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Pregledni članak

CERTAINTY IN AN UNCERTAIN WORLD – A CLINICIANS' VIEWPOINT OF SENSITIVITY AND PRECISION

IZVESNOST U NEIZVESNOM SVETU – STAV JEDNOG KLINIČARA O SENZITIVNOSTI I PRECIZNOSTI

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Summary: Clinical practice is evolving as research evolves from the bench to the bedside. Similarly, analytical technologies are improving on an annual basis. Rightly or wrongly, increased emphasis is now placed by clinicians on such investigations to the detriment of clinical history and examination. As people live longer, the prevalence of long-term conditions such as thyroid disease, cardiovascular disease and malignancies is increasing. Clinical biochemistry assays play an important part in the management (screening, diagnosis, prognosis and monitoring) of such conditions. This is reflected in the UK since 2004 by the primary care contract where over 100 of the 550 clinical points depend on clinical biochemistry assay results. Inter-assay results may differ due to bias, precision, assay specificity and assay sensitivity. To date, little emphasis has been placed on the potential clinical effect of precision. This presentation will explore the effect that assay precision can have on the management of important long-term conditions such as thyroid disease, cardiovascular disease and malignancies.

Keywords: functional sensitivity, imprecision, prostate specific antigen, thyroid stimulating hormone, troponin, AU3000i

Kratak sadržaj: Klinička praksa evoluira kao i istraživanja. Slično tome, analitičke tehnologije bivaju unapređene svake godine. S pravom ili ne, kliničari sve više pažnje pridaju takvim ispitivanjima na uštrb kliničke istorije i pregleda. Kako se ljudski vek produžava, u porastu je i prevalenca dugotrajnih stanja kao što su bolest štitne žlezde, kardiovaskularna oboljenja i maligniteti. Testovi zasnovani na kliničkoj biohemiji imaju važnu ulogu u tretiranju (skriningu, dijagnostikovanju, prognozi i praćenju) takvih stanja. U Ujedinjenom Kraljevstvu je to postalo očigledno 2004. godine, od kada u primarnoj nezi preko 100 od 550 kliničkih zaključaka zavisi od rezultata testova iz oblasti kliničke biohemije. Rezultati se mogu razlikovati između testova usled odstupanja, preciznosti, specifičnosti i senzitivnosti testa. Danas se malo značaja pridaje potencijalnom kliničkom efektu preciznosti. Ova prezentacija ispitaće efekte koje preciznost testa može imati na tretiranje važnih dugotrajnih stanja kao što su bolest štitne žlezde, kardiovaskularna oboljenja i maligniteti.

Ključne reči: funkcionalna senzitivnost, nepreciznost, prostata specifični antigen, tireostimulirajući hormon, troponin, AU3000i

Introduction

Clinical practice is evolving as research evolves from the bench to the bedside. Similarly, analytical technologies are improving on an annual basis. Rightly or wrongly, increased emphasis is now placed by clinicians on such investigations to the detriment of clinical history and examination. As people live longer, the prevalence of long-term conditions such as thyroid disease, cardiovascular disease and malignancies are increasing. Clinical biochemistry assays play an important part in the management (screening, diagnosis, prognosis and monitoring) of such conditions. This is reflected in the UK since 2004 by the primary care contract where over 100 of the 550 clinical points depend on clinical biochemistry assay results.

Inter-assay results may differ due to bias, precision, assay specificity and assay sensitivity. To date, little emphasis has been placed on the potential clinical effect of precision. This short communication will explore the effect that assay precision can have on the

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management of important long-term conditions such as thyroid disease, cardiovascular disease and malignancies.

Spencer et al. (1) were one of the first to introduce the concept of functional sensitivity for TSH measurements in order to clarify the lowest amounts of TSH that assays could reliably measure. This introduced the use of total precision as a way of differentiating a meaningful value from noise. Functional sensitivity has been applied to numerous other assays, such as Troponin, PSA, Estradiol and others, in order to provide a way of ensuring manufacturer's claims are standardised and low values have a degree of reliability. In late 2004 the CLSI introduced a specific guideline (2) that proposed a process for estimating three important concepts – limit of blank, limit of detection and limit of quantification. It is important to note that the EP-17 guideline does not specify the goal for uncertainty, this is left to the manufacturer or user so this should be questioned when comparing assays. This guideline is now being required by the Food and Drug Administration (FDA) for USA regulatory clearance of assays. It is therefore likely that this new terminology will be increasingly used by manufacturers especially for new assays, thus users will need to familiarise themselves with these new concepts.

The estimation of limits of blank, detection and quantification heavily depend upon precision measurements. Imprecision underpins all measurements and naturally the higher the imprecision, the less certainty of trueness is attached to the result. Metrologists are well aware of this and have recommended that the cumulated uncertainty is calculated and reported as an adjunct to the result (ISO 15189 and VIM); so far this has not been introduced by many (or even any) laboratories as it is widely believed that this would cause more confusion than it resolves.

In clinical practice, there are numerous situations where the result of a clinical biochemistry test is heavily relied upon and subsequently the result has dramatic consequences. Some examples of these are TSH, Troponin and PSA although this list is not exhaustive and many more exist.

