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7. Reference Intervals for Plasma Proteins

7.1 Principles for Estimation of Reference Intervals

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7.1.1 Models for Estimation of Reference Intervals

Models for estimation of reference intervals are either parametric or non-parametric and both have advantages and disadvantages. The parametric models are usually based on the Gaussian distribution directly or after transformations of data. The advantage of the parametric approach is the power of all estimates and thereby, a relative narrow confidence interval about each reference limit. The weakness is whether the underlying distribution (directly or transformed) is fitted by the parameters (mean and standard deviation) to a reasonable extent. If not, then the estimates are biased and the reference limits correspondingly wrong. The quality of the parametric fit can be validated by a number of tests for skewness and kurtosis or by Anderson-Darling-test or Kolmogorov-Smirnov-test as described in IFCC's recommendations (10). The advantage of the non-parametric approach is its independence of the shape of the underlying biological distribution, whereas the disadvantage is a considerable uncertainty of the estimated reference limits. Both approaches are vulnerable to so called *outliers* when sample size is small, and the effect is reduced by increasing the number of reference values for both approaches.

Ideally, the IFCC-recommendations (10) should have been applied using the computer programme REF-VAL for the estimations of reference intervals. The model, however, is very flexible of nature and the programme which optimize the fit of any distribution, lacks the graphical presentation (except from a histogramme). The model is optimal for estimation of reference intervals for one single crude sample (or mixed sample), which makes it irrelevant for comparing a great number of distributions. Linnet has given some critical remarks to the IFCC recommendations (9).

Due to the nature of our data and the purpose of the study with a considerable number of reference values, combined with the many subsamples to compare, we decided to use the visual presentation as a tool for decisions about combining or separating sub-groups in the material.

For this purpose Harris and Boyd (5) have made an interesting and useful concept, directly related to the separation of reference intervals according to subgroups. The idea in their paper is to focus on the fraction outside each reference limit. When reference intervals for two subgroups are considered, both the combined reference interval and each of the two are calculated. Then the fractions outside the common reference limits is calculated for each of the subgroups. Ideally they should all be 2.5 %, but with increasing deviations between means and standard deviations of the subgroups the fractions decreases or increases for each of the two times two fractions. The limits for these changes have to be decided (see below). Harris *et al.* have applied the concept with success to S-Creatine Kinase for sub-groups according to race and gender (6).

