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Please read this bit first

The HPCSA and the Med Tech Society have confirmed that this clinical case study, plus your routine review of your EQA reports from Thistle QA, should be documented as a "Journal Club" activity. This means that you must record those attending for CEU purposes. Thistle will **not** issue a certificate to cover these activities, nor send out "correct" answers to the CEU questions at the end of this case study.

The Thistle QA CEU No is: **MT00025.**

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February 2007

Interpretation of Clinical Chemistry Laboratory Data

Part 6 – Biological Variation

This is Part 6 in an eight part series – compiled by Dr Jim McCulloch *– VERY LOOSELY based on the excellent book of the same name by Dr CG Fraser, published by Blackwell Scientific Publications, ISBN 0-632-01579-9.*

6.1 Introduction

The results of clinical laboratory tests performed on individuals often change with time. These changes could well be related to disease states, or changes brought about by treatment of diseases. Such changes can be used to follow the efficacy of the treatment or predict prognosis, or the likelihood of further complications.

These are not the types of change that are the subject of this chapter.

6.2 Biological rhythms

Changes in the levels of body constituents do occur with time in situations other than disease. The results of certain lab tests change with age, especially seen during the neonatal period and old age. Other more regular rhythmic changes occur and these may be of daily, monthly or even seasonal in nature.

Daily rhythms

Many serum and urine constituents show changes during the day. These daily rhythms may be classified as diurnal, where the variation occurs depending on the time of day, or nychthemeral, where the variation is dependent on the sleep-wake cycle. The best described example of such daily rhythms is that of serum cortisol.

Serum cortisol falls throughout the day from its peak concentration, seen about 8am. This has obvious implications on the timing of sample collection, the preparation of reference ranges, and the interpretation of patient results. A similar situation arises with serum TSH, which can vary by a factor of 2 or 3 throughout the day, and in fact many hormones show such fluctuations, or biological variations.

Monthly rhythms
The most important monthly cycles are those found in women during the reproductive phase of life, for example LH, estradiol and progesterone. As for daily variations, it is essential to be aware of the monthly rhythms so that specimens can be collected at the most appropriate time for the required investigation, and for an appropriate interpretation of the results to be made.

Seasonal rhythms
There are many seasonal rhythms, but they are usually due to exogenous factors. Examples of this include the rise in LDH during the summer (most likely due to increased exercise), and the increase in serum cholesterol in winter (perhaps diet related).

The best documented seasonal variation are the marked changes in the vitamin D metabolite, 25-hydroxycholecalciferol, where levels are higher in summer (particularly in countries far from the equator), most likely related to the amount of UV-B radiation.

6.3 Random biological variation

In contrast to those examples mentioned above, most analytes measured in the laboratory do not show cyclical changes. However, there will be variation, both within a single individual, and between individuals. The simplest way to consider this is that each of has this random variation of analyte concentration, and that if it is plotted on a graph, it will resemble a Gaussian curve.

If the serum sodium was measured on six days in a young adult male, typical results would be:

Units	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
mmol/l	138	137	139	140	137	141

The variation in the numerical results is due to two sources of variation: analytical imprecision and biological variation (BV) of a random nature. The BV in due to intrinsic variation around the homeostatic set-point, as well as to the many preanalytical factors and sample collection factors, such as posture, previous food intake, exercise, and so on.

A second individual is similarly examined for six days, with the following serum sodium results.

Units	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
mmol/l	142	140	143	141	142	144

As before, the variation arises from analytical and biological sources.

It can be seen by visual inspection that the two individuals vary, but they have different inherent levels of serum sodium. The BV around their own individual homeostatic set-point is the intraindividual BV; and the difference between the set-point of individuals is called the interindividual BV.

6.4 Determination of components and variation

First, a small but statistically valid group of individuals is selected, ensuring as far as possible that they are healthy, not taking any drugs that could affect the test results, and maintain their usual lifestyle during the period of sample collection. A series of samples will be taken from each subject, with care taken to ensure that the collection procedure and sample handling steps are standardised.

Likewise, analysis must be carefully controlled, with samples from each individual run in a single batch, to minimise analytical variation. Each sample is then reanalysed, giving duplicate results for each sample.

From the duplicate analyses, the analytical imprecision (SD_A) can be calculated, as follows:

$$SD_A = \sqrt{\frac{\text{sum of (differences)}^2}{2 \times \text{no of pairs}}}$$

For each individual, a single set of data is used to calculate the SD, which is made up of analytical and intraindividual BV.

$$SD^2 = SD_A^2 + SD_I^2$$

Note that variance is used (squared SD, or SD^2) as it can be more easily manipulated mathematically. The square root of this is the average intraindividual BV.

The second calculation is designed to measure the average interindividual BV, as follows.

$$SD^2 = SD_A^2 + SD_I^2 + SD_G^2$$

Where SD_G is the interindividual SD. The square root of this SD^2 is the average interindividual BV.

6.5 Magnitude of biological variation

Table 6.2 is an example of some intraindividual and interindividual BV of some serum constituents, expressed as a CV.

Table 6.2

Serum constituent	Intra BV	Inter BV
Sodium	0.7	0.6
Potassium	5.1	4.4
Calcium	1.7	2.2
Urea	13.6	16.9
Creatinine	4.6	10.6
Cholesterol	5.5	14.8
Total proteins	2.7	3.7
Alkaline phosphatase	6.7	23.4
CK total	71.6	67.7

NB: There are many such tables available in the literature. The latest version can be found at ***www.westgard.com***, in articles by Carmen Ricos.

