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EDITORIAL

The Importance of Understanding Variation

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The role of the laboratory is to produce results that will be used by clinicians to diagnose disease and monitor response to treatment, but a key issue that is now becoming more apparent is that over ordering of tests can actually lead to patient harm [1]. Inappropriate investigations or treatments which can be invasive may follow results that are imprecise. Clinicians also need sufficient information to identify true change in the patient. For laboratories to reduce the likelihood of these poor outcomes they need to understand not just the imprecision of their assays, but also the variation introduced by collection, biological variation and disease and to communicate these to the clinicians. Laboratory staff must understand not only when to suggest a test may be useful in the differential diagnosis but also when a test should not be repeated because the test results will not be able to be interpreted [2].

Analytical Imprecision

Analytical imprecision contains both pre-analytical factors as well as analytical factors. We use the imprecision of our assays to monitor the performance of the analytical system. The quality control rules used are dependent on the accurate determination of this imprecision as it is used to flag when an assay is not performing satisfactorily. The rules are based on the fact that the error associated with the repeated measurement of a sample, in this case a quality control sample, follows a Gaussian distribution, and hence we are able to predict if a value obtained from a measurement of this same sample falls outside this distribution of expected values. When this situation arises we have an error.

This layer of variation is perhaps the simplest to understand and control as we are monitoring the variation in the repeated measurement of a known sample that follows simple statistical rules. This has allowed us to create many rules that identify the situation where a set of quality control rules occur in a pattern which is statistically unlikely and hence is indicative of a failed run.

Controlling this first layer of variation in the analytical process is one of the major focusses of a laboratory and the way a laboratory is able to successfully control this variation often is the way that external users assess the quality of an organisation. We express this imprecision in the form of an Uncertainty of Measurement and every laboratory should have calculated the UM for each test [3].

External users of laboratories often have to deal with more than one laboratory so they would be aware of variation in imprecision of different laboratories. Even more problematic for a user of several laboratories can be interpreting results from different laboratories on a single patient over time. If different laboratories have different levels of imprecision then a user of those results may misdiagnose on the false understanding that a change in result represents a change in the patient's true situation rather than variation between laboratories. This is a significant problem particularly when some clinicians are not aware of this variation. Patient safety could be and often is compromised when a change in laboratory precision is interpreted as a change in the patient's condition.

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Biological Variation

The next layer of variation we need to understand to interpret results in a patient is biological variation which is made up of age, sex, seasonal and diurnal variation. Biological variation provides us with other useful information as well. It allows us to assess if the imprecision (UM) for an assay is good enough for the assay to be useful clinically. For some analytes such as cortisol and alkaline phosphatase we are well aware of the normal biological variation and we have age related reference intervals or testing procedures that take this variation into account. With some other analytes such as cholesterol, amylase and CRP we are not as aware of the wide variation there is in patients. Seeing a sequential change in a result and being aware that this change is likely to represent normal biological variation and not regression or improvement in a condition is critical to effectively advising on test results. As well as this "normal" variation about a set value for a subject there is also change in many variables which occurs as a part of the ageing process. Cholesterol and PSA increases with age in many societies, whereas eGFR decreases with age. Perhaps these changes reflect underlying disease processes or just ageing processes related to diet and gradual decline. But they are fairly constant and predictable. It is important that PSA and eGFR are interpreted knowing the age of the patient as these will increase in the absence of clinical disease [4]. We can estimate when a result has truly changed or if the difference between two results is due to the combined effects of analytical and biological variation by calculating the Reference Change Value (RCV) [5]. The RCV becomes a valuable tool to interpret change.

The key tool we use to interpret results is the reference interval (RI), but the value of the RI is dependent on the Index of Individuality, which is defined to be intra-individual variation divided by the population variation [6]. When the population variation is large compared to the intra-individual variation, the RI is not as useful. Serum creatinine is an analyte where this is very apparent in that an individual can lose up to 50 % of functional renal mass and remain in the RI. In a case like creatinine therefore it is pertinent to consider more specific RIs such as age and sex or to look at individual change over time.

Variation in Disease

The third layer of variation is the variation that occurs in disease which is in fact the reason why we measure analytes. In the presence of disease some analytes increase their normal variation and indeed in different forms of the same disease the variation may differ, for example creatinine in various forms of renal disease [7]. So in disease states the 'normal' variation seen with an analyte may increase markedly, for example creatinine may vary by up to 15 % in renal disease compared to the variation in a healthy individual of approximately 5 %. Again calculating the RCV with the new disease variation allows us to intelligently interpret whether or not a true change has occurred.

Application of Variation

We cannot interpret a result in isolation we need either a reference interval, a decision point or a previous result on this patient. Decision points are determined externally by use of trials and expert groups but the method used to determine the cut-off may not be the one used in the laboratory where the patient's sample has been referred. The precision of the assay may be different as trials often go to great pains to reduce analytical error by carefully controlling imprecision. For example, let a cut-off value be 6.5 and the error about this value is 0.5. Thus any result between 6 and 7 may truly be either below the cut-off or above it. In this situation the best advice for the clinician would be to repeat the test at some later stage and not diagnose based on the result. Many clinicians would be aware of imprecision, but most would not be aware of how large it can be, particularly if there are other components of variation such as biological and ageing also at play.

Many analytes such as cholesterol, renal function and PSA vary in a predictable way with age [5, 8, 9]. Thus it is possible based on a few estimates and assuming there are no other comorbidities, it is possible to predict a subject's cholesterol, eGFR and PSA some years ahead. This prediction can inform the clinician what is the most appropriate time to retest. More importantly when a patient is on treatment such as a statin or warfarin and they are compliant and stable, it is possible to predict when there will be a true change based on normal variation. In the case of a statin this change may not occur for a number of years [10, 11].

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