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Reference Intervals Revisited: A Novel Model for Population-Based Reference Intervals, Using a Small Sample Size and Biological Variation Data

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Guest: Dr. Abdurrahman Coşkun from Acıbadem University School of Medicine in Istanbul, Turkey.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics & Laboratory Medicine. I'm Bob Barrett.

One of the most frequently asked questions in the practice of medicine is, "is this test result normal?" This question can mean many different things, but generally, the question can be rephrased as "does this result fall within the population based reference interval?" The most widely used approach requires ranking at least 120 results generated from a healthy reference population and defining upper and lower limits as the 97.5th and 2.5th percentiles.

While relatively straightforward, this process is time consuming, particularly when performed for each test performed by a clinical laboratory. As a result, manufacturer defined reference intervals are often used, but these may not be appropriate for all patient populations. Is there another way? Is it possible to harness the best of both worlds? A reference interval that accurately reflects a hospital's unique patient mix that can also be generated quickly and easily. A new research article appearing in the October 2024 issue of *Clinical Chemistry* proposes a new approach to reference interval calculation that requires substantially fewer data points than traditional methods.

In this podcast, we're pleased to speak with the article's lead author. Dr. Abdurrahman Coşkun is a Professor of Medical Biochemistry at Acıbadem University School of Medicine in Istanbul, Turkey. He's actively conducting research on biological variation and clinically relevant references for the interpretation of laboratory test results.

So Dr. Coşkun, first of all, why do physicians need reference intervals? Are they essential for medical practice?

Abdurrahman Coşkun: Yeah. Interpretation of laboratory data is a comparative process that requires reference values for comparison. For

healthy individuals, if the results of laboratory tests fall within a range known as the reference interval, they are considered normal. But if the results fall outside this interval, they are classified as abnormal.

This classification may lead to further investigation to determine the underlying cause. Additional tests are usually conducted to diagnose the potential diseases. So, we can say that reference intervals are essential in medical decision making since clinicians frequently rely on them when making their decisions.

Bob Barrett: So how are reference intervals for laboratory tests currently estimated, and are those estimates reliable?

Abdurrahman Coşkun: Yeah. Well, currently reference intervals are estimated using data collected from populations. According to international guidelines, reference intervals should be estimated using data from at least 120 reference individuals. This makes the current method of estimating reference intervals impractical. That's why laboratories often prefer using reference intervals provided by manufacturers or adopted from other laboratories. However, such practices can introduce additional variability and reduce the reliability of reference intervals. This can significantly impact the accurate interpretation of laboratory data.

Another crucial aspect to consider is that in our metabolism, the concentration of biomolecules fluctuates around a set point, and this is known as within-subject biological variation. Additionally, set points vary among individuals, and this is known as between-subject biological variation.

Conventional methods for estimating reference intervals do not incorporate these variations into their calculations. Consequently, it can be concluded that conventional reference intervals do not accurately reflect the metabolic variations observed in individual metabolism.

Bob Barrett: What about the challenges estimating reference intervals for laboratory tests? What are they, and how can they be addressed?

Abdurrahman Coşkun: Well, finding 120 reference individuals for estimating reference intervals for laboratory tests is particularly challenging, especially in pediatric or elderly populations. Additionally, for some laboratory tests, reference intervals are determined for different age groups, genders, and other factors. In such cases, it is necessary to find at least 120 reference subjects for each subgroup.

This requirement makes the process more challenging due to the need for a large number of reference individuals.

For example, if a laboratory test requires reference intervals for 5 subgroups. In this case, we would need 5 times 120, or at least 600 reference individuals. To overcome this challenge, a new method for estimating reference intervals is needed. This method should require fewer reference individuals and also incorporate metabolic variation into the calculation of reference intervals.

Bob Barrett: How about this new method developed by you and your colleagues? How does this estimate reference intervals, and is this reliable?

Abdurrahman Coşkun: Yeah. In this new method, we took a pragmatic approach by estimating reference intervals using data from smaller number of reference individuals. In the new method, the limits of the reference intervals are determined using biological variation data, which is the gaussian combination of within-subject, between-subject, and analytical variations.

Biological variation data can be obtained from the EFLM database, so it does not need to be calculated separately. And in the new method, the key requirement is the midpoint of the reference interval. And this can be reliably estimated using data from approximately 16 reference individuals. Now in contrast to conventional methods, which requires at least 120 reference individuals, this new method requires data from only 16 individuals. This makes it much more practical for laboratory use.

Additionally, by incorporating both within-subject and between-subject biological variations, this new approach is more reliable than the conventional reference intervals.

Bob Barrett: Well, finally Dr. Coşkun, what is the next step in estimating reliable reference intervals?

Abdurrahman Coşkun: Yeah, the next step. For reliable reference intervals, first of all, accurate biological variation data is essential.

In this regard, the EFLM Biological Variation Working Group and the Biological Variation Database Task Group are doing excellent work in providing reliable biological variation data.

And it should be noted that human metabolism is a dynamic process, and within-day variations are observed in the concentration of most biomolecules. However, unfortunately, current reference intervals are often based on data collected

from morning samples. This means that there are no reliable reference intervals for afternoon or evening times.

To enable accurate interpretation of laboratory data, we need dynamic and continuous reference intervals that cover a 24-hour period and also potentially include monthly and seasonal variations.

Bob Barrett: That was Dr. Abdurrahman Coşkun from Acıbadem University School of Medicine in Istanbul, Turkey.

He wrote a research article describing a new method to generate reference intervals using a small number of samples in the October 2024 issue of *Clinical Chemistry*. He's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.