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Relevance of analytical and biological variations to quality and interpretation of test results: examples of application to haematology

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Summary. - In the frame of a comprehensive quality system, the level of quality to be attained (analytical goals or quality specifications) need to be defined. Among the several approaches used for such a definition, that based on the (intra-individual) biological variation seems to be scientifically sound, and the most universally applicable. Data on biological variation are easily generated by applying specific experimental protocols. Once the biological variation is known, it can be combined to the analytical variation for the calculation of the critical difference. This is the difference between two repeated measurements in the same patient, due to the effect of both analytical and biological variation: when this difference is exceeded, a significant change in the patient's condition can be assumed. Some examples of calculation of the analytical goals and of the critical differences are shown, based on some quantities usually measured in the haematological laboratory.

Key words: analytical variation, biological variation, analytical goals, critical difference.

Riassunto *(Rilevanza della variabilità analitica e biologica ai fini della qualità e della interpretazione dei risultati di laboratorio: esempi di applicazione alla ematologia).* - Nell'ambito di un sistema di qualità completo, esiste la necessità di definire il livello di qualità che deve essere raggiunto per soddisfare determinate esigenze cliniche (traguardi analitici o specificazioni di qualità). A tale scopo si sono seguiti differenti approcci; tra di essi, quello basato sulla variabilità biologica (intra-individuo) appare il più solido scientificamente, nonché quello più universalmente applicabile. I valori di variabilità biologica per le differenti grandezze sono generati abbastanza facilmente mediante l'applicazione di protocolli sperimentali specifici. Quando i valori della variabilità biologica sono noti, essi possono anche essere utilizzati, in combinazione con quelli della variabilità analitica, a generare le differenze critiche. Queste rappresentano le differenze tra due misure consecutive nel medesimo paziente ancora imputabili all'effetto combinato delle due fonti di variabilità. Quando la differenza critica è sorpassata, si è verificata una variazione significativa delle condizioni del paziente. Vengono riportati alcuni esempi di calcolo dei traguardi analitici e delle differenze critiche per alcune grandezze comunemente misurate nel laboratorio di ematologia.

Parole chiave: variabilità analitica, variabilità biologica, traguardi analitici, differenze critiche.

A model for analytical quality

In the frame of a "quality system" designed to cover all aspects of laboratory work, aiming at assuring the production of the service in a "total quality" environment, the control of analytical quality still deserves considerable attention. In order for the overall service-producing process to be cost-effective the level of analytical quality to be achieved must be consistent with the expected use of the laboratory results. In this respect, a model for achieving the desired (or needed) level of analytical quality will be based on the cyclic interaction of three major elements [1]: a) specifications (of analytical quality); b) creation (of analytical quality); c) control (of analytical quality).

Ideally, at the start of the process (e.g. when introducing a new test in the laboratory routine), the cycle can be imaged to start from the specifications of the analytical quality, in order to fulfill the specific requirements of the medical situation requesting the information. Then, the quality nearest to the specified level is created, by proper selection of measurement methods, and an adequate "control" system is implemented. If the information thereby generated point to analytical performance not fulfilling the specifications, action is generated towards the "creation" phase; alternatively, the cycle is maintained as an ongoing procedure, to monitor the stability of the analytical process.

In the following discussion, the "specification" phase will be considered.

Defining the specifications

If information about the required performance is not available, quality can only be judged in comparison with other analytical procedures, in comparison with the performance at other analytical sites (e.g. other

laboratories), or according to a subjective feeling of what is good and what is poor. By contrast, defining the goals for a stated (medical or analytical) purpose, and defining the quality specifications accordingly, seems to be a more logical process. The concept that quality specifications are a necessary prerequisite for running a cost-effective service is not new, and for many years efforts have been devoted to the definition of such specifications [2-9]. The different approaches that have been followed can be grouped under three main headings:

- analytical approach;
- clinical approach;
- biological approach.

Briefly, in the analytical approach, the median quality achieved by a stated group of laboratories, or by a core-group selected among them, is taken as the quality level to be attained. In the clinical approach, the opinion of the clinicians about the difference between two measurements significant for a change of the patient's conditions is used as the basis for the calculation of quality specifications. Alternatively, the opinions of groups of experts (consensus conferences) are applied. In the biological approach, the quality specifications are anchored to objective biological quantities pertaining to individuals or populations, like reference intervals, and biological inter- and intra-invidual variation. Advantages and drawbacks of the several approaches have been discussed [6-10]. Although there is no absolute consensus, the biological approach is generally considered to be the most applicable and objective, and will be discussed here.