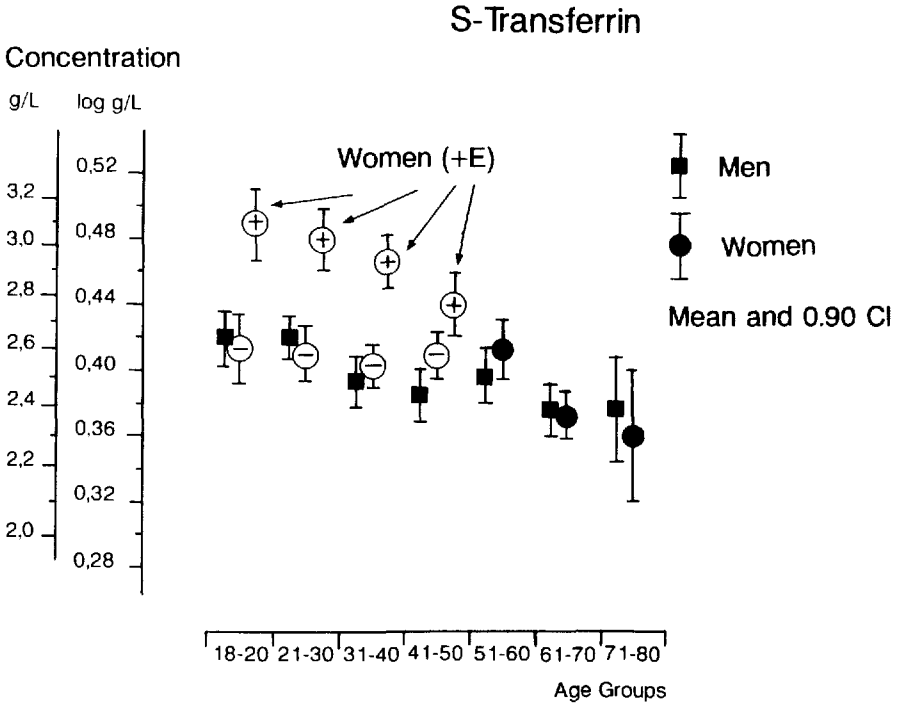

Fig. 7.1.1. *Mean concentrations with 90 % confidence intervals for each subgroup shown on a log-scale in relation to age for S-Transferrin. From Blaabjerg et al. (1) with permission.*

Further, the best visual presentation of distributions is the *probit-plot* (2 - 5, 7, 8) where the cumulated frequencies are plotted on a *standard deviate scale* as function of the concentration values. When Gaussian, the cumulated distributions show up as straight lines. For reference intervals the most relevant transformation of data is to take the logarithm, as many laboratory reference values are distributed close to log-Gaussian. The probit-plot has many advantages as many distributions can be shown simultaneously in the same plot without confusion and the judgement of the quality of the parametric fit to the data is better by the eye than by any statistical test. Further, when deviating from the parametric model, the plot can be used directly for non-parametric estimation. Detailed descriptions of probit-transformations and instructions for constructions and advises for interpretations are given by Gowans *et al.* (3) and Hyltoft & Hørder (8).

7.1.2 Levels of Sub-specification.

Three levels of specification can be applied:

* a very detailed where all subgroups are described, i.e. with specified reference intervals for each subgroup. This approach may be relevant for extremely large subgroups, but the uncertainty of each group is considerable as sample sizes decreases. We have decided to extract informations about biological tendencies from the graphical presentation of each subgroup as mean with a 90 % confidence interval in relation to age. As most of the distributions are log-Gaussian these values are presented on a log-scale.

* a rather detailed, but with combination of subgroups according to the Harris-Boyd-concept (5), using the limits for fractions outside the combined intervals 1.3 % to 4.4 % according to Gowans *et al.* (4). For the smaller sample sizes (below 120 individuals), however, the actual confidence interval calculated according to Bliss (2) was used. Moreover, a number of choices and decisions had to be taken, where a single subgroup differed, without biological or other explainable reasons (see below). This level can be considered the basic clinical chemical level.

* a third and more practical level may be a further simplification, where more semi-subgroups are combined, or groups are described by a percentage deviation from the main group. This has been adapted by some of the Nordic countries as shown in chapter 8, but these are national applications determined from practical or other reasons.

7.1.3 Example

The chosen example is S-Transferrin, where the mean values with 90 % confidence intervals are shown in relation to age in Fig. 7.1.1. Women below 50 years of age using estrogen show higher values than the rest - and should (as the first estimate) be treated as a separate group. For the rest there is a tendency to decreasing values for increasing age. From approx. 2.6 to approx. 2.4 g/L. For practical reasons we had decided to separate at 50 years - and as the numbers in the two oldest age groups are small, then the uncertainty is too large for a clear decision about separating in two different reference intervals above and below 50 years.

