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Review Article

Reference Intervals (RIs), Normal Values, Health Status and Clinical Decision Limits (CDLs), the Theory and the Practice

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Abstract

There was a clear distinction between healthy reference values measured in healthy populations or individuals and patient reference values measured in patients having various diseases. It is now commonly accepted that reference values describe fluctuations observed in healthy populations or individuals, which makes the definition of health or characterization of health status a critical step.

From 1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, in which it was recommended that each laboratory follow defined procedures to produce its own reference values. Although there were very important developments and implementations between the 1990s and 2008; the C28- A3 guideline, published in 2008 by Clinical and Laboratory Standards Institute (CLSI) and IFCC constituted the most significant step in the development of RIs and is still in current use

From its inception, and according to IFCC definition, reference values are measured in a well-characterized population of individuals selected according to predefined criteria such as age, sex, nutritional status, and diet. In addition, it is presumed that reference individuals are healthy, which raises the question of the definition of health. There is no accepted consensus on the definition of health.

The RIs are descriptive of a specific population and are derived from a reference distribution (usually 95% interval), whereas CDLs are thresholds above or below which a specific medical decision is recommended and are derived from Receiver Operating Characteristic (ROC) curves and predictive values. CDLs are based on the diagnostic question and are obtained from specific clinical studies to define the probability of the presence of a certain disease or a different outcome. These limits lead to the decision that individuals with values above or below the decision limit should be treated differently. CDLs are defined by consensus and vary among different populations. It is important that RIs are not confused with CDLs.

To avoid confusion, the C28-A3 recommended reporting decision limits or RIs but not both, with a clear indication of which has been used.

Keywords: Reference Intervals (RIs); Normal Values; Health Status and Clinical Decision Limits (CDLs)

Introduction

In the mid-20th century, Grasbeck and Fellman published a paper entitled 'Normal Values and Statistics' as an initial study in the field of reference intervals (RIs) [1]. This was followed by a presentation by Grasbeck and Sais on 'Establishment and Use of Normal Values'; intended to replace the more ambiguous concept of normal values and to ''establish a well-defined nomenclature and recommended procedures in the field [2,3].'

In this first publication [1], there was a clear distinction between healthy reference values measured in healthy populations or individuals and patient reference values measured in patients having various diseases. It is now commonly accepted that reference values describe fluctuations observed in healthy populations or individuals, which makes the definition of health or characterization of health status a critical step. In subsequent years it was realized

that the terminology of 'normal values' was not adequate, so the term 'reference values' came into use.

From 1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, in which it was recommended that each laboratory follow defined procedures to produce its own reference values [4-9]. Although there were very important developments and implementations between the 1990s and 2008[10-13]; the C28- A3 guideline, published in 2008 by Clinical and Laboratory Standards Institute (CLSI) and IFCC constituted the most significant step in the development of RIs and is still in current use [14].

This guideline entitled 'Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory' provides the necessary steps mainly for the selection of reference individuals, pre-analytical and analytical considerations, and analysis of reference values for a RI establishment study. In the C28-A3 guideline, in order to perform a multicenter RI study, criteria need to be satisfied described with the topics (i.e. a priori selection of reference subjects, clear definition of the pre-analytical phases, demonstration of traceability of results and standardization, and well-defined quality control program with clear criteria) [14]. In recent years, knowledge additional to the Guideline has come from the multicenter RI studies, especially those conducted by IFCC. RIs are derived from reference distribution, usually of 95% interval, and describe a specific population.The classical cascade is defined from reference individuals, a reference sample group, reference values, reference distribution, reference limits and RIs. The reference individuals form the reference sample group for measurement of the values from the reference population. Through statistical analysis of the distribution of the obtained values, the reference limits are calculated. These limits then define the RI [4].

The selection of reference individuals using a sample questionnaire is explained in detail in the CLSI/ IFCC document, C28-A3 [14].