Analytical and biological variations

In a stated system at a given time any measurable quantity has a "true value", which is unknown. The measurement procedure gives an estimate of such value, and the difference [(estimate) - (true)] is the analytical error, which includes a random component, and is responsible for the analytical variation ($V\underline{a}$). Furthermore, in any living organism, the value of each quantity at any given moment is the result of a steady-state equilibrium, which is set at a given homeostatic point. The value of the quantity itself oscillates around the homeostatic point, and this oscillation is the intra-individual biological variation ($V\underline{i}$). Therefore, any point-measurement can be regarded as an estimate of the homeostatic set-point, which is influenced by both the analytical and the biological variations. The difference between the estimate and the homeostatic point is the total error, which also includes a random component, and generates the total variation of the estimate ($V\underline{t}$), given by:

$$V\underline{t} = (V\underline{a}^2 - V\underline{i}^2)^{1/2}$$

Analytical goals based on the biological variation

Biology-based analytical goals can be calculated and applied to all the quantities, pertaining to the several sub-disciplines of laboratory medicine (e.g. clinical biochemistry, haematology, microbiology), that are measured and expressed in numerical form, as continuous variables [11]. The use of biology-based analytical goals has been mainly advocated in the field of clinical biochemistry [12, 13], but applications in the field of haematology have also been reported [14-18].

Before further discussing this biological approach, it is necessary to consider briefly the different medical contexts in which laboratory tests results are used rationally. Schematically, these may be considered to include [19]:

- single patient testing, to confirm or exclude illness;
- repeated testing in a patient, for monitoring purposes;
- testing a population, for epidemiological work.

It is generally recognized that the second situation (monitoring) is the one requiring for the most stringent analytical quality specifications. Therefore, this situation will be considered as a model for developing analytical goals: it may be speculated that if the requirements for monitoring are met, those for the other medical applications of laboratory tests also are. It is unlikely that, in most situations, a laboratory will measure the same quantity with different methods, characterized by different analytical quality, according to the expected use of the results.

Clearly, the point is to maintain $V\underline{t}$ as low as possible, in order to make any estimate as close as possible to the "true" homeostatic set-point being assessed, thereby minimizing the influence of the analytical quality on the medical decision. Assuming that the influence of all the so-called pre-analytical sources of variation has been minimized by adequate standardization, no action is possible to reduce the $V\underline{i}$, which is an intrinsic characteristic of each individual. The only way left to reduce $V\underline{t}$, is to lower the $V\underline{a}$ to a level where it does no longer add significantly to the $V\underline{i}$. It is easy to show that if $V\underline{a}$ is equal to half $V\underline{i}$, then $V\underline{t} = 1.12 \times V\underline{i}$. In other words, if such condition is fulfilled, the total variation is only about 10 % higher than the biological variation [5,11]. This is considered acceptable; in fact, Fig. 1 shows that even if $V\underline{a}$ is well below this limit, there is no substantial decrease of $V\underline{t}$, whilst for higher values of $V\underline{a}$, $V\underline{t}$ steeply increases. Therefore, the condition:

$$V\underline{a} \leq 0.5 \times V\underline{i}$$

is considered a realistic analytical goal, based on biological variation, applicable universally.

For the practical implementation of these biology-based analytical goals, it is necessary to know the values of the biological variation. Experimental protocols have

been suggested for the production of biological-variation data [5], and numerous applications to various quantities have been reported [20-23]. The protocols applied by the several researchers differ slightly in some details, with particular reference to the time-base [23, 24], and the sample-groups of subjects enrolled in the published assessments are not completely homogeneous. Nevertheless, the reported values for the biological variation often show an acceptable agreement. An additional observation is that the intra-individual biological variation shows differences, somewhat considerable, among the different individuals: patient-specific analytical goals for each quantity should therefore be considered. This is clearly impractical. However, if it is considered that analytical goals are target for improvement but are not inflexible limits [25], the use of some kind of weighed mean value, or of some other mathematical approach for estimating average biological variation values, mediating inter-individuals and inter-protocols differences of estimate, may be acceptable compromises [23, 26, 27].

Table 1 shows some analytical goals, based on biological variation, for quantities frequently measured in the haematological laboratory.

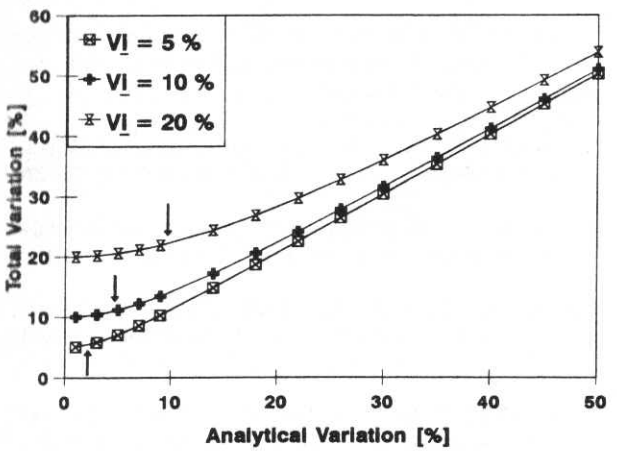

Fig. 1. - Total variation (Vt) as a function of analytical variaton (Va) for three values of intra-individual biological variation (Vi). The arrows show the analytical goals, where Va = 0.5 x Vi.