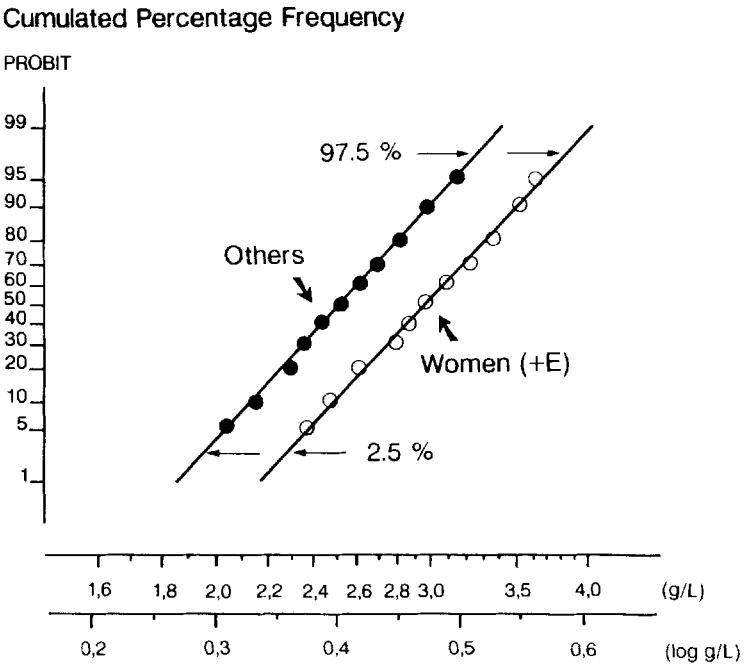

Fig. 7.1.2. Probit-plot with log-abscissa showing for S-Transferrin the distributions for women using estrogen and the remaining group (all others). From Blaabjerg et al. (1) with permission.

The two distributions, one for women using estrogens and one for all others, are illustrated in Fig 7.1.2, from which it is clear that both distributions are close to log-Gaussian (straight lines) and clearly separated.

From Fig. 7.1.1, however, there was also a tendency to decreasing values with age for women using estrogens with the age-group 41 to 50 as the lowest. In consequence this group was compared separately to the total group of women using estrogens. In Fig.

7.1.3 the broken line indicates the total group and the dotted line the age-group 41 to 50 with a total of 24 individuals. The latter distribution is not log-Gaussian (thin straight line) and converges to the total distribution in both ends. The 90 % uncertainty limits for a sample size 24 is shown around the total distribution (double curves). It is seen that the central values are below the confidence interval, but both ends are inside. As we find no biological evidence for separation, we have chosen to use the total reference interval.

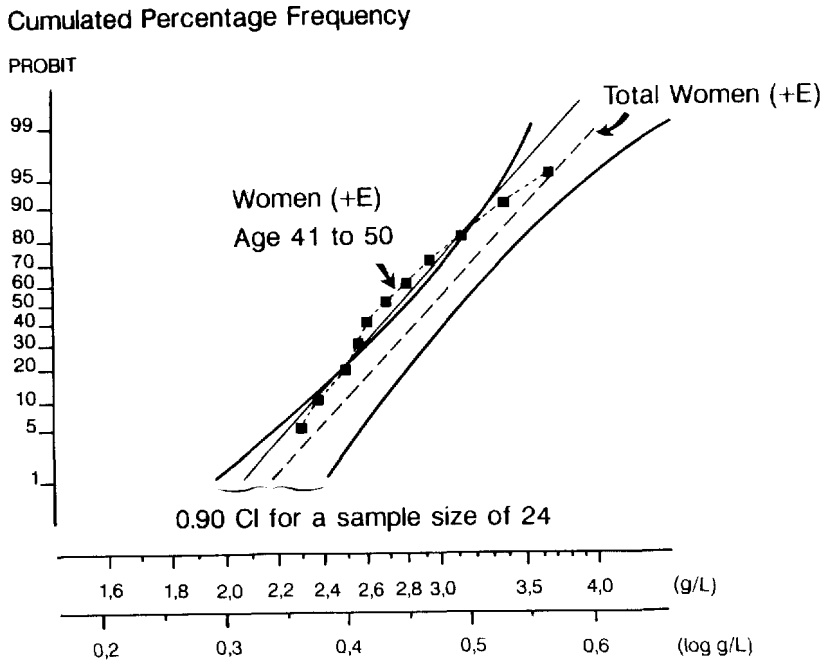

Fig. 7.1.3. *Illustration of a comparison between a subgroup and the total group. Probit-plot of S-Transferrin showing the total group of women using estrogens (dotted line) with a 90 % confidence interval corresponding to the subgroup (here 24) for each percentile. The subgroup (women between 41 and 50 years of age) is shown as the broken line and indicated parametrically by the thin straight line). From Blaabjerg et al. (1) with permission.*

However, when the distributions are separated according to high and low doses of estrogens, then the two groups separate clearly as seen from Fig. 7.1.4.

