Biological variation - what's it all about?

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Biochemical and hematological analyses are done in laboratories, clinics, general practices and in point-of-care settings such as ITU. Generally, numerical test results are generated.

Test results vary in individuals over time due to preanalytical variation, analytical imprecision and biological variation. The important question is whether changes seen in patients do infer clinical improvement or deterioration. To interpret serial results in an individual objectively, assessment against reference change values (RCV) is advocated.

RCV are easy to calculate from in-house imprecision and readily available data on biological variation. RCV should be widely available to all who interpret test results. Analytical imprecision influences RCV and, to minimize confounding effects, imprecision should be less than one-half the within-subject biological variation.

Analytical bias also affects test result interpretation when population-based reference values are used. Bias should be less than one-quarter of the group biological variation to allow the same reference values to be used in alternate sites.

It's another busy morning on the Intensive Therapy Unit (ITU). The patient in Bed 2, a 78-year-old man with severe sepsis, has his morning pH, blood gases and electrolytes done in the ITU mini-laboratory by the nurse looking after him.

This point-of-care testing (POCT) approach has been used for some years, the nurses and doctors undertaking the analyses, while the laboratory staff provide quality control and assurance, maintenance, training and advice. The results are captured and the updated cumulative result chart printed out for the ward round: part of the chart is shown as **Table I**.

On the ward round, the Consultant asked one of the junior medical staff for the latest results. The Registrar stated that the sodium was 143 mmol/L and the potassium was 4.2 mmol/L. He also followed this objective numerical statement with the comment that they had both "*risen since yesterday*".

Analyte	09/07/04	08/07/04	07/07/04	06/07/04	05/07/04	04/07/04	Reference Interval
Sodium (mmoL/L)	143	141	139	144	143	140	135-147
Potassium (mmol/L)	4.2	3.5	4.0	4.3	3.7	3.8	3.5-5.0

TABLE I.

The Consultant stated that the patient was stable, had not improved or deteriorated and had not had his therapy changed. He questioned why, then, it was thought that both results had risen.

The Clinical Biochemist attending the daily ward round took the chart and examined the results. He remarked that even casual inspection of the results would show that the variation was random in nature.

The Registrar asked why the results varied over time in patients who were stable and not improving or deteriorating. The Clinical Biochemist explained the nature of test result variation over time as follows, having studied a recent book on the subject [1].

There are a number of sources of variation. The first is the variation that occurs before the analyses are performed. The samples themselves may vary in an individual.

For example, if arterial blood and venous blood were taken from the same patient and glucose or pH and blood gases measured, the results would be different. If venous stasis was applied for different lengths of time, the results for analytes that become more "concentrated" on venous stasis, for example, hemoglobin, would differ from sample to sample.

Another source of test result variation is sample-handling technique. For example, if samples for pH, gases or glucose were taken and then left for varying lengths of time before analysis, the results would vary because the composition of the collected blood does not stay the same – ongoing metabolism results in changes in these parameters with time.

These sources of variation, both in sample collection and in handling, are termed "preanalytical variation" by laboratory professionals because they occur before the analysis is performed.

The Senior Nurse suggested that these sources of variation would not be significant in the Unit because, in conjunction with laboratory staff, Standard Operating Procedures (SOP) had been created for all aspects of sample handling and collection.

In addition, good training of new staff members, and continuous professional development of all staff, meant that these SOP were adhered to at all times.

The Clinical Biochemist agreed that it was vital to minimize preanalytical variation. He then explained that the analysis itself had inherent variation. He also explained that this was easy to see by simple examination of the results of the daily internal quality control (QC) sample analyzed in ITU and all other POCT sites. The QC sample was a single material but the results did differ from analysis to analysis.

This variation was correctly termed "imprecision" and, since random in nature, could be quantitated numerically by calculating the Standard Deviation (SD) or coefficient of variation (CV, calculated as SD × 100 / mean) which gave an objective measure of the magnitude of the dispersion of the results. Laboratory staff regularly calculated the CV of the QC material to see if the analyses were satisfactory or were becoming problematic.

The final source of random test result variation was "biological variation". A simple way of looking at this was to consider that every individual has their own "homeostatic setting point" for every analyte measured and that results, due to normal homeostatic mechanisms, vary in a random manner around this point.

For example, the patient in Bed 2 has homeostatic setting points of 142 mmol/L for sodium and 3.9 mmol/L for potassium. It is also clear that, as expected from

Sodium (mmol/L)	09/07/04	08/07/04	07/07/04	06/07/04	05/07/04	04/07/04	Reference Interval
Patient in Bed 2	143	141	139	144	143	140	135-147
Patient in Bed 4	137	136	137	138	137	135	135-147
Patient in Bed 5	145	146	144	143	143	145	135-147
Patient in Bed 9	140	138	139	138	140	139	135-147

TABLE II.

considerations of physiology, the variation of sodium is less than potassium.

In fact, all analytes have their own characteristic within-subject biological variations and these have, like imprecision, been quantitated in terms of SD and CV. However, unlike imprecision, these do not have to be worked out locally, because they have been very well studied and documented: compilations for hundreds of analytes are available in the literature and on the Internet [2].

The Clinical Biochemist requested identification of some of the other stable patients in the Unit and collated their sodium results: these are shown in **Table II**.

These four patients have different homeostatic setting points of 142, 137, 144 and 139 mmol/L, respectively. The difference among individuals is termed the "*between-subject biological variation*".

For most analytes, the within-subject variation is much less than the between-subject variation [2]. This has very interesting consequences for clinical investigation that are detailed elsewhere [1].

How is it used in everyday practice?

