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Opinion

From error to uncertainty: A search for variabilities in the laboratory reports

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With the advancement of science and technology the diagnostic modality has been gradually getting integrated with the health care, thereby evolving into a new discipline of lab medicine. Therefore, the role of lab is no more limited to analytical phase only but expanded to clinical decision making and management planning. This demanded better quality assurance in lab reporting. The advent of sophisticated technology in clinical biochemistry lab, the error in analytical phase is drastically reduced, However, the preanalytical including biological variations has emerged as the major source of variations in the lab report. Therefore, definite need has been felt to think beyond the total error paradigm of lab quality control, resulting into the concept of uncertainty which can accommodate all possible components of the lab medicine as a probable source of uncertainty in the lab result. The knowledge and implication of the measurement of uncertainty seems to be challenging for the lab professional as well as the clinicians due to apparent complexity of the statistical methods involved and also for the lack of accessibility and orientation regarding the requisite computer applications. The regulatory guidelines are endorsing uncertainty measurement. Hence, it might be imperative to embrace this system in near future.

Keywords: Bias, Errors, Total error, True error, Uncertainty

Prelude

Case study

A 37-year-old male patient with a history of hypertension presents with a complaint of pruritus, lethargy, and nausea. He also complained of mild oliguria, and pedal edema, weakness in peripheral extremities. On physical exam, the patient was well-nourished but was in moderate distress. His blood pressure was 145/95, pulse 82, respirations 25 and he was afebrile. His biochemical blood investigation results serum urea: 45 mg/dL (17-43 mg/dL), serum creatinine: 1.2 mg/ dL (0.7-1.2 mg/dL), serum uric acid: 6.9 mg/ dL (4.5-7.2 mg/dL), serum sodium: 139 mEq/L (137-145 meq/L), serum potassium: 4.8 meq/L (3.5-5.0 meq/L) and serum chloride: 100 meq/L (97-110 meq/L).

Despite a clinical suspicion of renal pathology, the biochemical investigation did not reflect such indistinctive renal profile parameters; serum creatinine value was within normal limits with only a minor elevation in serum urea level. All the other parameters like serum uric acid, sodium, potassium, and chloride are also within the normal limit. Although the later parameters particularly the electrolytes are not that much expected to change unless a severe renal pathology sets in, however, the reported concentration of serum creatinine, which is an early indicator and cardinal renal marker, being within the normal reference range appears to be paradoxical. To address this issue, the index of individuality was retrieved which is basically represented as variations in the estimation due to biological reasons from different studies, by the European Federation of Clinical Chemistry (EFLM)[1].

The analyte index of individuality (II) of the biochemical parameters are tabulated (Table 1); the example shows that biological variation in the population is much more than the individual variation for creatinine, which is quite below 0.6, a lower limit for considering the reference range to have clinical relevance. Therefore, it is not possible to interpret the clinical condition based on a single measurement value of this parameter. Similarly, urea is also having an II value just around the above-mentioned limit, and hence poses a chance to be misleading.

The existing model of error detection methods focuses on the analytical measurement process and its results. Bias is estimated as the difference between the mean of replicate measurement results and the true

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Table 1 — The analyte index of individuality (II) of the biochemical parameters

Analytes	CV_I	CV_G	II
Urea	13.9	21.0	0.66
Creatinine	4.5	14.1	0.32
Uric Acid	8.3	22.4	0.37
Sodium	0.5	1.0	0.50
Potassium	4.1	4.2	0.97
Chloride	1.1	1.3	0.84

[II = CV_I/CV_G (CV_I is biological variation within subject. CV_G is biological variation between subjects)]

value. In a clinical lab setup, the combination of bias and imprecision which is expressed as the total error, has gained importance. However, multiple factors that may actually influence the measurement results should be accounted for, including biological variation, preanalytical variation, analytical variation, and post-analytical variation[2].

Diagnostic uncertainty of a measurand in laboratory medicine is the combined uncertainty of all such relevant sources of uncertainty when using the measurand for diagnostic purposes.

Evolution: From Error to Uncertainty

In the early 19th century, C.F. Gauss introduced the error paradigm to understand the true information in a measurement result. Errors are classified as systematic and random. Systematic errors can be corrected, but random errors can only be reduced by repeated measurements. However, the true value of a measurement cannot be known exactly due to various factors and actually beyond absolute traceability. This makes it difficult for the error paradigm including Total Error (TE) and Analytical Total Error (ATE) as an oversimplified model to explain an actually rather complex system. This can also lead to misconceptions like equating repeatability and reproducibility with overall measurement uncertainty (MU)[3].

