Mini-Review

Calculated Chemistry Parameters – do they need to be harmonised?

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Abstract
In clinical chemistry, harmonisation of the testing process is a global initiative with the purpose of improving patient safety, allowing better integration of research data and enabling the use of national electronic heath records. In Australia, as in other countries, the initial focus has been on the harmonisation of the more commonly measured analytes. There are also a number of calculated parameters, derived from these measured analytes, which could also be considered for harmonisation. Calculated parameters that are reported by laboratories and used for clinical decision-making should undergo the same robust process of harmonisation as is the case for the measured analytes. Aspects that should be considered for harmonisation are: terminology, the formulae used and where possible the use of common reference intervals. To investigate pathways towards the harmonisation of calculated parameters, three commonly reported parameters are considered. Calculated osmolality, the anion gap and albumin-adjusted calcium are all derived from common analytes which have individually been considered for harmonisation. They present different methodological, measurement uncertainty and terminological hurdles to harmonisation and are likely to require different pathways and solutions.

Introduction

Why Harmonise?

Since 2012, the Australasian Association of Clinical Biochemists (AACB) has held annual scientific workshops to assess the viability and support for harmonised reference intervals for common biochemical analytes. Laboratories are now operating, or soon will be, in the era of the electronic health record where both clinicians and patients may question unexplained reporting variations between laboratories.

Some analytes have proven straightforward to harmonise using available evidence, including method traceability data, external quality assurance (EQA) information, a priori reference interval studies, expert review and data mining of laboratory patient databases.[1] Other analytes, although still considered important, were more challenging and set aside for additional investigation and review.

Harmonisation - Consistency in reporting improves patient safety

Miller et al. have emphasised the need for agreement in results between laboratories: "Results between different clinical laboratory measurement procedures (CLMP) should be equivalent, within clinically meaningful limits, to enable optimal use of clinical guidelines for disease diagnosis and patient management".[2] Any failure in this process can result in wrong clinical, financial and technical decisions as well as an inability to properly aggregate data from research trials.[2] As a result there has been a concerted effort globally to harmonise the testing process. Although originally the focus was on the standardisation of the analytical methods,[3] there has been a recent widening of the lens with an acknowledgment of the importance of the total testing process, including pre- and post-analytical phases.[4,5]

The reporting of calculated parameters, derived from measured parameters, are a part of the post-analytical phase of the testing process.[5] Numerous different formulae may be used for the calculations.[6] However the formulae and reference intervals may not necessarily be appropriate for the analytical methods used.[7,8]

Calculated parameters like measured parameters are reported by laboratories and, importantly, they are also used by clinicians for clinical decisions.[9,10,11] It follows that the same rigorous approach used to harmonise the measured parameters

should be followed for the calculated parameters.

The International Consortium for Harmonization of Clinical Laboratory Results, an initiative of the American Association for Clinical Chemistry (AACC), has produced a toolbox for the process of harmonisation of a measurand.[12] Briefly it outlines the following process:

- Determination of the measurand
- Assessment of the properties of the measurement system
- Assessment of the applicability of harmonisation
- Consideration of the benefits, risks and the will to harmonise
- Implementation of post-harmonisation assessment

For calculated parameters the same process can be used. The consideration of the formula used is equivalent to the first step (determination of the measurand). Many formulae may be in use for a single calculated parameter. Whatever the reason for this may be, the decision to adopt a single standard formula should involve a robust decision process based on good evidence. The next two steps in the process involve assessment of the properties of the individual measuring systems and potential for harmonisation of the component measurements. Ultimately, the question of whether to harmonise a calculated parameter rests on these steps and the clinical utility of doing so.

A fundamental tenant of the harmonisation process is to aim for initial consensus on key issues. Harmonisation of any parameter is also an opportunity to seek input from clinicians on their perceptions on utility and optimal reporting. Consensus is most likely where laboratories and clinicians collaborate to own the agreed outcome.

Contentious issues may need to be set aside for future investigation and discussion, but where possible that process must not slow the elimination of illogical or dangerous variations in reporting that have been identified. For some calculations, various formulae exist, each with their pros and cons. The reason for different formulae indicates that none are perfect. It may be difficult to decide on a preferred formula, especially if this decision needs to be based on scientific principles.

Common Reference Intervals

The ultimate goal of harmonisation is the implementation, where possible, of harmonised reference intervals. Differing reference intervals may compromise patient care, create difficulties when integrating research data, inhibit the creation of electronic record keeping and add to the expense of assay implementation for individual laboratories.[13]

Inter-laboratory comparison programs, using commutable material, such as The International Measurement Evaluation Program (IMEP) and the bias program of the Royal College of Pathologists of Australasia (RCPA) and the AACB have shown that inter-laboratory biases within Allowable Limits of Performance should not be an impediment to the use of common reference intervals for many analytes.[14,15]

It seems reasonable that if common reference intervals are applicable to all the component analytes of a formula then the same should be possible for the calculated parameter. It is important to note, however, that with calculations the inaccuracies of the various components are additive, and this may result in an unacceptably large cumulative inaccuracy.