Health is a relative condition lacking a universal definition. The designation of good health and determination of normality for a candidate reference individual may involve a variety of examinations, such as a history and physical and/or certain clinical laboratory tests. The exclusion and partitioning criteria can be implemented appropriately through a well-designed questionnaire. Ideally, RIs are determined on the basis of a healthy population using direct methods [15].

However, indirect methods, which are also known as data mining, based on previous laboratory data can also be useful [15]. Various methods may be used for the selection of a group of healthy individuals from a general hospital population and reference values are calculated from hospital data using statistical methods, such as Bhattacharya analysis [16] and some modifications of the method [17,18]. Pre-analytical and analytical aspects must be taken into consideration in the implementation of a RI study. Generally, the pre-analytical considerations involve biological (i.e. sampling time in relation to biological rhythms, fasting or non-fasting and physical activity) and methodological factors (i.e. sample collection techniques, type of additives, with or without tourniquet and sampling equipment, specimen handling, transportation, time and speed of centrifugation, and storage conditions).

For reproducibility and standardization, it is essential that the pre-analytical aspects are accurately defined and described as the preanalytical phase is known to have the highest errors in the total test process [19]. Because of the importance of harmonizing pre-analytical phase of the total testing process, an effort has been made by the European Federation for Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE) to support the worldwide harmonization of color coding for blood collection tube closures. EFLM, WGPRE believes that such harmonization would reduce pre-analytical errors and substantially improve patient safety [20,21].

Analytical aspects include the analytical variability of the method used for the measurement, equipment/ instrumentation, reagents, calibration standards, and calculation methods. Different commercial methods may be used in a trueness-based approach to the reference measurement system providing results traceable to the system and thus, comparable results can be produced in clinical laboratories. When performing a RI study, the reference measurement systems and standard reference materials are of great importance to ensure traceability of the test results in comparisons [22].

In the IFCC publication in 1987 (136) it was recommended that reference limits should always be presented together with their 90% confidence intervals (CIs). The CI is a range of values including the true percentile (e.g. the 2.5th percentile of the population) with a specified probability, usually of 90% or 95%, as the 'confidence level' of the interval. In the C28-A3 guideline, non-parametric Cis are given from the observed values corresponding to certain rank numbers from Reed et al. [23] Although one can theoretically determine 95% RIs with a lower number (as few as 39 samples), it is clearly recommended that at least 120 subjects are required to calculate the CIs of the lower and upper RIs in this guideline [13].

Horn and Pesce [24] proposed a 'robust method' method based on transformation of the original data according to Box and Cox [25] followed by a 'robust' algorithm giving different weights to the data, depending upon their distance from the mean. This method can provide the reference limits from a limited number of observations using only 20 subjects [26]. However, a robust method with such a small number of reference subjects (e.g. N = 20) cannot

provide an acceptably narrow set of confidence limits. A small number of subjects can lead to uncertainty of calculated reference limits revealed by the width of its CIs. To calculate the 90% CIs around the limits, it is possible to use 'the bootstrap method' which is a 'resampling' method and creates a 'pseudosample' from the data. The RI is derived from each pseudosample and the process is repeated many times (1000 - 2000) yielding a distribution of lower and upper RIs [24].

From this distribution, 5th and the 95th quantiles may be used to determine the 90% CI for each limit. A critical drawback of this approach is that the 90% CIs can be very wide if the sample size is small (at least 80 individuals are needed to obtain acceptably small 90% CIs) [27].

The C28-A3 guideline allows for subjective validation of a RI by laboratory assessment of population demographics and pre-analytical and analytical parameters. This guideline recommends that each laboratory adopts existing RI values by performing an analysis to validate the transference of a RI reported by a manufacturer or other donor laboratory. The acceptability of the transfer may be assessed by examining a small number of reference individuals (N = 20) from the receiving laboratory's own subject population and comparing these reference values to the larger, more adequate original study [14].

If no more than 2 of the 20 samples (or 10% of the test results) fall out of the range of the existing RI, it may be adopted for use, at least provisionally [14]. If more than 2 of the 20 samples fall outside these limits, a second 20 reference specimens should be obtained. If no more than 2 of the 20 samples fall out of the range of the existing RI, it may be adopted for use. If three or more again fall outside these limits, the user should re-examine the analytical procedures used and consider possible differences in the biological characteristics of the two populations sampled [14].