The Westgard concept of Total Analytical Error (TAE) was introduced in 1974, when the major source of uncertainty or the so called 'error' was recognized to be generated from the analytical process relying on mostly manual or semiautomated methods. Based on measured analytical results with defined confidence levels, this concept estimates the limits around a 'true value'. For a given test, it calculates a range of acceptable results by taking both systematic and random errors into account[4]. However, with the advent of high-end instruments and precise methodologies, the analytical component of the testing chain delivers reasonably error-free results, shifting the focus to other components of the diagnostic process for possible error inputs that might affect the outcome. There is also another problem that bias and imprecision are incompatible types of error. The bias is measured as a vector quantity, while the precision is measured as a standard deviation. These two values add up to create TE as a scalar value (one sided linear distance from the 'true value'), which makes it difficult to estimate exact total errors. In addition, the TE method relies largely on multiple replicate observations of a single measurand during the analytical phase only. Due to this limitation, this approach is gradually becoming less reliable, particularly because as just mentioned above, with the technical advancement in the analytical process, the error probability from this phase has greatly been reduced. Therefore, error detection process focused on this phase become relatively redundant. A recent account of the distribution of contributions from different phases of the diagnostic testing process categorically displayed a very meagre share of the analytical error as opposed to those generated from other phases[5]. This highlights the significance of a more widespread account of the whole process in a more holistic manner.

Therefore, to take an effort towards such transition, an error-based approach to uncertainty and uncertainty measurement was published in 1993/1995, called the Guide to the expression of uncertainty in measurement (GUM). With the GUM approach, U + b represents the expanded uncertainty with assumed bias, while b represents the assumed bias of zero. This proposition is directly analogous to the total error as described by Westgard and others, incorporating both a bias and an imprecision component. Uncertainty in the reported value entail both the uncertainty due to random errors and the uncertainty due to any requisite corrections for systematic errors (usually that is assumed to be zero for a standardized process; b=0). Manufacturers can use this method to identify errors in analytical systems, improve analytical systems, and establish traceability[6].

Various aspects including pre-analytical and post-analytical, biological, matrix, and interference were incorporated as source of uncertainty during the 2008 GUM and Bayesian statistics was considered in the probabilistic approach[7]. Finally, in 2012,

the "Expression of Measurement Uncertainty in Laboratory Medicine" was published; and it is the only global guidelines for the determination of measurement uncertainty. This guideline suggested a "top-down" approach that utilizes intermediate-term quality control data to estimate measurement uncertainty. This approach simplified the determination of measurement uncertainty for laboratories and is in alignment with ISO 15189 guidelines[8]. However, this method is tedious and tend to overestimate, hence may be less practical. Therefore, a more realistic bottom-up modelling approach integrating all the components has been contemplated. Although it is apparently simple, however, the expanded uncertainty demanded comprehensive evaluation of each steps in the analytical process and also derivation of a mathematical model to include the component inputs and its specific association with the ultimate output variation. Hence, the GUM did not receive very welcome acceptance due to practical difficulty for its regular use in medical laboratories. Interestingly in 1988, one error estimation method attempted to actually evaluate uncertainty in alkaline phosphatase isoenzymes by selective inhibition through Monte Carlo simulation (MCS) method. The basis for the evaluation of uncertainty of measurement in MCS exploits the propagation of distributions through a mathematical model of measurement[9]. Complexities of uncertainty calculations involving technically demanding mathematical procedures can be evaded by standard spreadsheet applications and hence can be applied for most medical laboratory setup using a readily available spreadsheet like Microsoft Excel. This development has paved the way of popularization of the uncertainty paradigm into practice.

However, still the overall status is inclined towards the total error-based quality assurance system in the traditional way due mainly to an inertia vested onto the existing internal quality control system to assess the imprecision and external quality control schemes to detect any emergence of undesirable bias. In contrast to this well-known and rather simpler frequency based statistical methods used in total error deduction, the requisite of the probabilistic with possible Bayesian statistical principles in uncertainty calculations still kept it in abeyance for popular use.

Errors *vs*. uncertainty

A conflict between the conventional and the reformist approach is almost eternal. However, besides this simple temporal and developmental distinction, there is need to understand the basic tenet and objective of these two methods before selection or rejection to make it a mere replacement of the old method by the new one.

Measurement uncertainty (MU) methodology has the main advantage of combining uncertainty estimations from different sources to estimate the uncertainty of a larger process. Combined uncertainties from pre-analytical, analytical, and post-analytical phases could be used to estimate the uncertainty of the whole diagnosticprocess[8]. In the pre-analytic phase, the most important consideration is the individual biologic variability of a patient, which can be really large for some analytes. For example, the biologic variability for cholesterol can be as high as 6% compared to a typical method coefficient of variation (CV) of 2-3%[10]. This highlights the importance of taking patient-specific factors into account when evaluating measurement uncertainty. The initial example of the clinical vignette regarding the issue of the individual versus group variation, echoes the same fact.

