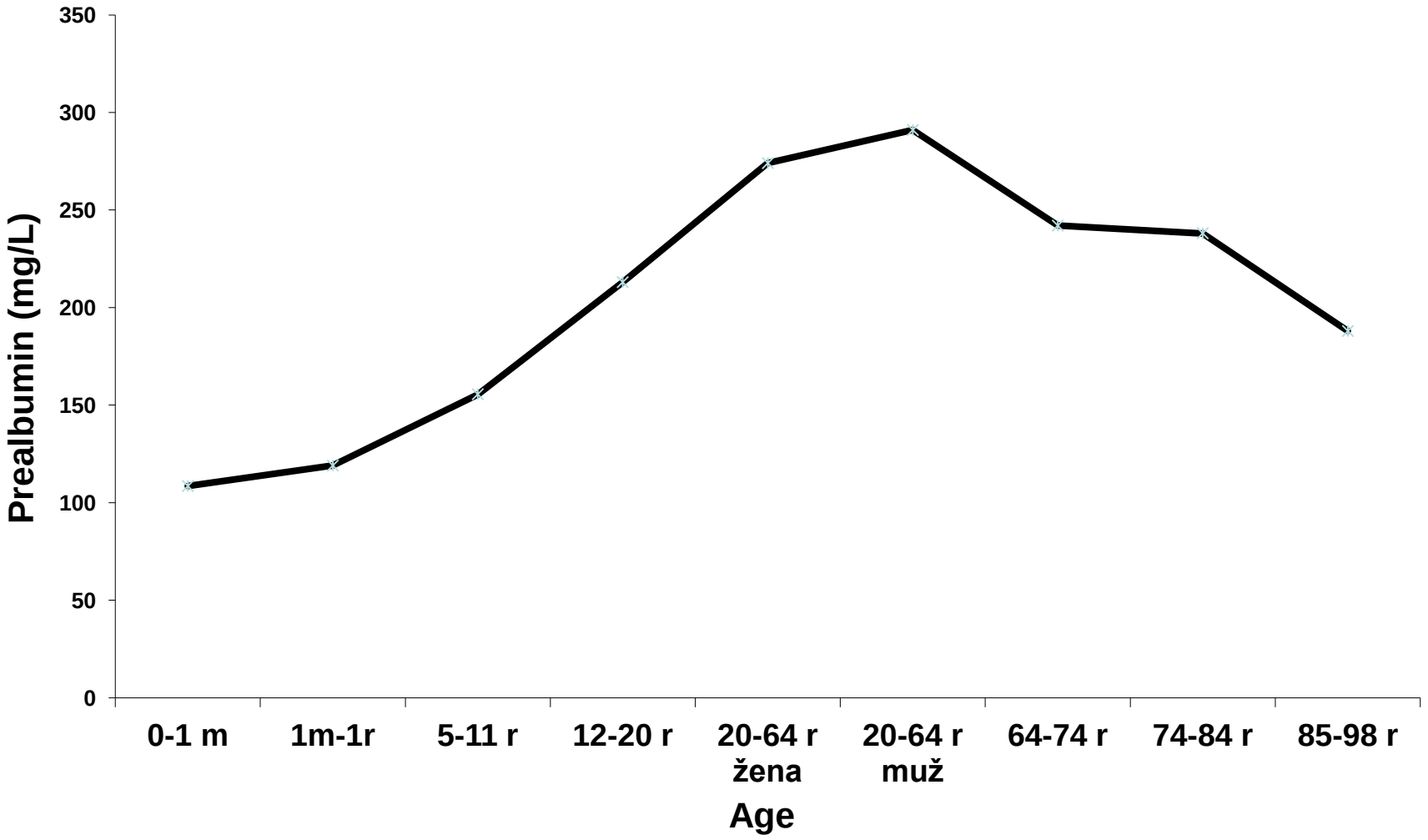
- Distribution of the data – normality, semilog. (TSH, HDL-chol, calciurie), bifasic

- Overlapping of healthy and ill population

Sources of biological variability

- Biorhytms (circadian, seasonal) – eg. Fe, TSH – 50% difference between 8 am and 2 pm
- Age (gestational, postnatal)
- Homeostasis (eg. prepubertal, postmenopausal, menstrual cycle)
- Sex and gender, transG (eg. creatinin)
- Etnicity (eg. amylase in Asians, CK in Black)
- Pathology (eg. IgM – acute bronchopneumonia)
- Stimuli: meal, physical activity, tea,coffee, nikotin, ethanol, drugs etc.

