Editorial

Rainer Haeckel

Indirect approaches to estimate reference intervals

<https://doi.org/10.1515/labmed-2021-0003>

Medical laboratory results must be accompanied by biological reference intervals where applicable, decision limits and/or interpretation of results where appropriate [1]. Clinical decision limits are related to a special disease or, independent of a special disease, reference intervals identify 95% of a subpopulation which probably has no disease (consists of non-diseased individuals). The term non-diseased means „healthy“ or „normal“. However, healthiness and normality are difficult to be defined.

The present special issue of the *Journal of Laboratory Medicine (JLM)* deals with reference intervals (RIs). Direct and indirect methods compete with each other. Both approaches have their benefits and disadvantages as outlined in the review of Haeckel et al. [2]. This review is based particularly on those of Sikaris [3], Jones et al. [4] and Farrell [5] and add more recent developments.

As shown by all contributors to this special issue, estimating reference intervals with indirect methods require less resources than direct methods and have more potentials for stratification according to several biological variables, such as age and sex. But their major disadvantage is the practicability. Older methods as Bhattacharya and Hoffmann can be performed without computers because they were developed in the pre-computerised area. They require subjective judgements, even if they are performed with newer software tools. Recent approaches as truncated maximum likelihood (TML) [6] and truncated minimum chi-square (TMC) [7] can only be performed by more or less complicated software programmes which, however, are available gratuitously [8]. These software tools have already implemented automatic stratification according to age and sex, calculation of confidence limits, diurnal variation, and many other features. They also allow estimation of RIs simultaneously for several measurands. These techniques will probably be used more widely if they

are implemented in commercially available laboratory information system (LIS). Examples are already realised [9].

A special problem provide measurands with a diurnal variation as e.g., random plasma glucose concentrations. Although this phenomenon is known since many years, it was neglected in the derivation of RLs as pointed out by Özcürümez et al. [10]. Because of the observed diurnal variations, indirectly estimated upper RLs of random glucose concentrations should be stratified for sample collection during early morning (e.g., 2:00–6:00 am) and late morning (e.g., 6:00–12:00 am). This is particular important if directly determined upper RLs are compared with indirectly estimated upper RLs [11]. Directly determined upper RLs are usually derived from samples collected during late morning. Furthermore, the authors proposed to extent their approach for the characterization of daytime dependent variations to other measurands with known or suspected diurnality.

TMC can also be used for data distributions of measurands with a relatively high percentage of results at or below the detection limits as e.g., high sensitive cardiac troponins [12], C-reactive Protein or prostate-specific antigen (PSA) and was also proposed as a tool to investigate the prevention of glycolysis in the pre-examination phase of random glucose concentrations [13].

Indirect methods are also suited for establishing common RIs. For stratification purposes, the number of contributing values may not be sufficient, especially at both ends of human life span. Then, the values from several laboratories can be combined if the pre-analytical and analytical conditions and the sub-populations are comparable [14]. Bohn and Adeli [14] address the problems of combining RIs from various laboratories to common RIs. „Combining statistical outcomes with existent clinical knowledge can facilitate the comparison of centre-specific RIs and the decision to harmonise. Analytic specific measurement uncertainty and associated allowable performance limits can also be helpful in this regard“. The latter concept is favoured in the review of Haeckel et al. [2].

Special problems are the verification of the various indirect approaches. Most studies compare the applied indirect approach with a direct method for the estimation

Rainer Haeckel, Bremer Zentrum für Laboratoriumsmedizin, Klinikum Bremen Mitte, 28305 Bremen, Germany, Phone: +49 412 273446, E-mail: rainer.haeckel@gmx.net. <https://orcid.org/0000-0001-8671-962X>

of RIs. Good agreement of both models are reported for many measurands e.g., by Bohn and Adeli [14] and for platelet indices by Hermann et al. [15]. In many other studies direct RIs taken from the literature were used which have the problem of transference which are usually neglected. For comparing the diagnostic efficiency of the various indirect methods simulation studies are most appropriate and allow statistical confidence analysis [2].

Although recent indirect approaches have reached a far advanced state, some questions still remain to be studied, as e.g., the minimum number of data required, prevalence of pathological values, effect of overlapping distributions or avoidance of binding effects. The last problem was studied by Arzideh et al. [16].

Many biological variables limit the accuracy of RIs determined by indirect but also by direct approaches. These limits received more awareness recently when the assumptions for indirect methods were studied in more detail as summarised by Sikaris [17]. Considering all variables influencing the estimation of RIs would improve the accuracy of RIs and in consequence the diagnostic efficiency of RIs. However, most laboratories are presently poorly informed by their requesters of laboratory tests and have no access to these information although they are probably available in many hospital information systems. Despite this situation, RIs indirectly estimated of unselected sub-populations are comparable with directly determined RIs in many cases [6, 7, 12, 14, 15, 17].