Total analytical error (TAE) model, which focuses solely on the analytical phase, considers only bias and imprecision, but does not consider the sources of variation present in the pre- and post-analytic phases, which can lead to an underestimation of the total measurement uncertainty. Therefore, the MU methodology is a more comprehensive approach[11]. In contrast with error methods which depend on a true value, uncertainty methods do not question the existence of a true value, but it definitely questions its ambiguity due to obvious lack in the exact knowledge about the true value. Error methods employ the simple addition of orthogonal variances. However quite justifiably simple addition of variances from different sources with varied probability distribution can’t be appropriate, rather more sophisticated methods of Law of Propagation, as used in uncertainty calculation, is needed. Moreover, by nature the statistical method for describing bias and imprecision employs a simple frequentist approach by a usual gaussian with central tendency and measures of its dispersion respectively. On the other hand, the concept of uncertainty defines the observed value to be a conditional probabilistic function of all its variates with a defined limit of confidence, obviating any dependence on a hypothetical true value. While, the MU seems to enjoy a clear advantage over TE premise from rationalistic viewpoint, it is also to be

remembered that both these methods have different objectives and thus has distinct target. MU is more comprehensive and encompasses all the components of the diagnostic chain and hence for obvious reason it has more application in lab medicine directly providing the information of total possible discrepancy in the reported value of a measurand for the clinician. On the contrary, the total error provides succinct yet complete feed-back of the analytical procedure to the lab personnel to take appropriate action.

Another aspect of the disagreement is that although ought to be clinically more useful, the uncertainty measurement is quite unfamiliar to the clinicians and at times can prove to be more perplexing than the total analytical error and the allowable error which is rather simpler to realize for regular use.

Measurement Uncertainty: The process

Uncertainty defined by the GUM is ‘‘a parameter, associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand’’[12]. This parameter is usually a standard deviation, or the width of a confidence interval. This uncertainty can be expanded by a factor (e.g., 1.96): this is interpreted as an interval in which the value of the result of a measurement resides with a defined probability. As explained before, this method has the inherent benefit of recognizing and accounting for all sources of uncertainty and provides a detailed understanding of the measurement process, so as to take appropriate rectification measures, if any.

The non-empirical, realistic bottom-up approach in measurement uncertainty is a method to determine the uncertainty of a measurement result by identifying all the individual sources of uncertainty linked to the final results. This is done by first determining the model equation for the measurand, then creating an uncertainty budget, and combining all uncertainties through error propagation. The process involves establishment of a functional relationship expressed as a mathematical equation for describing the measurand (output) as a function of the relevant inputs. When we get the input uncertainty estimate derived statistically from serial repeated measurements like internal QC dataset, then it is ascribed as Type A evaluation with an expected normal or gaussian probability distribution. Whereas, type B evaluation refers to the uncertainty estimate that has been obtained from an authentic report or a calibration certificate. This type of evaluation is usually represented as a rectangular probability distribution unlike the former. However, a reported Type B uncertainty based on earlier repeated measurements, may also be considered as a special case designated as ‘fossilized’ Type A uncertainty with an assumed normal distribution[10]. These types of distributions of the various input uncertainties should all be expressed as standard uncertainties and not as expanded uncertainties. The expanded uncertainty refers to an additional 95% coverage interval as a good approximation.

For medical laboratory applications with respect to uncertainty in measurement, normal, Student’s ‘t’, the rectangular and symmetrical triangular distributions are particularly relevant. When the number of observations is relatively small, the Student’s ‘t’ distribution with its associated degrees of freedom should be considered. The ‘t’ distribution applies in situations where high-accuracy analysis is undertaken with a limited number of observations. A rectangular or uniform probability function is used as a model in situations where the probability of obtaining any value between two stated limits is equal to the probability of obtaining any other value between these limits[13].

As mentioned above, MCS provides a practical and arguably a better alternative to the GUM modelling approach and is a suitable tool for assessment of total uncertainty. Modern computational advancement provides widely available spreadsheet software for utilization in most laboratory medicine applications. In contrast to the theoretical modelling, MCS may be regarded as experimental statistics. The MCS procedure uses algorithmically generated random numbers which are then steered towards a prescribed probability distribution. For each input, the MCS procedure creates a random numeric value drawn from its respective probability density function; a single numeric value is generated as output from all such inputs having known functional relationship. This process is reiterated for several times so as to produce a set of simulated results as output; the mean and standard deviation of these output results are designated as respective values of the measurand and its standard uncertainty. In contrast to the original GUM procedure which only results to develop the basic skeleton (‘forms the bare bones’; the mean and standard uncertainty of the measurand), the MCS