The medical, nursing and other professional staff on the ward round all understood the concept that variation in test results in individuals was due, not only to improvement or deterioration, but also to preanalytical variation, analytical imprecision and within-subject biological variation.

However, they wondered why clinical biochemists had spent so much time and effort generating numerical biological CV for so many analytes.

The Clinical Biochemist replied that numerical data on biological variation had many uses and that these were very well documented [1].

As far as everyday practice in ITU was concerned, there were two main applications, firstly, in deciding on the significance of change in serial results and, secondly, in setting desirable analytical performance targets.

Is a change in serial results significant?

The Clinical Biochemist explained that, since test results do vary because of preanalytical variation, analytical imprecision and biological variation, a change in an individual patient must exceed these sources of variation to be significant.

As already agreed, preanalytical variation can be minimized by adherence to SOP for sample collection and handling and with good and ongoing training. Thus, changes in serial results are significant only if they exceed the inherent analytical imprecision plus biological variation.

This sum, called the "*reference change value*" (RCV), can be easily carried out because analytical imprecision is known from QC analyses, and biological variation can be found in the published database [2].

However, he reminded the group that addition cannot be done by simply adding the CV. Variance, which is CV^2, has to be used in calculations. If the imprecision is termed CVa and the within-subject biological variation

is termed CVb, then the total random variation (CVt) can be calculated.

The total variation is the square root of the sum of the squares of the component variations, $CVt = (CVa^2 + CVb^2)^{1/2}$. To calculate the RCV, CVt is multiplied by $2^{1/2}$ because there are two samples, and then by a factor, conventionally called Z, which is equal to the number of standard deviations appropriate for the probability selected.

Very commonly, $P < 0.05$ is used as "*significant*" and the appropriate Z is 1.96. Sometimes, "*highly significant*" is used, $P < 0.01$, and the appropriate Z is 2.58.

Thus, $RCV = 2^{1/2} \times Z \times (CVa^2 + CVb^2)^{1/2}$, and this is easy to calculate for each analyte. This can be used to assess the significance of changes in serial results in an individual. For example, for potassium, the analytical imprecision in ITU (taken from the QC) is CV = 2.1 %.

The biological variation (taken from the Internet database) is 4.8 %. Thus, RCV for $P < 0.05$ is $2^{1/2} \times 1.96 \times (2.1^2 + 4.8^2)^{1/2} = 14.5$ % – so only changes in potassium greater than 14.5 % are significant. Changes less than this are expected from the inherent random sources of variation.

This calculation could be done for all the analytes commonly performed in POCT settings and a short table placed beside the analyzer. The Clinical Biochemist promised to do this for the analyses done in the POCT setting of the ITU.

However, it was noted that much more sophisticated approaches were possible with modern IT.

The Clinical Biochemist reminded those present that the laboratory actually did this assessment for many analytes and flagged significant and highly significant changes in analytes on both electronic and printed reports with * and **, respectively (see example in reference 1).

He commented that the laboratory also used the RCV in quality management for delta-checking – assessing whether a test result in an individual has changed so markedly that it is likely that a serious error or blunder has been made.

The laboratory also used RCV for auto-validation – electronic reporting, without detailed inspection by professional staff, of results that fall within reference intervals and have not changed significantly.

How good should analyses be?

Earlier discussion noted that all analytical techniques have inherent random variation, the analytical imprecision. An interesting question to the Clinical Biochemist was how low imprecision has to be to facilitate good clinical decision-making.

He noted that there have been many publications in the literature of laboratory medicine on this topic. A consensus conference [3] had followed up a published editorial [4] and strongly recommended that specifications for desirable imprecision should be derived from data on the components of biological variation [5].

One basic concept was that, in clinical monitoring, RCV should be kept low so that changes in test results in an individual were significant. Imprecision is the important analytical characteristic here. Biological "*signal*" should not be confounded by "*noise*" due to imprecision.

Theoretically, imprecision should be less than one-half the within-subject biological variation. If this quality specification is achieved, then only a small amount of noise is added to signal.

The Clinical Biochemist advocated that the data generated on the imprecision of the POCT analyses done in the ITU should be compared against the quality specifications derived from biological variation to see how much "noise" was actually added on a day-to-day basis to the biological "*signal*".

For potassium, since the imprecision was 2.1 %, and the biological variation was 4.8 %, the amount of noise added was indeed small, less than 10 %. As long as

the imprecision was less than 2.4 %, the analytical technique would be satisfactory.

This analysis was done for all the POCT analyses performed in ITU on an ongoing basis by laboratory staff.

The Clinical Biochemist then extended the discussion into general interpretation of numerical laboratory results. He noted that population-based reference values are often used. Since patients often had tests done by the laboratory as well as using the POCT in ITU, the reference values should be the same for both sites.

To ensure that this was so, the laboratory and ITU analytical techniques had to have similar levels of bias. Theoretically, bias should be less than one-quarter of the group biological variation, that is, bias $< \frac{1}{4} \times (CV_{within\text{-}subject}^2 + CV_{between\text{-}subject}^2)^{\frac{1}{2}}$. Again, the data are easy to find on the Internet [2].

For potassium, the bias had to be less than 1.8 % to use the same reference values on the laboratory reports and as interpretation criteria for the POCT in ITU.

The Clinical Biochemist reminded the group that, when the POCT system had been commissioned, paired sample data had been obtained to assess the difference in the results obtained by the two approaches (the comparative bias between the main laboratory and POCT analyzers), and the quality specifications for bias were surpassed.

He also noted that inspection of the IQC values over time was a very useful tool in the ongoing assessment of bias as well as providing a quantitative measure of imprecision.

All agreed that this had been an interesting and educational morning.

References

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