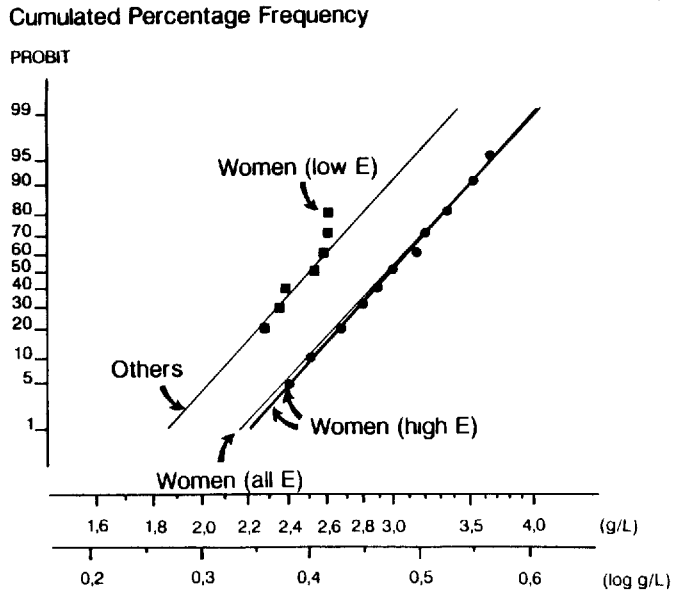

Fig. 7.1.4. *Distributions of S-Transferrin for women using high and low doses of estrogens.*

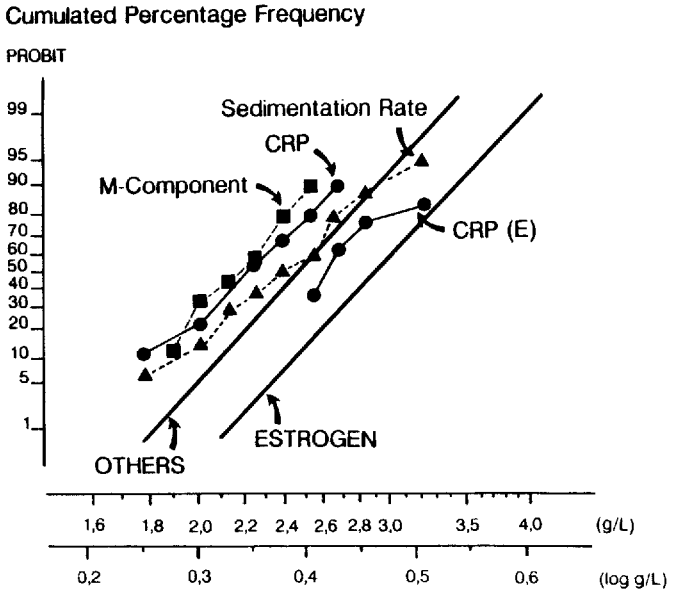

Fig. 7.1.5. *Illustration of the discharged values due to elevated B-Sedimentation Rate, S-CRP or presence of M-Component, and compared to the accepted reference distributions.*

Further, the individuals discharged from the main groups due to elevated values of B-Sedimentation Rate, S-CRP or due to the presence of M-Components can easily be displayed on the probit-plot, and thereby be compared to the accepted reference individuals as illustrated in Fig. 7.1.5, even though they are of very limited sample sizes.

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