All of these analytes have »established« critical decision points at low levels. In TSH the term »undetectable« is often used although 0.15–0.2 mIU/L is the classical lower limit of the reference interval. For PSA 4 μg/L is the currently accepted cut-off in the screening use of this assay although multiple workers have attempted to change this. During the monitoring of patients post treatment extremely low levels are reported and any alteration in the levels is significant and can result in alteration of the management of the patient. However, changes in PSA concentration are important and thus even small changes need to be seen with clarity to facilitate appropriate decision making. The precision of a PSA at low levels is equally critical to that seen around the cut-off.

Another example is troponin, an analyte unique to the world of clinical biochemistry. Never before has a single assay had such clinical emphasis placed upon it. An elevated troponin level is a clear indication of myocardial damage (although not its cause) and invariably results in some form of clinical intervention or further assessment. More importantly, a false negative result can lead to inappropriate discharge of the individual and the consequences of this are often unfavourable. The cut-off for this assay has been widely debated and today is inextricably linked to its precision profile (IFCC). Many assays today have upper limits of normal (99th percentile) that exceed the limit of quantification, thus there is effectively a grey zone where interpretation of a result is dubious (3).

Of course, we can never forget in an immunoassay that there are a number of factors that directly influence the result. Heterogeneity of the analyte has lead to the requirement for equimolarity, especially in PSA (free v. complexed) and troponin (ITC, IC and free forms), standardisation is often complex and even the introduction of international standards there are large differences between methods and often commutability is less than ideal. There are numerous interferences awaiting the unwary, HAMA, heterophilic antibodies, auto-antibodies, sample collection devices or additives, drugs, endogenous substances, to name but a few. All of these factors can directly influence a result and mislead the clinician (4–6).

Thyroid Stimulating Hormone

In a recent study, we sought to statistically compare the TSH functional sensitivity using the Olympus 3000i and the Bayer Advia Centaur.

We conducted an evaluation of the functional sensitivity of the ADVIA Centaur and the Olympus AU3000i by plotting the total CV as a function of the TSH concentration. Single reagent lots were employed for each analytical platform and n = 18. Using the best fit reciprocal curve derived on MS Excel, the concentrations corresponding to a CV of 20% were obtained for both analysers (*see Figures 1* and *2*). The F-test was also employed to statistically compare the precision profiles of the assays.

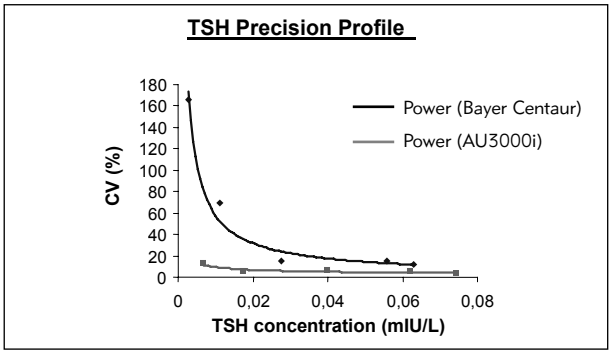

Figure 1 TSH precision profile. TSH, thyroid stimulating hormone.

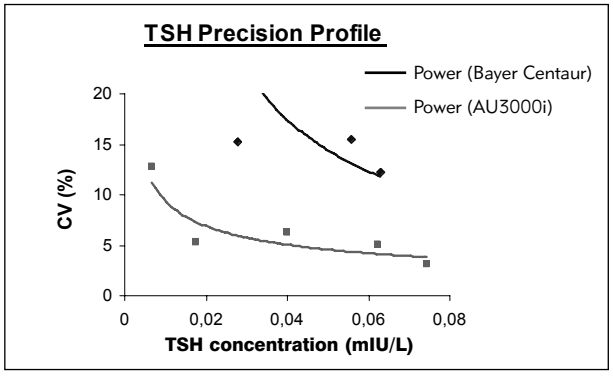

Figure 2 TSH precision profile. TSH, thyroid stimulating hormone.

The functional sensitivity for the AU3000i was 0.0019 mIU/L while the cut-off for the ADVIA Centaur was 0.0341 mIU/L. Using the F-test, $p < 0.01$, and thus the precision profiles were statistically significant with $p < 0.0002$ for all 5 pools.

Precision rises significantly below the functional sensitivity of TSH assays. According to the 2006 UK guidelines (7) Laboratories should use a TSH method with a functional sensitivity of <0.02 mIU/L as »the measurement of TSH using an assay with a functional sensitivity of <0.02 mIU/L is a desirable early stage in the diagnosis of hyperthyroidism«. This is especially important when diagnosing hyperthyroidism in non-thyroidal illness, pregnancy and when optimising thyroxine replacement therapy.

In this study we concluded that the Olympus AU3000i but not the ADVIA Centaur meets the UK specifications.

It is evident that more sensitive assays such as the Olympus AU3000i should provide a more reliable distinction between the profoundly suppressed serum TSH concentrations of primary hyperthyroidism and suppression thyroxine doses in thyroid malignancy from the less pronounced suppressed serum TSH in typical thyroxine replacement therapy or euthyroid patients who present with non-thyroidal illness.

