Gender-dependent Reference Limits

Sir,

In a recent article [1] Weiss et al. determined the reference interval for three adhesion factors in serum. The statistical methods need some comments, which may be of interest.

The number of reference persons were 30 males and 30 females. The approved recommendations from the IFCC [2] state that the number of individuals should be at least 120, and that this number should apply for each subclass of partition, e.g. gender. Furthermore, in the same recommendations the Anderson-Darling test is preferred before the Kolmogorov-Smirnov, because the former is more likely to reject the hypothesis of a Gaussian distribution. The reference interval should include the central 95 % of the reference population, i.e. 2.5 % of healthy individuals shall display values outside this interval. This means that at least 40 individuals are needed to find one such person on each side.

These facts prompted me to further scrutinize the statistical methods of the article [1]. Generally, variables displaying a small biological variation compared with the mean value tend to form reference distributions close to Gaussian, whereas otherwise a positive skewnes is frequently seen. For the three analytes, sCD44, sCD44(v5), and sCD44(v6) the range/mean ratios were 0.9, 1.3, and 1.6 respectively, indicating that the first of them (sCD44) might be randomly sampled form a Gaussian distribution, whereas the others probably should reveal a positive skewness if the number of observations was increased sufficiently. The great range/mean ratios indicate that the authors may have made an Type II error stating that sCD44(v5) and sCD44(v6) were Gaussian distributed.

The gender differences of the reference limits can be tested statistically. The reference range using a parametric design is the mean ±1.96 SD. For a true Gaussian distribution the standard deviation S_r of this limit is:

$$s_r = \sqrt{\frac{3s^2}{n}}$$

where s is the sample standard deviation, and n is the number of observations [3]. Differences in reference limits can therefore be tested by a Student's t-test:

$$t = \frac{|r_1 - r_2|}{\sqrt{s_{r1}^2 + s_{r2}^2}}$$

where r_1, r_2, sr_1, and sr_2 are the reference limits and their standard deviations, respectively.

Applying this test on the upper limits (the lower limits may not be of clinical interest) in Table 1 of the study [1], no gender difference was found for sCD44 (t=0.96, n.s.), whereas for sCD44(v5) and sCD44(v6) the differences were statistically significant (t=4.99, and t=4.56 respectively, p <0.001). This fact supports the conclusion of the authors, that there is a gender difference for the latter two quantities, but not for the first. However, note that the test infers a Gaussian distribution, which may be false.

Determinations of reference intervals are important in clinical chemistry, but many (especially new) methods are expensive, and it is tempting to use too small a number of reference indivduals. The reasons for using a sufficient number are clearly stated in the approved recommendations from the IFCC [2]. Therefore no exceptions should be made from this rule, unless sampling from healty individuals may be controversial (e.g. lumbar puncture) and/or the method is very expensive.

References

1. Weiss S, Jung K, Lein M, Schnorr D, Loening S. Referenzbereiche der löslichen Adhäsionsmoleküle CD44 (standard), CD44(v5) und CD44(v6) im Serum gesunder Erwachsener. Lab.med. 1995;19:373-6.
2. Solberg HE. Approved recommendations (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determinations of reference limits. J Clin Chem Clin Biochem 1987;25:645-56.
3. Larsson L, Öhman S. Serum calciumion activity. Some aspects on methodological differences and intraindividual variation. Clin Biochem 1979:12:138-41.

Sten Öhman
Department of Clinical Chemistry
University Hospital of Linköping
S-581 85 Linköping, Sweden.
Fax: +46 13 22 32 40
E-Mail: sten.ohman@klk.uil.lio.se

Reply to the Comment "Gender-dependent Reference Limits"

Sir,

Dr. Öhman takes our publication on CD44 proteins in serum [1] as an opportunity to comment upon the determination of reference intervals. There is no question that every author is glad when attention is paid to his articles and they spark off discussion. We are therefore grateful to Dr. Öhman for having addressed this theme, which is always topical. No objections can be made to his general comments and the stipulation that all reference intervals should be determined according to the IFCC guidelines [2]. Owing to the ex-

