Historical Control Data (HCD) or Reference Interval of Clinical Pathology Parameters: How to Create and Interpret?

Introduction

Historical control data (HCD), also called as the 'reference intervals'. HCD constructed with values obtained from the reference individuals. In most cases, reference individuals are control animals of previous studies and clinically healthy animals that have not received treatment.

The value of reference intervals for data interpretation sometimes is overestimated, and potential for their improper use is more. While historical reference intervals can be helpful for establishing some perspective concerning what is typical or expected conditions of every toxicology study are unique, and it is inappropriate to use a reference interval as the primary reason for dismissing an apparent difference between the control and treated animals as being incidental or biologically insignificant. It can be equally inappropriate to use the reference interval as the primary reason for determining that an apparent difference is real or adverse.

Investigators must understand the limitations of reference intervals with respect to the data interpretation. By themselves, reference intervals do not determine whether an apparent difference is real or adverse. They are simply an adjunct to sound scientific judgement.

In their consensus document published in 1996, the Joint Scientific Committee for International Harmonization of Clinical Pathology Testing states "The concurrent control data are more appropriate than historical reference ranges for comparison with test material treatment groups" (sic).

How Do I Create Historical Control Data (HCD) Using Microsoft Excel?

Historical Control Data (HCD) is prepared from values of individual control animals of previous studies. Data can be sorted out as per the age, strain, supplier, site of blood collection, use of anaesthesia, type of anaesthesia, diet, fasting status, time of sample collection, sample matrix (e.g., serum or plasma), analyser/instruments used, the vehicle used, route of administration, etc., provided the data is available for enough number of animals. These values are subjected to statistical treatment for the elimination of 2.5% of the values at both ends of distribution (top/upper and bottom/lower), resulting in the central 95% reference interval.

The procedure for the elimination can be done using Microsoft Excel. Data from the previous studies of control animals is compiled in an excel sheet. In that, one row/column has data of one parameter. For the elimination of bottom 2.5% values, "PERCENTILE" from "Formulas" option on the toolbar is selected. A range of rows or columns in "Array" is selected and 0.025 in "K" is entered". The process is the same for the elimination of the top 2.5% except value entered in "K" (which is 0.975). Thus, we get two values, i.e., 2.5th percentile and 97.5th percentile forming central 95 percentile range. The N (total number of animals) is important to know the strength of data.

Use of Historical Data for Interpretation

A routine statistical analysis between the values of the treated groups and the control group is carried out. Though historical data is mainly referred for parameters showing statistical significance, it is not a rule. For this exercise, individual values from all groups are compared with the central 95 percentile range, i.e., how many values from the control group and the treated groups are beyond historical ranges. It is to be kept in mind that as the range itself is central 95 percentile, theoretically 1 in 20 values can be out of the historical range. If a greater number of animals from the High Dose are beyond range (in one direction, i.e., either increased or decreased), and none or few numbers from the Control group, the effect is more likely due to the test item treatment. It is to be noted that there is no thumb rule, and each case is different.

There are multiple factors to be considered viz. How large was the difference? Was it consistent over time? Was it consistent between sexes? Was it dose-dependent? Were there correlative anatomical pathology findings? Correlative clinical pathology findings? Correlative in-life observations? Was the difference statistically significant? Did the 'difference' exist before the treatment was initiated? What age were the animals? How did the data for each animal change over time? About specific tests, how much inter-animal and intra-animal variability is expected for the species tested? How much analytical variability is typical? What was the route of administration? What was the test material? What was the vehicle or excipient? Were the animals randomised for blood collection? What was the site of blood collection? Which type of anaesthesia used for blood collection? How much did the animals bleed during the study?

Certainly, there are other factors too. On the other hand, if results of treated animals are within the reference interval, it is not always safe to conclude that the effect is not adverse, it is entirely possible that individual animals with significant health problems have results that are within the reference interval.

Final Say

Conditions in every safety assessment study are unique. It is inaccurate and misleading to suggest that animals used for establishing historical reference intervals are a representative sample of a larger population that encompasses any given safety assessment study. The concurrent control animals are the only true sample of the "population" that encompasses the treated animals. Apparent differences between the control and treated animals, with respect to results of the clinical pathology tests, must be evaluated in the full light of all the conditions and findings associated with the study in question. Historical reference intervals add little to that evaluation.

Regardless of many pitfalls affecting their use for data interpretation, historical ranges do have other important functions. They serve as a nonspecific measure of quality control that can detect changes overtimes in assays, study conditions, or animal characteristics. Historical ranges also serve as a nonspecific measure of analyte variability. The cause of seemingly excessive variability might be inadequate assay precision, non-standardised preanalytical procedures, or true inter-animal variability. Reference intervals are valuable only when treated animals are tested at an unscheduled interval because of the signs of toxicity or when very few animals are used for small investigational studies with no concurrent control group. Although pre-treatment baseline data are more relevant to the interpretation of potential effects, it may be discovered that a few animals acquired for a small study have pre-existing problems or a typical with respect to historical data.

References

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About The Author

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He has been pivotal for interpretation of NOAEL, NOEL or LOAEL and writing reports of various toxicity studies consisting of carcinogenicity, two generation, extended one generation, pre or post natal developmental, chronic, sub-acute studies etc. He has professional experience of more than 13 years in the CRO industry. He is instrumental in maintaining the GLP in the Pathology and Clinical Chemistry Section of the JRF.

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