LETTER TO THE EDITOR

Harris & Boyd's Test for Partitioning the Reference Values

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Dedicated to Eugene K. Harris, in memoriam

Sir,

One of the steps in the establishment of reference intervals recommended by the International Federation of Clinical Chemistry (1) is to decide upon the need for partitioning the reference values according to sex, age or other relevant influences.

To decide upon the need for partitioning, Student's t test or Mann-Whitney's U test are usually applied to compare the reference samples under study. But these tests may be too sensitive for this purpose: if the sizes of the two subgroups compared are large enough, small differences between subgroups without clinical importance may become statistically significant. For this reason, Harris & Boyd developed a special statistical test in order to find out the need for partitioning under a diagnostic point of view (2–3).

Having two subgroups of reference values, the statistical criteria proposed by Harris & Boyd are:

\[
0.7 \leq \frac{s_1^2}{s_2^2} \leq 1.5
\]

or

\[(s_1 - s_2)(s_1/n_1 + s_2/n_2)^{1/2} \geq 5 \cdot \left[ \left( \frac{n_1 + n_2}{2} \right)^{1/2} \right],
\]

for \(n_1 \geq 50\) and \(n_2 \geq 50\) and \(s_1, s_2, s_1, s_2\) being the means and the standard deviations and \(n_1, n_2\) the number of subjects in subgroups 1 and 2, respectively, it is necessary to estimate reference limits in both subgroups separately.

Among the different influences on the variation, sex has been most frequently taken into account for partitioning, and among the different biochemical quantities, the substance (or mass) concentration of iron(II+III) in serum (or plasma) has been one of the properties most frequently partitioned by sex, as may be seen in prestigious books of clinical chemistry (4, 5).

We have applied the Student's t test and the Mann-Whitney's U test and Harris & Boyd's test to two subgroups of reference values of iron(II+III) substance concentration in serum: one subgroup of 100 healthy reference women, aged between 18–45 years (\(x = 34\) years), and another subgroup of 52 healthy reference men aged between 18–45 years (\(x = 32\) years). All results were obtained with a BM/Hitachi 911 analyser (Boehringer Mannheim, Mannheim, Germany) using the ferrozine method (Boehringer Mannheim, Ref. 1553704).

The mean and the standard deviation of the quantity \(y\) are 15.2 μmol/l and 6.3 μmol/l, respectively, in the women subgroup, 18.5 μmol/l and 5.6 μmol/l, respectively, in the men subgroup. The Student's t test and the Mann-Whitney's U test detect significant differences between the two subgroups (\(P = 0.002\) and \(P = 0.001\), respectively). However, Harris & Boyd's test concludes that partitioning is not necessary for diagnostic purposes.

Finally, the reference limits for the mixed subgroups estimated parametrically – for the reference values obeying the Laplace-Gauss' law according the Anderson-Darling test (6–7) – are 4 μmol/l (confidence interval 90%: 3–6 μmol/l) and 29 μmol/l (confidence interval 90%: 27–30 μmol/l).

As it is very difficult (more reference individuals) and very expensive (more measurements) to partition we conclude that it would be very convenient to apply the Harris & Boyd's test rather than traditional statistical tests to decide upon the need for partitioning.

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¹) According to ISO, IUPAC and IFCC recommendations the comma is used as the decimal sign.

References


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LETTER TO THE EDITOR

Storage of Serum for the Determination of Ionized Magnesium

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Sir,

About 1.5 years ago Ising et al. (1) performed a thorough study about the methodological influences on the determination of the ionized magnesium concentration in serum (iMgf\(^{2+}\)). One of the conditions they investigated was the effect of storage. According to their paper, serum samples can be stored for 4 weeks at 4 °C with a maximum change in iMgf\(^{2+}\) of 10%.

In a pilot study performed in our laboratory and a study reported in the literature (2) good results were obtained when the serum samples were stored frozen. Therefore, a study was started to investigate the effect of storage at +4 °C, −20 °C and −80 °C on iMgf\(^{2+}\), in serum of healthy volunteers.

Blood was drawn anaerobically from 5 healthy volunteers. The serum of each volunteer was collected, mixed and the iMgf\(^{2+}\) was measured. The remaining serum was stored in separate, airtight, rubber-sealed sample tubes (0.8 ml; Sarstedt, Nümbrecht, Germany) which were filled with serum as completely as possible. For each different point in time an individual aliquot was used to determine iMgf\(^{2+}\) using the ion-selective electrode Kone Microlyte 6.0 (1, 3) (Kone, Espoo, Finland). Results are expressed as the relative change in iMgf\(^{2+}\) in relation to iMgf\(^{2+}\) in the fresh sample (day 1). As suggested by I.e. (1) the samples with a pH exceeding 7.80 were excluded. As can be seen in figure 1a, the determinations of the iMgf\(^{2+}\) measurements in samples stored at 4 °C increase constantly. After 2 weeks the increase already exceeded 10% and after 4 weeks 16%. In our opinion the most important reason for this increase is a steady increase in pH in the same period (the correlation coefficient between iMgf\(^{2+}\) and the pH is 0.81 (P < 0.0001)).

When the samples were stored at −20 °C the determinations of the iMgf\(^{2+}\) measurements remain within the 10% change for 3 months (fig. 1b), with a minimal increase in pH. Since after 331 days the pH exceeded 7.80, these iMgf\(^{2+}\) measurements were excluded.

By storing the serum samples at even lower temperatures, −80 °C, the samples can be stored for minimal 331 days (almost 1 year) with a maximal change in iMgf\(^{2+}\) measurements of 10% (fig. 1c) and a minimal change in pH.

In conclusion, in contrast to Ising et al. (1), we recommend to store serum samples frozen in airtight, rubber-sealed, as-completely-as-possible-filled tubes, even when the ionized magnesium has to be determined within one week. The most common temperature to store serum is −20 °C, which is sufficient when the determination of iMgf\(^{2+}\) is performed within 3 months after the collection of the serum. When samples have to be stored much longer a storage temperature of −80 °C is recommended.

Fig. 1 Effect of different storage temperatures on the determination of ionized magnesium in serum samples of healthy volunteers: +4 °C (a), −20 °C (b) and −80 °C (c). Results are expressed as the relative change in ionized magnesium concentration in relation to the concentration on day 1 (fresh serum sample).

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