Bob Barrett: This is the podcast from *'Clinical Chemistry,'* I am Bob Barrett. Defining pediatric reference intervals is not an easy task. The continuously changing physiology of growing children makes their laboratory values a moving target. One has to consider the inter-individual variation, not only of the homeostatic point, but also of the timing with which the development of the individual occurs and the first year of life and puberty are the most critical periods. In addition, ethnic and behavioral differences may also cause variations. The only way to deal with such complexity is to gather large populations of reference individuals. However, the ethical problems involved in obtaining specimens from a healthy newborn or child further complicate this already demanding effort.

In the May 2012 issue of *'Clinical Chemistry,'* a paper published by a Toronto-based group led by David Colantonio sheds new light on pediatric reference intervals. In the accompanying editorial published in the same issue, Dr. Ferruccio Ceriotti, from the San Raffaele Hospital in Milan, Italy, provided a careful evaluation of the study and came away impressed.

Dr. Ceriotti is our guest in this podcast. Dr., why is there so much interest in reference intervals?

Dr. Ferruccio Ceriotti: Okay, as Horn and Pesce stated years ago, reference intervals are probably the most frequently used medical decision tool. They are not the only tool and far less than perfect, but certainly represent a first indication of the health status of an individual.

Until few years ago, but still nowadays, each laboratory was requested to define its own reference intervals. This activity is expensive and increasingly difficult, an Institutional Review Board approval is needed and, due to rapidly changing technology, it becomes a never ending issue.

Moreover, the ISO Standard 15189 requires a documentation of the source of the reference intervals in use and their continuous monitoring.

Performing this check, many clinical laboratories realized that not all the reference intervals in use were obtained according to scientifically valid approaches.

Also, manufacturers are now more concerned about the problem because the European Directive on in vitro medical devices put under their responsibility the definition of reference intervals.

Finally, the higher mobility of the patients and their use of Internet resources put in evidence the great heterogeneity of reference interval among different laboratories and raised the question if these differences were always justified.

Bob Barrett: Dr., which are the most critical aspects in the definition of reference intervals and why are they so difficult to obtain?

Dr. Ferruccio Ceriotti: Certainly, the most challenging problem is the recruitment of a sufficient number of individuals. IFCC and subsequently CLSI defined the magic number of 120 individuals.

To make a long story short, this number represents the minimum number of data required to calculate the 90% confidence interval around a limit when using nonparametric techniques.

Most of the distributions of reference intervals, are non-Gaussian. So the nonparametric approach is the only one that does not require complex mathematical data transformation.

Calculation of 90% confidence interval is important because it allows understanding the uncertainty around the limits and consequently, the degree of trust that we have in them.

If the distribution is highly left skewed and we want to obtain a low uncertainty around the upper reference limit, we will need far more individuals.

Moreover, due to the need for partitioning by gender and age, this number has to be multiplied by 2, 4, 10, or even far more, if ethnicity or life habits introduce differences.

Bob Barrett: Why are pediatric reference intervals so problematic?

Dr. Ferruccio Ceriotti: The problem is strictly connected to the previous one.

When physiological concentration of a certain analyte is continuously changing with the growth of the child, you will need an incredibly high number of individuals to define correct reference intervals for the different ages. To understand if males and females are different, you need also to partition by gender.

As the work of Colantonio and coworkers well demonstrated, fortunately, the gender differences are rare in children, but they manifest themselves at puberty. The problem is that males and females develop puberty at different ages and the pubertal spurt occurs at very different ages in the various subjects.

This fact is not relevant only for the sexual hormones, but also for very common analytes like inorganic phosphate, creatinine, uric acid, or alkaline phosphatase. So there is a partitioning problem and difficult decisions on how to define the age classes.

The best possible solution is the application of sophisticated mathematical algorithms that allow a calculation of a continuous distribution with age of the upper or lower limits.

Bob Barrett: In your editorial you underscore the relevance of traceability, standardization and harmonization of analytical methods. Why are they so important?

Dr. Ferruccio Ceriotti: I have the impression that these aspects are highly underestimated when defining reference intervals. When the analytical method used for the definition of reference interval has an adequate specificity and its correct standardization allowed the demonstration of the methodological traceability to higher order methods or material, the reference data obtained are applicable as they are by any other laboratory using analytical methods of proved traceability, if the population served has similar characteristics.

Standardization and traceability are the basis for the definition of common reference intervals, but also for population specific reference interval, if genetics, ethnicity, or lifestyles influence reference intervals. Continuously repeating the costly exercise of redefinition of reference intervals for any modification of the analytical methodology is nonsense.

For well standardized analytes, only population differences should justify different reference intervals.

Bob Barrett: Okay, Dr., well, finally let's look ahead. Which future developments do you foresee for reference intervals in laboratory medicine?

Dr. Ferruccio Ceriotti: Quite difficult question. I believe that reference intervals as we term them now will be at least partially abandoned in the future to be replaced by decision limits specific for the various clinical questions.

But the definition of reliable decision limits have two prerequisites: the complete understanding of reference intervals and a very high standardization level. These two prerequisites will allow the calculation of clinical specificity and clinical sensitivity of the test in the different clinical settings and consequently the choice of the decision limits based on ROC curves.

A possible further development, with improvement of the reliability of the analytical techniques and of the possibility of information technology will be the definition of individual reference intervals.

Bob Barrett: Dr. Ferruccio Ceriotti is an expert on reference values and a researcher at the San Raffaele Hospital in Milan. He has been our guest in this podcast from '*Clinical Chemistry.'*

I am Bob Barrett. Thanks for listening!

Total Duration: 8 Minutes