Prealbumin – age dependent



Creatinin (umol/l)

Postnatal age	Gest.age 28 w	Gest.age 36 w
2 days	40 – 220	23 – 143
7 d	23 – 145	16 – 98
14 d	18 – 118	12 – 80
21 d	16 – 104	11 – 71
28 d	15 – 95	10 – 64

Intraindividual biological variability

- Homeostatic point (value)
- Variation coeficient of intraind. BV
- S-Na 1 % (vs. 135 – 145 mmol/l)
- S-creatinin 10 % (vs. 40 – 80 umol/l)
- S- osteocalcin 50 %
- Index of individuality: 0.6 – 1.4

Analytical variability

- Analytical var. coef. smaller than var. coef. of intraind. BV
- Eg. VC S-Na = 1 %
- Method dependent:
- S-creatinin Jaffé, VC = 10 %
- S-creatinin enzymatic method, VC = 4.5 %
- Analytical VC similar in various high values: S-creatinin 100 vs. 500 umol/l

Delta check and critical difference

- Answer to question: Is the change of two following results in the same patient clinically significant taking in account analytical and biological variability of the test?

- Eg. S-creatinin 100 vs. 118 umol/l
- Eg. cholesterol (therapy), PSA (relaps), cTnI (dg. AIM)

- Delta check – simple absolute or relative (%) difference (hs-cTnI, creatinin)
- Critical difference – calculated from variation coeficients of analytical and biological variability (on 95% level of probability)

Cut-off value, decision limit

- Replacing reference intervals to reach better sensitivity or specificity of the test and appropriate diagnosis
- Eg. TSH newborn screening (sensitivity – nearly 100 %), PCT sepsis (specificity nearly 100 %), PSA, NSE, cTnI
- Depends on benefits for patients, changes according progress in diagnostic procedure and therapy
- Arbitrary cut-off:
- P-glucose, S-cholesterol, S-LDL-cholesterol

Biological halflife - examples

- Glycated Hb HbA1c (diabetes mellitus), 60 days (Cave! Variant and pathological Hb have different T1/2)
- PTH (intraoperative measurement), 5-8 min
- Tumor markers, postoperative measurement (AFP 5 days)
- CDT(carbohydrate deficient transferin) (abusus of ethanol), 8 days

Special terms - examples

- Reflex testing
  (TSH – fT4, paraprotein – immunofixation)

- High (ultra) sensitiv methods:
  hs-Tg, hs-CRP, hs-PSA, hs-cTnI etc.

- Lipemic, hemolytic and icteric index (possible interferences)

- TAT (total turnaround time)

Quantifying Biological Variation

How are you going to quantify biological variation?

You have to dissect out the components of variance: -

$$\sigma^2_{total} = \sigma^2_{Analytical} + \sigma^2_{Individual} + \sigma^2_{Group}$$

Evaluating the significance of change in serial results.

- **Critical Difference or Reference Change value indicates the value by which 2 serial results must differ to be considered statistically significant: -**

  $$CD = 2^{½} * Z * (CV_A^2 + CV_I^2)^{½}$$

  Probabilty = 95% Z = 1.96

  Probability = 99% Z = 2.58

- **Only valid if the variance of $\sigma^2_{Individual}$ is homogenous.**

  (Costongs J Clin Chem Clin Biochem 1985;23:7-16)

Multipliers for $(CV_A^2 + CV_I^2)^{½}$ to Obtain Critical Difference at Different Levels of Probability

Multiplier $(2^{½} * Z)$	3.64	2.77	2.33	1.81	1.47	1.19	0.95
Probability of false alarm	0.01	0.05	0.10	0.20	0.30	0.40	0.50
Probability	99%	95%	90%	80%	70%	60%	50%

Significance of Change?

63 year old patient: Cholesterol 1 = 6.60 mmol/L
Cholesterol 2 = 5.82 mmol/L

Significant change ?

CVa = 1.6% CV_I = 6.0%

$$\mathbf{RCV = 2^{½} * Z * (CV_A^2 + CV_I^2)^{½}}$$

$$\mathbf{95\%RCV = 1.414 * 1.96 * (1.6^{½} + 6.60^{½})^{½} = 17.2\%}$$

$$\mathbf{99\%RCV = 1.414 * 2.58 * (1.6^{½} + 6.60^{½})^{½} = 22.6\%}$$

Actual Change = ((6.60 – 5.82)/6.60)*100= **11.8%**

Dispersion $= Z^* (SD^2_A + SD^2_I)$

Dispersion of first result = result ± 1.96 SD : -

95% level 6.60 = 5.80 –7.40

99% level 6.60 = 5.54 – 7.66

Dispersion of 2 result

95% level = 5.82 = 5.11 – 6.53

99% level = 5.82 = 4.89 – 6.75

Overlap: therefore neither significantly or highly significantly different

Can use the formula to ascertain the probability that change is significant. Calculate Z using the (((6.6-5.82)/6.6)*100%) as RCV and look up in tables. 82% in this case.

Assessing the utility of reference intervals.