Manufacturers of analytical systems are obliged to provide RIs by several directives. If external derived RIs are transferred to a laboratory, many aspects must be considered [2, 4, 5]. The transference responsibility remains with the customer who often does not receive the necessary support from the manufacturers. The transference problem would disappear if laboratories would determine their own RIs. Then, the laboratory would again have sole responsibility for its RIs, and its local role in the medical decision process would regain its importance due to the professional expertise required [18].

Present concepts of RIs are confined to one measurand. It appears promising to combine the influence of two or several related measurands on estimating RIs which may be closer to decision limits than to RIs. These limits are termed functional reference limits and are reviewed by Sezgin et al. [19].

Research funding: None declared.

Author contributions: The author has accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The author states no conflict of interest.

References

1. International Standard Medical laboratories – requirements for quality and competence, ISO 15189-2012(E). ISO Copyright Office: Geneva, Switzerland; 2012:1–39 pp.
2. Haeckel R, Wosniok W, Streichert T. Review of potentials and limitations of indirect approaches for estimating reference limits of quantitative procedures in laboratory medicine. *J Lab Med* 2021;45:35–53.
3. Sikaris K. Physiology and its importance for reference intervals. *Clin Biochem Rev* 2014;35:3–14.
4. Jones GRD, Haeckel R, Loh TP, Sikaris K, Streichert T, Katayev A, et al. Indirect methods for reference interval determination – review and recommendations. *Clin Chem Lab Med* 2019;57: 20–9.
5. Farrell CJL, Nguyen L. Indirect reference intervals: harnessing the power of stored laboratory data. *Clin Biochem Rev* 2019;40: 99–111.
6. Arzideh F, Wosniok W, Gurr E, Hinsch W, Schumann G, Weinstock N, et al. A plea for intra-laboratory reference limits. Part 2. A bimodal retrospective concept for determining reference limits from intra-laboratory databases demonstrated by catalytic activity concentrations of enzymes. *Clin Chem Lab Med* 2007;45: 1043–57.
7. Wosniok W, Haeckel R. A new estimation of reference intervals: truncated minimum chi-square (TMC) approach. *Clin Chem Lab Med* 2019;57:1933–47.
8. German Society of Clinical Chemistry and Laboratory Medicine. Decision limits/guideline values. Available from: www.dgkl.de/arbeitsgruppen/entscheidungsgrenzen-richtwerte [Accessed 18 Dec 2018].
9. Amodeo B, Schindler A, Schacht U, Wahl HG. Calculation of reference intervals from existing laboratory data based on the Reference Limit Estimator integrated in the OPUS:L information system. *J Lab Med* 2021;45:131–4.
10. Özcürümez M, Arzideh F, Figge A, Haeckel R, Streichert T. The influence of sampling time on random glucose indirect reference limits, decision limits and the estimation of biological variation. *J Lab Med* 2021;45:111–9.
11. Torge A, Haeckel R, Özcürümez M, Krebs A, Junker R. Diurnal variation of leucocyte counts affects the indirect estimation of reference intervals. *J Lab Med* 2021;45:121–4.
12. Haeckel R, Wosniok W, Torge A, Junker R. Reference limits of high-sensitive cardiac troponin T indirectly estimated by a new approach applying data mining. A special example for measurands with a relatively high percentage of values at or below the detection limit. *J Lab Med* 2021;45:87–94.
13. Haeckel R, Wosniok W, Torge A, Junker R, Bertram A, Krebs A, et al. Age and sex dependent reference intervals for random plasma/serum glucose concentrations related to different sampling devices and determined by an indirect procedure with data mining. Urgent plea for studying the diagnostic efficiency of various concepts proposed to improve the pre-examination phase for determining blood glucose concentrations. *J Lab Med* 2021;45:95–101.

14. Bohn MK, Adeli K. Application of the TML method to big data analysis and reference interval harmonization. *J Lab Med* 2021;45:79–85.
15. Hermann W, Risch L, Nydegger UE, Sakem B, Imperali M, Renz H, et al. Reference intervals for platelets indices in seniors and frequency of abnormal results in a population based setting – a comparison between directly and indirectly estimated reference intervals. *J Lab Med* 2021;45:125–9.
16. Arzideh F, Öczüzümez M, Albers E, Haeckel R, Streichert T. Indirect estimation of reference intervals using first, last results or results of data from patients without repeated measurements. *J Lab Med* 2021;45:103–10.
17. Sikaris K. Dealing with disease in indirect reference limit determination. *J Lab Med* 2021;45:55–68.
18. Haeckel R, Wosniok W, Arzideh F. A plea for intra-laboratory reference limits. Part 1. General considerations and concepts for determination. *Clin Chem Lab Med* 2007;45:1033–42.
19. Sezgin G, Loh TP, Markus C. From distribution-based reference intervals to functional reference limits: a case study of serum ferritin. *J Lab Med* 2021;45:69–77.