

Review Article

Pediatric Reference Intervals for Clinical Laboratory - Challenges and Opportunities

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Abstract

In hematopathology and biochemistry laboratories, reference intervals are usually provided along with numeric test results, to assist clinicians to interpret patient's test results. To establish reference intervals, the recruitment of at least 120 healthy reference individuals per partition group is usually required, in order to achieve an acceptable statistical confidence. This poses particular challenges for pediatric population as the establishment of reference intervals should take into account age, gender, and ethnic origin, the variables affecting reference intervals in children. As a result, initiatives with multi-center involvement are currently in progress in the United States, Canada, and European countries. In such studies, both pre-analytical factors and analytical processes should be clearly defined and closely monitor to assure valid test results and reliable reference intervals are produced. Further validation is required for the transference of a published reference interval to assure its validity to be used by a different laboratory.

Reference intervals - definition and requirements

Reference intervals are values that assist clinicians to interpret their patient's laboratory test results. In the field of clinical pathology, reference intervals are usually required when the test results are provided as numeric values, such as in hematopathology and biochemistry laboratories. Current guidelines define reference intervals as the lower and upper limits, within which 95% of results from apparently healthy children would fall. Based on guidelines published by the Clinical Laboratory Standards Institute (CLSI) a single reference interval study performed de novo requires the recruitment of at least 120 healthy reference individuals per partition group, in order to achieve an acceptable statistical confidence [1]. The possibility that separate intervals are desired for defined subclasses of subjects, for example different age groups, should be considered before the actual process of securing and analyzing subject specimens.

Pediatric reference intervals - additional considerations and challenges

Because child development and growth profoundly influence the reference intervals for many of the disease biomarkers measured, the establishment of reference intervals should take into account age, gender, and ethnic origin [2,3]. One can easily appreciate the significant undertaking in terms of time, resources, and costs to each laboratory in order to abide by this requirement for each and every analyte measured. This

is especially difficult for younger pediatric population which usually has rapid physiological changes, and is also due to the impact of repeated blood draw on their health.

To establish reliable pediatric reference intervals, it is important to carefully consider pre-analytical variables that are difficult to control in children, such as health status, fasting or not fasting, sampling time in relation to biological rhythms, sampling and phlebotomy technique. Although it is preferable to recruit healthy volunteers to participate in such studies, it often becomes a challenge, especially for younger children, due to the invasive nature of the study. An alternative approach is to recruit children admitted in the hospital for minor surgical or medical conditions, which have no or minimal impact on the analytes being studied. However, partition criteria for reference individuals should be well defined based on their health status and the potential impact on the study. Additional statistical tool may be needed to further inspect the data, for example using mixture analysis [4]. In addition, phlebotomy for infants and children is technically challenging, and the technique and collection sites may differ among institutions. Thus, the proper training should be provided to the staff involved in order to standardize the sampling collection process.

Collaborative opportunities and the need to standardize the study and analysis process

Collaboration among institutions is particularly desirable for pediatric reference interval studies. First, it allows expansion

of patient pool from multiple centers in order to meet statistical requirement. Second, valuable patient samples could be analyzed across various instrument platforms to provide instrument-specific reference intervals and benefit larger patient population. This requires that patient partition criteria, sample collection procedure, and sample storage conditions are optimized and standardized within the study group. Furthermore, quality control processes should be closely monitor to assure the validity of testing method and comparability of results.

Current status and progresses

The clinical laboratories world-wide have been actively involved in the establishment of pediatric reference intervals. In Canada, CALIPER (Canadian Laboratory Initiative on Paediatric Reference Intervals), is a nation-wide initiative to establish a comprehensive database for both traditional and emerging biomarkers of paediatric disease [5]. In addition to many publications, CALIPER also make the pediatric reference intervals available at <http://www.caliperdatabase.com>. In the United States, National Health and Nutrition Examination Survey (NHANES) is a program of studies designed to assess the health and nutritional status of adults and children in the United States. Although the survey results are available at http://www.cdc.gov/nchs/nhanes/about_nhanes.htm, the data are not provided in reference interval format which means further analysis is required to utilize the data. And, certain tests and age groups are not included in their database. In the United States, there is also the "Children's Health Improvement through Laboratory Diagnostics" initiative. This initiative has an extensive list of biomarkers under their assessment. The targeted age groups vary based on the tests, ranging from 1 to 17 years of age [6]. In Europe, the German Health Interview and Examination Survey for Children and Adolescents (Ki-GGS) was started in 2003 and the data collection for KiGGS Wave 1 came to an end in June 2012 [7]. Nordic Reference Interval Project (NORIP) is a Nordic project with the goal to recommend serum/plasma reference intervals for common quantities used in clinical chemistry, and the pediatric reference interval is an critical component of the project [8].

Clearly, as more age, gender, and even race specific pediatric

reference intervals become available, clinical laboratories will have easy access to reference intervals appropriate for their patient population, with the transference of a reference interval reported. CLSI guideline requires that a user or receiving laboratory must ensure comparability of the reference population and pre-analytical/analytical variables, by examining a smaller number of reference individuals ($n = 20$) from the receiving laboratory's own patient population [1]. If no more than two of these new results fall outside the reported reference limits, the latter may be considered acceptable for use in the receiving laboratory. However, if three or more again fall outside the limits (or if five or more in the original set fall outside the limits), the user should reexamine the analytical procedures used, consider possible differences in the biological characteristics of the two populations sampled, and consider whether the receiving laboratory should develop its own reference interval.

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