- **Utility of population based reference data?**
- **Ratio of Within to Between subject variances.**

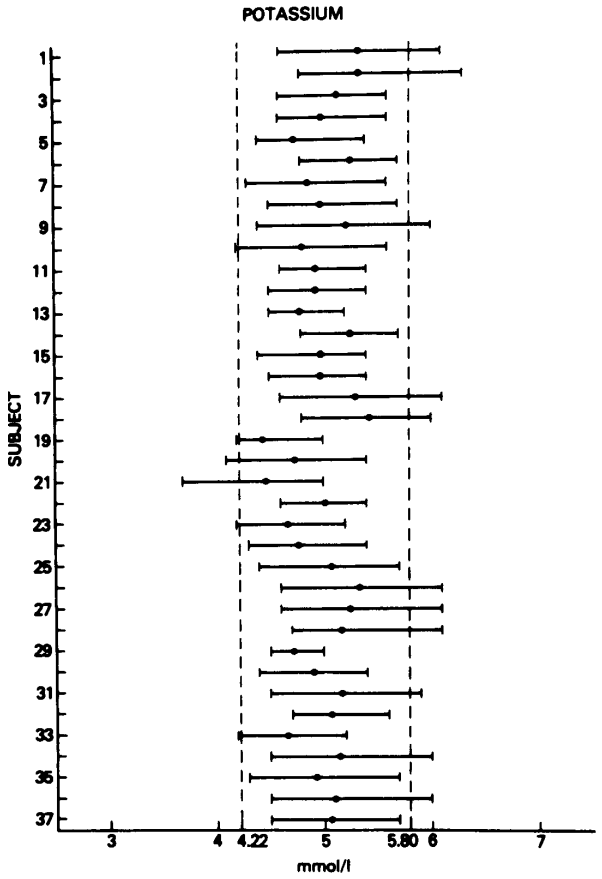
Index of Individuality = CV_I / CV_G

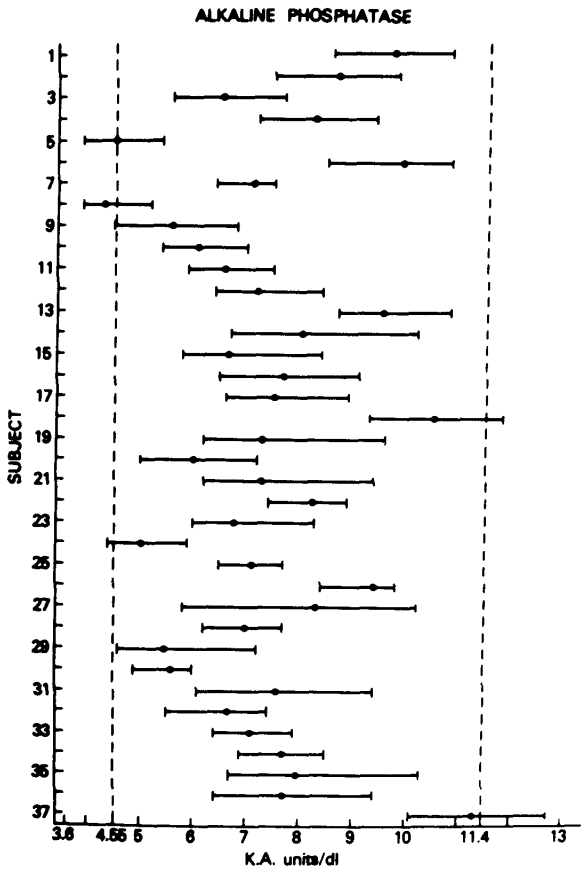
Population Ref Intervals: -

Index $\leq$0.6 = Limited in Value

Index $\geq$1.4 = Applicable

Biological Variation &Utility of Reference Intervals





Number of specimens required to estimate homeostatic set points: -

Cholesterol testing

How many samples (n) required to estimate set point within ±5% given: -

CV_I = 4.9% CV_A = 3% (Recommended)

Substitute equation: -

$$n = (Z. CV_{A+ I}/D)$$

$$n = [1.96 \cdot (3^2 + 4.9^2)^{½}/5]^2 = 5.07$$

RCV at 95% and Number. of Specimens Required to Assess the Homeostatic Set Point at Different Levels of Imprecision

CV_A (%)	CV_I (%)	RCV[a] (%)	Number of specimens[b]
2.0	4.7	14.1	4
3.0	4.7	15.4	5
4.0	4.7	17.1	6
5.0	4.7	19.0	7
6.0	4.7	21.1	9
7.0	4.7	23.4	11
8.0	4.7	25.7	13
9.0	4.7	28.1	16
10.0	4.7	30.6	19
15.0	4.7	43.5	38
20.0	4.7	56.9	65

[a]RCV ($p \leq 0.05$) = $2.77 (CV_A^2 + CV_I^2)^{1/2}$, assuming no statistical evidence of heterogenity

[b]Number = mean result is within ±5%of homeostatic set point$1.96^2 \times (CV_A^2 + CV_I^2)^{1/2}/25$.