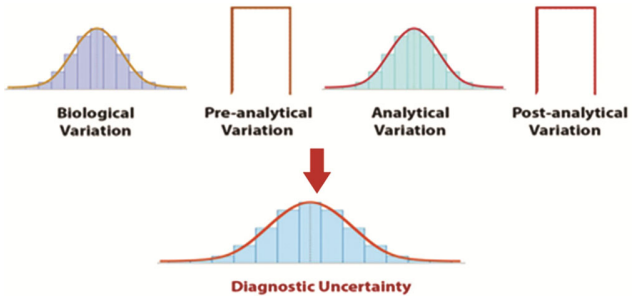

Fig. 1 — A schematic view of the output derived by MCS platform showing various components of uncertainty in the diagnostic chain with their respective probability distribution functions and their integration through the law of propagation to generate the total diagnostic uncertainty output as a gaussian distribution

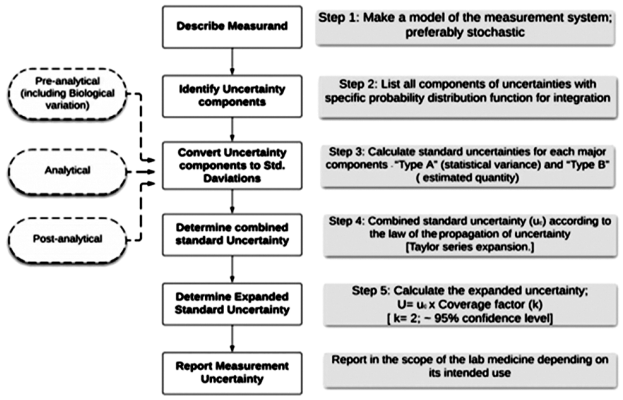

Fig. 2 — Modelling protocol to be followed in laboratory to incorporate all possible sources for measuring total probable uncertainty.

procedure provides the body shape ('adding fleshes further'; the complete distribution with an output dataset) developing the actual probability density function of the measurand which contains enriched information (Fig. 1). Since MCS procedure performs random sampling from the predefined probability function of the input variables and integrate them into a simulated output dataset with any required coverage interval (e.g., for medical laboratory applications with a normally distributed measurand, this is typically 95% for a coverage factor of 1.96), hence the overall process might be considered as propagation of distributions. Moreover, all nonlinearities in the functional relationship are taken into consideration in the MCS procedure and for correlated inputs, combined simulation of a bivariate distribution is provided by this procedure. The modelling protocol to be followed in laboratory to incorporate all possible sources for measuring total probable uncertainty (Fig. 2). Finally, the requisite of intricate mathematical skills is largely reduced by use of simplified computational process.

Translational potential

As explained above, that emergence of diagnostic modality as an integral part of patient management process demands its role and establishes it as Lab Medicine. Hence, every component of the diagnostic lab service should be taken into account for possible source of error[14]. The currently popular total error paradigm, being based on analytical error only, fails to accommodate other potential source of errors particularly the most significant component of pre-analytical including the otherwise under-represented biological variations. Therefore, in clinical perspective, uncertainty measurement can provide more decisive edge and thus has obviously better translational impact to add more certainty in medical management system. It can be envisaged that to complement the modern concept of precision medicine there is direct need for a commensurate precision lab medicine which is only possible with overall uncertainty assessment[15].

As stated by William Osler, Medicine is a science of uncertainty and an art of probability. Therefore, medical diagnosis demands knowledge and skills in medicine along with the process to recognize and address the uncertainties[16]. The error-based method is currently in practice because it can be cost-effectively estimated by simple measurements of a control sample in laboratory, despite the fact that the uncertainty estimate is more comprehensive and definitely better than the error detection approach involving the analytical phase only. However, the error approach and the uncertainty approach are rather complementary for an appropriate evaluation of the diagnostic report; errors in the measurement are the integral component of the entire sources of measurement uncertainty[17].

Conclusion

The impact of the value addition by the Law of propagation and Bayesian calculations has not yet been fully explored in lab medicine. Towards this end, there is need for further empowerment of the personnel through awareness and orientation and provision for the access to the digital advancement through availability of better computing facility in the lab. In future, quite justifiably to meet the need of better and more holistic diagnostic and health care

management, the uncertainty approach must be embraced. Therefore, it is very important to explicitly recommend and clearly categorize both error and uncertainty approaches with their specific purposes in the Revised versions of the Guide to the expression of Uncertainty in Measurement (GUM) and International Vocabulary of Metrology (VIM) guidelines.

Conflict of interest

Authors declare no competing interests.

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