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pensive test methods, 120 males and 120 females could not be included in each group in our study. As Dr. Öhman confirms, this is also the reason why the IFCC accepts smaller groups. Our study did not claim to determine reference intervals in strict compliance with the IFCC guidelines. Our remark about having proceeded according to the IFCC rules referred only to the central 95% reference interval used for determining the gender-specific reference limits. We believe that all other statistical evaluation methods used have been adequately described in the paper, so that there is actually no reason for us to comment on the remarks of Dr. Öhman. However, to prevent further misunderstandings we would like to make the following observations with regard to Dr. Öhman's comments:

1. The IFCC guidelines do not by any means rule out a determination of reference limits on the basis of smaller numbers of probands [2]. This is referred to in the paragraph on „intuitive assessment" [2]. Nevertheless, in such a case the tentative nature of the limits obtained should be mentioned. Although our number of test persons of 30 men and women in each group implies this, we should have emphasized the preliminary nature of the reference limits in our study more strongly.

2. The method of examining the distribution according to Kolmogoroff-Smirnoff, which was criticized by Dr. Öhman, is described in the IFCC recommendation as a simple and quick testing procedure. A compelling reason for using the Anderson-Darling Test is not given in the IFCC recommendation, although it is mentioned that this test is more powerful than the Kolmogoroff-Smirnoff test. Also, in textbooks on statistics [3], the Kolmogoroff-Smirnoff test is recommended for smaller and intermediate numbers of test persons, so that there is no reason for objections in principle to this method. In addition, the Kolmogoroff-Smirnoff test (in contrast to the Anderson-Darling procedure) is part of many statistical computer programs and thus is readily available.

3. Dr. Öhman concludes from the range/mean ratios of 0.9, 1.6 and 1.3 for sCD44std, sCD44(v5) and sCD44(v6), respectively, that there is perhaps no Gaussian distribution for both the variants v5 and v6. Our calculations with the Kolmogoroff-Smirnoff test do not suggest such an assumption. A correct evaluation with the help of the range/mean ratios requires separate calculations for each gender if there are gender dependent values as proved in our study. The gender-dependent differences for all the three CD44 proteins examined were demonstrated in the parametric as well as in the non-parametric tests.

4. Dr. Öhman proves that the calculations of the gender dependence of the upper reference limits show gender-dependent differences for CD44 variants v5 and v6, but not for the standard form CD44. In doing so, he does not take into consideration that, especially with respect to adhesion factors, lowered values could also be of diagnostic interest. Using the formula mentioned by Dr. Öhman, it can be demonstrated that the lower reference limits for sCD44std show gender-dependent differences, but that the reference limits for variants v5 and v6 do not. Since we had the opportunity to determine the soluble CD44 proteins for only a limited time and as its diagnostic value is still unexplored, we have intentionally indicated the upper and the lower limits of the central 95% reference intervals. The IFCC recommends "that the fractiles should be preferably accompanied by confidence intervals, e.g. 0.90 confidence intervals around the reference limit" [2]. Thus it appears, with CD44std for instance, that the confidence intervals of the lower reference limits for women (136-250 µg/l) and men (285-375 µg/l) do not overlap. Misunderstandings would have been avoided if we had indicated these intervals in our publication.

5. Reprints of our article were much in demand. This shows a general interest in this parameter. Despite the limited numbers of test persons, we still do believe that the reference ranges indicated for sCD44 molecules in serum are a valuable addition to the instructions for CD44 determination. If this public disputation again draws attention to general problems of reference value determination, it is surely consistent with the intention of Dr. Öhman.

References

1. Weiß S, Jung K, Lein M, Schnorr D, Loening S. Referenzbereiche der löslichen Adhäsionsmoleküle CD44(standard), CD44(v5) und CD44(v6) im Serum gesunder Erwachsener. Lab.med. 1995;19:373-6.
2. Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determinations of reference limits. J Clin Chem Clin Biochem 1987;25:645-56.
3. Sachs L. Angewandte Statistik. Berlin:Springer-Verlag, 1991.

Klaus Jung, Susanne Weiß
Klinik für Urologie
Universitätsklinikum Charité
Schumannstraße 20/21
D-10098 Berlin
Fax: +49-30-2802-1402
Email: jung@rz.charite.hu-berlin.de