Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Biological Variation

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Slide 1:
This Pearl is presented by Danni Li at the University of Minnesota, Department of Pathology and Lab Medicine.

Slide 2:
Biological variation is an important concept for clinical chemists to understand, because it helps answer many questions that clinical chemists encounter on a daily basis. For example, it can be used to determine whether the changes that occur in an individual's results are statistically significant or not. It can be used to determine whether population-based reference ranges are helpful for interpretation of individual patients' test results. It can also be used to calculate the reliability coefficient used in epidemiology.

Slide 3:
In this lecture, I would like to start with the definitions of two types of biological variations: within-subject and between-subject variations, and explain how they are determined. I will use a case study to illustrate the situations in which the concepts of reference change value (RCV) and index of individuality (II) may be helpful. Then I will show you how to calculate RCV and II using biological variations.

Slide 4:
There are two types of biological variations: within-subject and between-subject. Within-subject variation is the random fluctuation around a homeostatic point. In individuals, the homeostatic points usually vary. The variation between individuals is called between-subject biological variation.

Certain analytes have predictable biological rhythms or cycles. For those analytes, a patient sample must be collected at the time in the cycle that is appropriate for the clinical purpose to which the test result will be applied. However, most analytes do not have cyclical rhythms that are of major clinical importance.

Slide 5:
Within-subject and between-subject biological variations are determined by conducting a biological variation study. The first step of the study is to recruit study subjects of interest. The study subjects can be healthy or patients with stable diseases depending on the contexts of the study. The second step is to take serial samples from study subjects at regular intervals that are consiste nt with the intended use of the tests. Next, the samples are analyzed in duplicates, and statistical techniques such as analysis of variance (ANOVA) are used to determine the analytical, within-subject, and between-subject biological components of variations.

For most of the common analytes, biological variation studies have been done. Therefore, by doing a literature search, we could find within-subject and between-subject biological variation values for most of the common analytes. However, the caveat of using the published biological variation values is that they are method- and study subject-dependent, and therefore, may not be universally applicable.

Slide 6:
Now let's start our case, and discuss the situations that Reference Change Value (RCV) and Index of Individuality (II) may be helpful.

A 63-year-old female returned for evaluation and treatment of hypercholesterolemia despite management with triple lipid-lowering therapy (fibrate, statin, and cholesterol absorption inhibitor). Her family history was significant for hypercholesterolemia. She also had type 2 diabetes.

Slide 7:
This slide shows her lipid profiles in October 2009 and January 2010. In Octob er, her total cholesterol was 225 mg/dL, LDL cholesterol 146 mg/dL, HDL-cholesterol 40 mg/dL, and triglycerides 197 mg/dL. In January, her total cholesterol was 273 mg/dL, LDL cholesterol 181 mg/dL, HDL-cholesterol 44 mg/dL, and triglycerides 138 mg/dL. The question is whether the lab results in October 2009 were significantly different from the ones in January 2010.

Slide 8:
The test results of an individual person vary over time due to three factors: pre -analytical variation (CV_P), analytical variation (CV_A), within-subject biological variation (CV_I). Pre-analytical variation is influenced by preparation of the individual for sample collection, sample collection itself, and conditions that the collected samples experience after collection prior to the samples being measured. Analytical variation is the imprecision of the measurement. Within-subject variation, as I explained earlier, is the random fluctuation around a homeostatic point.

Slide 9:
Reference Change Value (RCV) will help us to understand whether the change of an individual's serial result is statistically significant. RCV is determined by Z score, analytical variation (CV_A), and within-subject variation (CV_I), assuming that pre-analytical variation (CV_P) is negligible. Z score is defined as a number of standard deviations that a result is from the mean. Z score of 1.96 indicates a probability of

95%, statistically significant. When Z score is 2.58, it indicates the change would have a probability of 99%, therefore statistically highly significant. Whether Z score of 1.96 or 2.58 should be used depends on the clinical contexts. In the rest of this lecture, Z score of 1.95 or RCV of 95% will be used.

Slide 10:
For total cholesterol, RCV of significant change is calculated to be 17.5%, using the analytical variation of 2.0% and within-subject variation of 6.0%. The within-subject variation of total cholesterol is taken from the book *Biological Variation: From Principle to Practice* by Callum G. Fraser. The actual change of total cholesterol was calculated to be 21.3%. The actual change was larger than the RCV of significant change, and therefore the change of total cholesterol level from 225 to 273 mg/dL was significant.

Slide 11:
For the other three analytes, calculated RCVs and actual changes are shown in this table. Actual changes of all the other three analytes are less than their RCVs of 95%. Therefore, the changes were not statistically significant.

Slide 12:
In an effort to decrease this patient's LDL cholesterol, Niaspan was added. However, as the patient was a diet-controlled diabetic, the addition of Niaspan may increase her blood glucose level. So it was decided to add a conservative dose of Niaspan at 1000 mg and closely follow her glucose levels.

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After addition of Niaspan, the patient came back in March and had a lipid profile. The actual changes of the analytes were -26.7% for total cholesterol, -29.8% for LDL cholesterol, 6.8% for HDL cholesterol, and -46.2% for triglycerides. Her total cholesterol change was more than the RCV of 95%, so the change of her total cholesterol was significant. So was LDL cholesterol: the actual change was 29.8%, and the RCV of 95% was 24.4%.

Slide 14:
Since starting Niaspan, the patient developed gout for the first time in her life. Although synovial fluid examination is used for diagnosis, uric acid is used as surrogate marker for gout. Her uric acid was 5.3 mg/dL in April-10. Reference range for uric acid is 2.4-6.4 mg/dL. Given that this patient was diagnosed to have gout, why was her uric acid level, a surrogate marker for gout, still within the reference range?

Slide 15:
Traditionally, population-based reference range is determined as 90% confidence intervals for the 95th percentile reference limits at the 2.5th and 97.5th percentiles. Population-based reference ranges are often used to interpret patients' lab results.

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Comparison of the population-based reference ranges and individual range usually show two distinctive patterns: A and B. In pattern A, within-subject variation is larger than between-subject variation; in pattern B, within-subject variation is less than between-subject variation. Intuitively, we know that if an analyte has the pattern A for the population-based reference ranges and individual ranges, population based-reference range would be helpful in interpreting patients' results. If an analyte has the pattern B for the population-based reference ranges and individual ranges, population-based reference range would not be helpful in interpreting patients' results.

Slide 17:
Index of individuality calculates the ratio of the total within-subject variation to between-subject biological variation. Most often, it is simplified to the ratio of within-subject variation to between-subject variation.

Slide 18:
This table shows the index of individuality of common analytes. Studies have shown that if the index of individuality is less than 0.6, population-based reference range is of little use. If the index of individuality is above than 1.4, population-based reference range is of significant use. For uric acid, the index is 0.5, indicating that population-based reference range would not be useful as homeostatic reference range for individuals, as for the patient in our case.

Slide 19:
Recently, the index of individuality for cardiac Troponin I was published. Both within- and between-day index were less than 0.6, indicating that cardiac Troponin I has very high individuality. This shows the importance of serial sampling of Troponin in a patient for diagnosis of acute myocardial infarction.

Slide 20:
In summary, biological variation is an important concept for clinical chemists to understand, because it helps answer many questions that clinical chemists encounter on a daily basis. Within-subject biological variation can be used to calculate Reference Change Value for determination of whether the changes that occur in an individual's results are statistically significant or not. Within- and between- subject variations can be used to calculate index of individuality for determination of whether population-based reference ranges are useful for interpretation of individual patients' test results.