Reference intervals (RIs), health status and clinical decision limits (CDLs)

It is now commonly accepted that reference values describe fluctuations observed in healthy populations or individuals, which makes the definition of health or characterization of health status a critical step. From its inception, and according to IFCC definition, reference values are measured in a well-characterized population of individuals selected according to predefined criteria such as age, sex, nutritional status, and diet. In addition, it is presumed that reference individuals are healthy, which raises the question of the definition of health. There is no accepted consensus on the definition of health.

The RIs are descriptive of a specific population and are derived from a reference distribution (usually 95% interval), whereas CDLs are thresholds above or below which a specific medical decision is recommended and are derived from Receiver Operating Characteristic (ROC) curves and predictive values [28]. CDLs are based on the diagnostic question and are obtained from specific clinical studies to define the probability of the presence of a certain disease or a different outcome. These limits lead to the decision that individuals with values above or below the decision limit should be treated differently. CDLs are defined by consensus and vary among different populations. It is important that RIs are not confused with CDLs [29].

To avoid confusion, the C28-A3 recommended reporting decision limits or RIs but not both, with a clear indication of which has been used. Lastly, reference interval can be distinguished by accurate and reliable reference intervals of a clinical laboratory testing [30]. Reference interval is crucial for disease screening, diagnosis, monitoring, progression and treatment efficacy. Clinical chemistry reference intervals are also important tool for identifying abnormal laboratory results and ultimately guiding patient management decisions [31] (Figure 1).

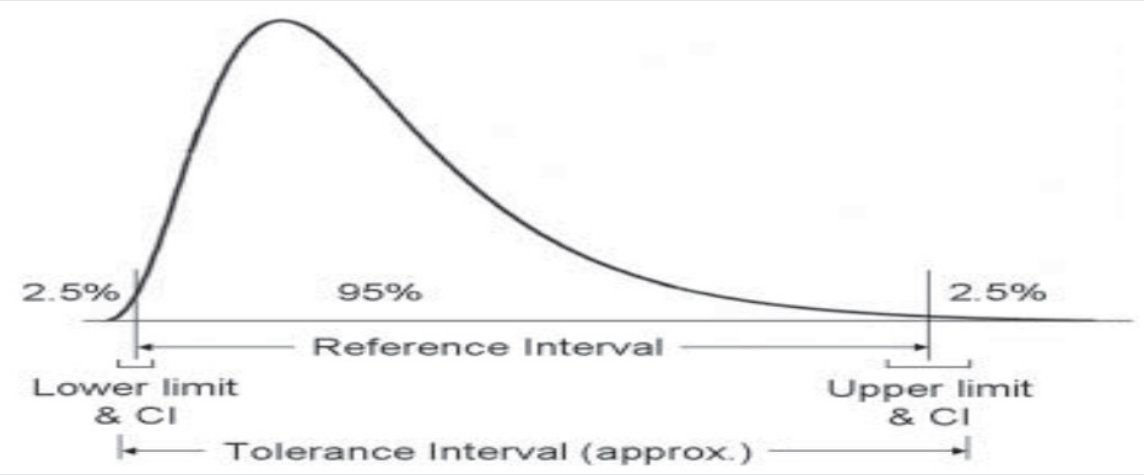

Figure 1: Reference Interval Normal Distribution (usually 95% interval)) [27].

Reference intervals are typically established by assaying specimens from a sample group of people who meet carefully defined criteria [32-35]. Reference interval is usually defined as the values encompassing the central 95% of specimens, equating to 2 standards deviations on either side of the mean [31,36]. Producing reference intervals for a general population is a major challenge, as it requires selecting the appropriate reference population and recruiting individuals who represent relevant demographic groups that meet the inclusion criteria; collecting, processing and testing specimens; and finally, calculating reference values with possible stratification of the data into subgroups.

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