Prostate Specific Antigen

For Prostate Specific Antigen a similar situation exists. Stamey (8) discussed at length the confusion caused by detection nomenclature and proposed the residual cancer detection limit (RCDL) as a way of comparing true clinical detection limits. However, the measurement of this requires specific highly defined patient samples, which requires informed consent and thus is not widely performed. The EGTM reviewed the impact of precision on results around the cut-off by examining the effect of within individual biological variation (9) another form of precision that has a direct bearing on the interpretation of an individual's result. This imprecision in conjunction with analytical variation complicates the interpretation of results around the established cut-off of 4 μg/L (10). The effect of biological variation is often overlooked and can have profound consequences, the most notable being CRP which has been hotly debated for the use as a cardiovascular risk marker. The large variation of this analyte in vivo means that single measurement of CRP may have little value and multiple measures are required to accurately establish a baseline.

An important application of PSA is the monitoring of patients post prostatectomy where very low levels are expected, functional sensitivity (as defined for TSH) is critical and the inter-assay CV of 20% again is used to determine the lowest reliable estimate of PSA. At present the evidence is equivocal as to whether measuring extremely low levels (<0.4 μg/L) although there is some evidence to suggest certain treatments may be most effective whilst the PSA level is very low. In a recent yet unpublished study the inter-assay precision profile of the Olympus AU3000i PSA assay was studied by analysing serum pools at a range if very low concentrations persisted over 20 days. Using the best fit reciprocal curve derived on MS Excel, the concentrations corresponding to a CV of 20% were obtained (see *Figures 3* and *4*). This study produced a functional sensitivity in the order of 0.0012 μg/L at a CV of 20% and whilst these extreme low levels are still debatable, the overall precision of this assay down to 0.01 μg/L is better than 5%, which ultimately means these low levels are being measured with a high level of confidence, thus any alterations in PSA at these levels are detected with a high degree of confidence.

Taking into account the biological variation, it is considered that a clinically significant change in serially collected samples is between 20 and 30% (11–13). Whether this applies to such very low levels remains to be seen, but newer generation assays are now able to see these levels with increasing clarity and thus the certainty of these measurements are increased. The danger is that patients who see these results may be alarmed by a PSA changing from 0.2 μg/L to 0.04 μg/L as this is a doubling of the PSA levels and is often

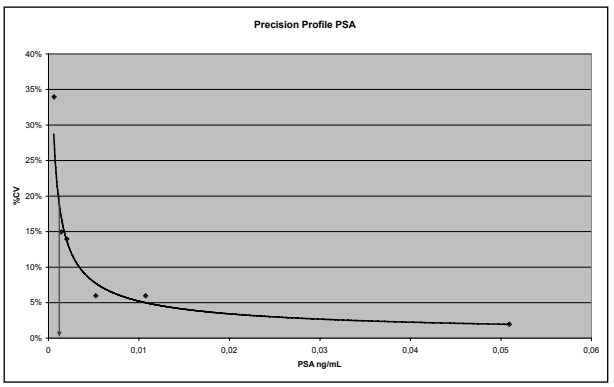

Figure 3 PSA precision profile. PSA, prostate specific antigen

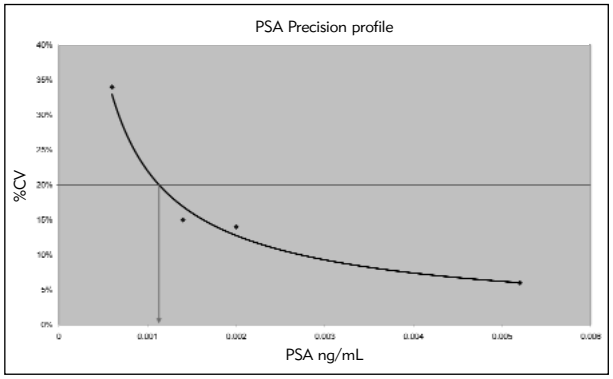

Figure 4 PSA precision profile. PSA, prostate specific antigen.

used as a diagnostic measure. These low values, whilst analytically valid, require consideration clinically before reporting routinely.

This discussion can continue for numerous other analytes where low levels are significant, Troponin, CRP, PTH and so on. The introduction of guidelines to harmonise the way detection limits are set is necessary, however the EP-17 guideline does not specify the goal for uncertainty, this is left to the manufacturer or user (EP).

In conclusion, the concept of precision and sensitivity is not new, however as technologies improve and reliance on results becomes more absolute, laboratorians and clinicians need to be extremely vigilant when introducing new systems or methods. Reporting and interpreting results especially at very low concentrations in assays such as TSH, PSA and troponin should be carefully considered and communicated. Clinicians should be made aware of the limitations of methods and clinical laboratories must communicate with users of this service and supply information about the uncertainty of their results of measurement when applicable; this information may be attached to each patient's result, may be contained in the user's handbook or may be available on request. Complete and informative reporting and interpretation can only lead to better decisions in healthcare (14).

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