A Way Forward

A possible pathway to harmonisation of calculated parameters may consist of the following:

- Definition of the parameter
- Consensus for the terminology and reporting format used
- Selection of a common formula based on evidence and consensus
- Consideration of a common reference interval
- Implementation of a common reference interval if possible
- Progress assessment

As a starting point in this edition of the Clinical Biochemist Reviews (CBR) two articles investigate the harmonisation of three calculated parameters. There are of course many more parameters but the following three serve as good examples. Choy et al. propose the harmonisation of the formula for calculated osmolality whilst Hughes et al. discuss the possibility of harmonised reference intervals for the three calculated parameters: calculated osmolality, anion gap and adjusted calcium.

Anion Gap

The anion gap reflects the presence of unmeasured anions in serum and is primarily used in the investigation of acid-base disorders although this is not its sole use.[10] Two formulae can be used for the calculation of the anion gap:

1. Sodium + Potassium – Chloride – Bicarbonate
2. Sodium – Chloride – Bicarbonate

The second formula reflects the small absolute variation in potassium concentration in healthy patients. The RCPA Manual states the reference interval for the anion gap without potassium to be 4 to 13 mmol/L and 8 to 16 mmol/L with potassium.[16] As both formulae are commonly used, a consensus approach may be best in harmonising to the use of one formula.

Albumin-Adjusted Calcium

Although automated analysers measure total calcium concentration it is the ionised calcium that represents the biologically active fraction.[17] This can lead to misinterpretation in hypo- and hyperalbuminaemic states since albumin is the major calcium binding entity.[18] For this reason many laboratories provide an albumin-adjusted calcium (or 'corrected' calcium) alongside the total calcium result. Albumin-adjusted calcium is perhaps the preferred term since under certain conditions (acid-base disorders or severe hypoalbuminaemia) the "corrected" calcium may be misleading. Clinical Biochemistry Reviews and the Annals of Clinical Biochemistry have started to use the terminology "albumin-adjusted calcium" although the Standardisation of Pathology Units and Terminology (PUTS) project of the RCPA continues to use the terminology "calcium corrected for albumin".[1,19,20] It would be desirable for the terminology to be harmonised.

The following formula to adjust the total calcium is used frequently by laboratories and clinicians:

Albumin-adjusted calcium = Total Calcium – 0.02 x (40 – Albumin)

This formula was developed with albumin measured using the dye binding bromocresol green (BCG) method. Albumin is also measured using bromcresol purple (BCP), another dye binding method that is more specific for human albumin resulting in slightly lower values. A number of studies have shown that the above formula may not be applicable for laboratories measuring albumin with BCP.[21,22] These studies indicate that a slope of 0.012 or 0.015 respectively would be more accurate when using the BCP method. This would suggest that harmonisation would be limited to method-specific formulae.

Calculated Osmolality

Over several decades, there have been multiple attempts and publications proposing new formulae for calculated osmolality primarily for the purpose of assessing significant presence of toxic alcohols and other osmotically active substances. The formulae range from a simple '2 x sodium' to complicated formulae involving multiple analytes, factors and constants to account for unmeasured osmotically active substances in normal serum. As will be described in a separate article in this edition of CBR, the Smithline-Gardner formula [calculated osmolality = 2(Na) + Glucose + Urea] gives calculated osmolality close enough to measured osmolality in various patient cohorts. The formula is simple, applicable across most major analysers, and amenable to either rapid bedside or automated laboratory information system (LIS) calculation.

Uncertainty of Measurement

The National Institute of Standards and Technology (NIST) has outlined that "a measurement result is complete only when accompanied by a quantitative statement of its uncertainty. The uncertainty is required in order to decide if the result is adequate for its intended purpose and to ascertain if it is consistent with other similar results."[23] The uncertainty of measurement (UM) is defined by ISO 15189 as "a parameter associated with the result of a measurement that characterises the dispersion of values".[24] Imprecision is the major component of UM. For harmonisation, the accuracy (and not analytical imprecision) of the assays is relevant. With calculations the inaccuracies of the various components are additive, and this may result in an unacceptable large cumulative inaccuracy (the opposite may also be the case). Thus, while the individual assays may each be acceptably standardised (frequently not perfect), the calculations (with their cumulative bias) may not be. This cumulative bias may also negatively impact on harmonisation of the reference intervals. As in the case of the harmonisation of reference intervals of individual assays, calculations will have to be closely compared and reviewed in practice. On the other hand, the (large) cumulative analytical imprecision may allow harmonisation of intervals irrespective of the potential larger bias.

Conclusion

For the anion gap and calculated osmolality there do not appear to be any significant obstacles to the harmonisation of their respective formulae. With sufficient evidence there may also be a case for common reference intervals. It is likely that for albumin-adjusted calcium there may need to be method-based harmonisation until such a time as there is uniformity in the methods used to measure albumin.

As suggested in the introduction, there are many more calculated parameters used in clinical chemistry reports. Parameters such as the estimated Glomerular Filtration Rate (eGFR) are already well harmonised in practice. However, eGFR has only one test variable. As previously mentioned, the potential cumulative bias increases with each additional test variable in the calculation. Calculations based on one assay that is harmonised should, by implication also be harmonisable. However, this does not apply to "multiple assay formulae" such as Free Androgen Index (FAI) because of the effect of cumulative bias. It is our belief that, as with measured parameters, there should be a thorough approach to the harmonisation of all calculated parameters in use.

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