6.6 Biological variation in hospitalised patients

It should be understood that as shown above, BV is calculated on healthy subjects, while most samples coming to us will be from those who are unwell or suspected of being unwell. Fortunately, the few studies that have been performed show that BV applies equally to hospitalised patients. The point can be made that, while the homeostatic set-point will vary between sickness and health, in general the variation itself remains virtually unchanged.
As a result, the data available on BV can be applied validly in the clinical situation.

6.7 Uses of biological variation data

BV data can be used to (i) assess the significance of changes in serial tests on patients, (ii) determine the usefulness or otherwise of patient-based reference values, and (iii) delineate the minimum acceptable performance standards required for lab tests.

Significance of changes

The probability that two test results from a single patient are statistically significantly different can be based on the imprecision (SD) of the test. Table 6.4 below shows the probability of a significant change based on SD.

Table 6.4

Difference between results	Probability that results differ
1.5 SD	< 70%
2.0 SD	< 90%
2.5 SD	< 95%
2.8 SD	= 95%
3.0 SD	> 95%
3.5 SD	> 98%
4.0 SD	> 99%

However, this simplifies the situation somewhat, as the difference between two results is not only due to analytical variation, but also due to BV.

As discussed previously, to be 95% certain those two results differ, the **combined** SD (that which comprises BV **and** analytical variation), must be multiplied by 2.8.

The following table shows the effect of this for some common chemistry analytes, and the analytical SD that has been used is that of an average UK lab.

Table 6.5

Serum analyte	Units	Level	Change required
Sodium	mmol/l	140	6
Potassium	mmol/l	4.2	0.6
Calcium	mmol/l	2.40	0.19
Chloride	mmol/l	100	6
Bicarbonate	mmol/l	26	4
Urea	mmol/l	5.0	2.1
Creatinine	µmol/l	60	21
Urate	mmol/l	0.26	0.07
Bilirubins	µmol/l	10	10
Cholesterol	mmol/l	5.8	1.6
Triglycerides	mmol/l	1.2	0.9
Glucose	mmol/l	4.6	1.6
Albumin	g/l	40	5
Alk Phos	IU/l	60	22
AST	IU/l	20	13
LDH	IU/l	125	57

As an example of the use of such data, consider the results shown below, in Table 6.7, from a 58 year-old male. The results highlighted by an asterix (*) are statistically significantly different from day 1 to Day 2. The results not highlighted in this may do not show a statistical difference between Day 1 and Day 2 results. The fact that results are different does not necessarily mean that the patient is improving or deteriorating.

Table 6.7

Analyte	Units	Day 1	Day 2	Difference
Sodium	mmol/l	140	144	4
Potassium	mmol/l	4.2	4.9	0.7*
Chloride	mmol/l	101	103	2
Bicarbonate	mmol/l	24	29	5*
Urea	mmol/l	6.2	6.8	0.6
Creatinine	µmol/l	105	115	10
Calcium	mmol/l	2.25	2.47	0.23*
Alk Phos	IU/l	82	106	24*

It must be appreciated that the data shown in this section makes several assumptions, namely that the lab achieves this “UK average” precision, and that each patient has the same BV.

NB: Considering that in Africa we do not know either the average lab precision – if such an entity as an average exists – and have no idea if European BV data applies to our various populations here, such assumptions cannot be blindly accepted. However, the concept of knowing your own precision as assistance to those using our data for patient care is essential.

Reference values
A study in 1977 (Pickup et al) showed that although the potassium mean values from individuals always fall within the reference range, it was possible for some results from healthy individuals to fall outside the range. The fact is that we all have our own BV for potassium, and for all other analytes, and sometimes this will cause results to appear “abnormal”.

When the BV within individuals is greater than that between individuals, the use of reference ranges will be more valuable. It can be stated more simply: when results vary little between individuals, we can all then be compared to the same reference ranges more usefully.

It must be understood that the reference ranges we use are a combination of many variables, most notably BV, both within and between individuals, and analytical variation. If a reference range is wide, say creatinine compared to sodium, it shows either a wider BV or a poorer analytical performance.

Laboratory performance standards
It has become more common lately that goals for analytical performance be based on BV, on the premise that analytical imprecision should have a minimal effect of reference ranges. As a result, if BV is small, a better analytical performance is required.

It is generally stated by experts, that the analytical SD should ideally be equal to or less than half the intraindividual BV.

6.8 Summary

Changes take place in numerical lab results for a variety of reasons, and not simply because of changes in the disease state. Knowledge of these changes is essential for collection of specimens at the correct time, as well as to facilitate the better use and interpretation of lab test results.

Most variation is random biological variation (BV) about which a considerable body of data has been accumulated. BV is a useful concept with many applications.

CPD Questions:

1. How would you go about establishing BV for your own laboratory patient population? Prepare a full protocol for implementing this plan.
2. Consider Table 6.2. Why is the inter BV (the between patient BV) smaller than the intra BV (within individual BV) for both sodium and potassium? Does this make it easier or more difficult for reference ranges to be used for sodium and potassium?
3. Compare the BVs from Table 6.2 to the values used by Thistle QA to assess if your results are “Acceptable” or “Poor”. These acceptable ranges are shown on every Thistle QA report. Why do you think some of the Thistle QA acceptable ranges differ from BV?
4. You should know your own lab’s imprecision – the SD you use on your Levey-Jennings, provided of course that you have worked this out for your own lab! Now look at Table 6.5. Work out your own “change required” for some of these analytes and compare your figures to that in Table 6.5. Are there any differences? What does that mean about your lab’s performance?