

Size and Sources of Sweat Chloride Variability

The background is a solid teal color. In the bottom right corner, there is a silhouette of a mountain range with several peaks, rendered in a slightly darker shade of teal.

Sources of Error

- ◆ Analytical variation is readily estimated by internal and external Quality assurance.
- ◆ Actual uncertainty is less well defined
- ◆ Uncertainty may be affected by
 - collection procedures
 - biological variation

Study 1

Intra-individual variation in sweat chloride concentration over time

- ◆ 4 healthy adult volunteers
- ◆ Sweat was collected between 8 and 12 times over 2 years
- ◆ Methodology
 - Wescor Macroduct collection system
 - Dilution recovery measurement of Na and Cl by direct ISE on the Vitros 250
- ◆ Intra-individual biological variation was calculated from the equation:
$$CV_t = \sqrt{[(CV_a)^2 + (CV_w)^2]}$$

	Subject I		Subject II		Subject III		Subject IV	
Laboratory	A		A		A		B	
Sex	M		F		F		F	
Age	55-60		25-30		55-60		38-40	
Δ F508 carrier status	N/A		N/A		N/A		NEGATIVE	
Sweat electrolyte	Na	Cl	Na	Cl	Na	Cl	Na	Cl
n	8	8	8	8	8	8	12	12
Mean (mmol/L)	27.8	12.8	22.0	15.5	54.3	36.4	59.5	38.4
Range (mmol/L)	20-34	7-18	12-28	8-23	30-70	17-48	52-67	32-50
SD (mmol/L)	4.9	3.3	5.0	5.1	13.9	10.6	5.1	5.5
CV _a (%)	<2%	<2%	<2%	<2%	<2%	<2%	≤3%	≤2%
CV _w (%)	17.5	25.7	22.6	32.8	25.5	29.1	8.0	14.2
CV _t (%)	17.6	25.8	22.7	32.9	25.6	29.1	8.6	14.3
95% Subject CI mmol/L	18-37	7-19	12-32	6-24	27-81	16-57	49-68	28-49

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- ◆ Consider subject 3

 - Mean Chloride=36.4 mmol/L

 - SD=10.6 mmol/L

 - 95% Confidence interval is 16-57 mmol/L

- ◆ At a level of "50mmol/L" using these statistics

 - 95% Confidence interval is 30 - 70 mmol/L

- ◆ Both 'normal' and 'abnormal' results could be obtained due to intra-individual variability

Study 2

Variation of chloride from 2 collection sites

- ◆ Sweat was collected simultaneously from 2 sites by pilocarpine iontophoresis using the Gibson Cooke method
 - usually from both forearms
 - 295 patients
 - Chloride was measured by a mercuric thiocyanate colourimetric method

Results

Whole group Chloride $CV_a = 4.1\%$
 $CV_t = 20.2\%$

- ◆ Comparable CV_t persisted across the age ranges
- ◆ Most patients are clearly normal or clearly abnormal, but 47 individuals were in the intermediate range

Using this data at the decision cut-offs

- ◆ Chloride = 40 mmol/L there is 95% probability of the result lying between 31-49
- ◆ Chloride = 60 mmol/L there is 95% probability of the result lying between 51-69
- ◆ If only site was used up to 48% of patients with at least one intermediate value may be misclassified

These values only reflect simultaneous testing and do not take account of differences in an individual over time.

Study 3

Biological variation in sweat conductivity

- ◆ Sweat conductivity was measured weekly for 5 weeks in
 - 15 healthy adults
 - 20 healthy infants
 - 20 known CF patients (1-45 years)
- ◆ Westcor Macroduct collection and analysed on the Wescor Sweat-Check analyser
- ◆ $CV_a = 1.32\%$ at a concentration of 40mmol/L
- ◆ The intra-individual CV in their healthy infant group was 18% which is very similar to the previous studies

Study 4

Relationship of Sweat Chloride with mutation analysis

- ◆ Over 2 years 773 sweat tests conducted on subjects aged 6 months to 50 years
- ◆ 91 subjects had borderline sweat test results (31 to 59 mmol/L)
- ◆ 66 of these had complete CFTR gene scanning requested by the clinician
- ◆ All sweat tests were performed on both arms
- ◆ Every borderline or positive test was repeated

Results

- ◆ The 66 borderline subjects were investigated with standard panels and, if necessary, complete scanning of the CFTR gene
 - 16 carried one mutation
 - 26 carried two mutations
 - 24 tested negative
- ◆ 39mmol/L showed the best sensitivity for the sweat test with respect to genotype
- ◆ There is a 4-fold increase in risk for sweat chloride $\geq 39\text{mmol/L}$ than for a result $< 39\text{mmol/L}$
- ◆ The positive predictive value was 77.1%
- ◆ The negative predictive value was 54.8%

Summary

- ◆ There are over 1500 variations in the CFTR gene
- ◆ Some show variable phenotype and borderline or normal sweats
- ◆ CF could be considered as a continuous spectrum disorder
- ◆ Wide intra-individual variation also blurs the boundaries of normal, intermediate and raised electrolyte values.
- ◆ Rigid cut-off values may not be appropriate.
- ◆ The diagnosis of CF must take into consideration not only the sweat test and mutation results, but a critical evaluation of the clinical presentation of the patient.