Deacon's Challenge

No 105 - Answer

Recent draft UK national guidelines for the use of newer agents in the treatment of Type 2 Diabetes have recommended that thiozolidinedione drugs (e.g. pioglitazone) should only be continued if their introduction results in a reduction in HbA1c of at least 0.7 HbA1c units (%). If the biological within-subject variance is 0.01, what analytical precision must the assay achieve in order to be able to detect a true fall of 0.7 with greater than 95% certainty?

FRCPath, Spring 2009

If x_1 is the initial HbA1c result, x_2 the result after treatment and s_1 and s_2 their respective standard deviations then differences between the two results $(x_1 - x_2)$ can be considered as a normally distributed variable with mean $m_{1\text{-}2}$ and standard deviation $s_{1,2}$. If x_1 and x_2 are not significantly different at the 5% level of probability then their difference $(x_1 - x_2)$ would belong to a distribution with a mean of zero and combined standard deviation of $s_{1\text{-}2}$ and on 95% of occasions these differences would fall within the mean ± $2s_{1,2}$ range (or more precisely the mean ± $1.96s_{1,2}$ range). A *z* -score can be calculated for any value of $(x_1 - x_2)$ in order to determine the likelihood that this value is significantly different from $m_{1\text{-}2}$ at any desired level of probability:

$$z = \frac{(x_1 - x_2) - m_{1\text{-}2}}{s_{1,2}}$$

A *z* -score of 1.96 (approx 2 standard deviations) corresponds to a probability of 0.95 (i.e. 95%) that the *change* in HbA1c is significant.

The combined variance for two results which are subtracted (or added) is the sum of their individual variances:

$$s_{1,2}^2 = s_1^2 + s_2^2$$

We can reasonably assume that s is the same at both concentrations so that $s_1 = s_2$ (which we can call *s*) so that:

$$s_{1,2}^2 = s^2 + s^2 = 2s^2$$

Therefore $s_{1,2} = \sqrt{(2s^2)} = \sqrt{2} \times s = 1.414s$

Substituting $m_{1\text{-}2} = 0$ $z = 1.96$ and $s_{1,2} = 1.414s$ gives:

$$1.96 = \frac{(x_1 - x_2) - 0}{1.414s}$$

which can be rearranged to give the very useful expression:

$$\boxed{(x_1 - x_2) = \mathbf{2.8}s}$$

This means that for a change of $(x_1 - x_2)$ in two results to be statistically significant at the 95% level of probability they must differ by at least 2.8 standard deviations.

In this question we need to find a value for *s* which exactly fulfills this condition by substituting $(x_1 - x_2) = 0.7$ and solving for *s*:

$$s = \frac{(x_1 - x_2)}{2.8} = \frac{0.7}{2.8} = 0.25\%$$

This is the combined analytical and within-subject standard deviation (s_{Total}) which is related to the individual standard deviations by the expression:

$$s_{Total}^2 = s_{Analytical}^2 + s_{Within\text{-}subject}^2$$

Substitute $s_{Within\text{-}subject}^2 = 0.01$ and solve for $s_{Analytical}$:

$$0.25^2 = 0.01 + s_{Analytical}^2$$

$$s_{Analytical} = \sqrt{(0.25^2 - 0.01)} = \sqrt{(0.0625 - 0.01)} = \sqrt{0.0525} = \mathbf{0.23}\% \text{ (2 sig figs)}$$

A strict interpretation of the wording *"to detect a true fall of 0.7 with greater than 95% probability"* means that only the *negative* side of the distribution curve should be used. With 95% limits, 2.5% of results will be more than 0.7 HbA1c units above *and* 2.5% of results will be more than 0.7 HbA1c units below the initial value if no true difference exists. Therefore to detect a fall of 0.7 HbA1c units below the initial value with greater than 95% certainty requires a *z* score of 1.645 which corresponds to 90% limits i.e. only 5% of results will be more than 0.7 HbA1c units below the initial value if no true difference exists so that we can be 95% certain that there is a difference. This results in the alternative expression $(x_1 - x_2) = 2.33s$ so that the result for $s_{Total} = 0.301\%$ and $s_{Analytical} = \mathbf{0.28\%}$.

Question 106

A serum from a patient with a prolactinoma has a prolactin value beyond the analytical range of the assay. The endocrinologists have asked for a numerical value to provide a baseline for monitoring the patient. In an attempt to preserve the matrix upon dilution, 0.1 mL of the sample is mixed with 2.0 mL of a serum from another patient which has a prolactin value of 400 mU/L. If the assay result for the mixture is 1050 mU/L, calculate the prolactin concentration in the serum from the prolactinoma patient.