The critical difference

When two consecutive test results from a single patient are compared, the medically significant information is whether any observed difference between the two measurements has an acceptably high probability to indicate a change in the homeostatic set-point of the specific quantity (due to modification of the physiopathological state or to the influence of therapy). Since the two measurements are estimates, as mentioned above, they may differ simply by the combined effect of the biological and analytical variations, even in the absence of any real change of the homeostatic set-point. The critical difference (Dcr), also referred to as the reference change [26], is the highest difference between two consecutive measurements, that, at a chosen level of probability, may still be due to the combined effect of the analytical and biological variations [5, 11]. It is given by:

$$Dcr = k \times (Va^2 + Vi^2)^{1/2}$$

where k depends on the chosen probability. At p = 0.95:

$$k = 1.96 \times 2^{1/2} = 2.77$$

Even if the analytical variation is 0, the critical difference may still show a fairly high value, since it equals 2.8 times the biological variation; if the analytical variation is adjusted at the goal (0.5 x Vi), the critical difference equals 3.1 times Vi, which represents only about 10 % increase. However, if the ratio Va/Vi increases above 0.5, a steep increase of the critical difference over the biological variation is observed (Fig. 2).

Table 1. - Analytical goals (%) based on intra-individual biological variation for some haematological analytes

Analyte	Reference				
	17	18	14	15	16 (*)
Haemoglobin	1.2	1.2	0.0	1.2	1.4 - 1.3
Haematocrit	1.3	1.1	0.0	1.3	1.4 - 1.2
Erythrocyte count	2.1	1.0	0.7	-	1.2 - 1.0
Leukocyte count	6.7	4.7	4.5	7.8	5.8 - 5.5
Platelet count	3.9	4.9	0.0	3.3	5.0 - 4.0
MCV	1.1	0.4	-		0.6 - 0.6
MCH	0.8	0.6	-		0.4 - 0.4
Lymphocyte count	5.4	2.2	5.4	5.5	4.7 - 4.7
Monocyte count	7.0	12.3	8.2	8.1	7.2 - 4.6
Neutrophil count	10.9	7.8	3.2	6.2	8.7 - 9.7

(*) separate values for males and females, elderly

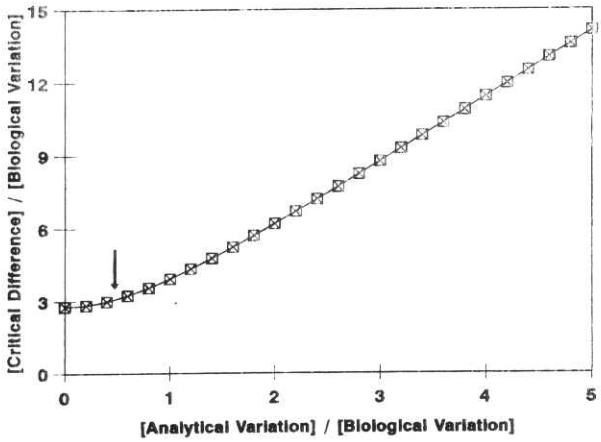

Fig. 2. - Critical difference as a function of analytical variation (Va), both normalized to the biological variation (Vi). The arrow shows the analytical goal (Va = 0.5 x Vi): if the analytical variation increases above this value, the critical difference shows a steep rise.

Table 2. - Critical differences for some haematological analytes, calculated on the basis of mean values for the biological variation, and for analytical variation values corresponding to the analytical goals

Analyte	Mean biological variation (*) %	Critical difference (**) %
Haemoglobin	2.10	6.50
Haematocrit	2.10	6.50
Erythrocyte count	2.40	7.43
Leukocyte count	11.67	36.13
Platelet count	7.03	21.78
MCV	1.35	4.18
MCH	1.20	3.72
Lymphocyte count	9.30	28.80
Monocyte count	15.80	48.93
Neutrophil count	17.53	54.30

(*) mean values from Table 1
(**) calculated for Va = 0.5 x Vi

Table 2 shows the values for the critical difference of some haematological quantities.

Much of the discussion about the analytical variation refers mainly to imprecision, in the assumption that either the inaccuracy is zero, or the repeated measurements are taken under stable analytical conditions, were the (unknown) inaccuracy is constant over time. However, a similar reasoning may apply in the presence of a known bias, to take care of the total error [10]. Also, it should be considered that calculation and use of the critical difference, as mentioned above, refer to pairs of measurements. When accurate estimation of changes is relevant to health care, the effect of the biological variation on the uncertainty of the measurements can be reduced by increasing the number of repeated sampling. The appropriate number of sampling for achieving the desirable accuracy can be established on the basis of biological variation [28, 29].

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