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7.5 Common Reference Intervals for Plasma Proteins in the Nordic Countries

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It is a philosophic question whether it is possible to establish common reference intervals valid for different ethnic groups and geographical areas. From a practical viewpoint, however, reference intervals are in current use and seem to be the best tool for a general validation of the first results from persons consulting the health care system - when clinical strategies with clear interpretation of results are missing. The reference individuals should be selected and the measurements and calculations should be performed according to IFCC (1, 3) or equivalent (cf. section 7.1). An important point, however, is the decisions about dividing and combining different reference intervals as investigated by Harris and Boyd (2). According to them the problem is mainly a statistical problem, but as discussed in chapters 7.1, 7.2 and 7.3, it is also a question of judgements based on biology.

Presumptions for Sharing Common Reference Intervals

Three main conditions have to be fulfilled for establishing common reference intervals:

- The analytical quality must be common
- The biology must be common, and
- The interpretation of data must be common.

The analytical quality must be common

In principle it is sufficient just to have the same analytical quality in all the laboratories involved, but in practice this can only be obtained by using either the same method (calibrator, reagents, equipment, instructions etc.) or by using specific methods with first class calibrators with values traceable to the highest level of trueness. Only the last solution is of interest for plasma proteins. The IFCC/CAP/BCR 470 reference preparation for plasma proteins makes it possible to obtain the needed standardization. The Nordic Calibrator has concentration values traceable to this reference preparation (cf. chapter 5). The main problem of plasma protein analyses, the unspecific reactions from turbid samples for nephelometric methods can be solved by ultracentrifugation of the samples (cf. chapter 6). So, the analytical prerequisites are available for establishing common reference intervals.

The biology must be common

We have investigated two ethnic groups living in different geographical areas in the Nordic countries. We did only find a difference for S-Haptoglobin of such an importance that we decided to specify different reference intervals in Finland and Denmark for this protein, probably due to differences in genetical subtypes of this protein. The results for immunoglobulins indicated variations, which may be of importance, but the tendencies between the two sexes were conflicting and could not justify any splitting up in Finnish and Danish reference intervals, as the groups used for the comparison were too small in size and only related to the age group between 30 and 40 years of age. The question about the immunoglobulins may be clarified by Swedish investigations where no increase in S-IgA with increasing age was found (personal communication). Thus, differences in biology may be disclosed by large scale investigations designed according to more specific questions.

The interpretation of data must be common

We have not used the IFCC recommendations for estimation of the reference intervals as we wanted to use the probit display in order to validate possible combinings of reference intervals for different groups (cf. sections 7.1 and 7.2). The results, however, are valid as the distributions are close to log-Gaussian (sections 7.2, 7.3, and 7.4).

The comparison between Finnish and Danish showed that in the Nordic countries it could be possible to establish common reference intervals for the seven proteins, with S-Haptoglobin and S-IgA as exceptions - and with the methodological exceptions for S-α_1-Antitrypsin (cf. section 7.3).

Reference Intervals for Nine Plasma Proteins

Table 7.5.1	Plasma Proteins (conc. in g/L) (95 %-intervals)					
Protein	**Group**	**Reference interval**	**Group**	**Reference interval**	**Group**	**Reference interval**
Prealbumin Transthyretin	M (all) W (>50) W (+E)	**0.26-0.45**	W (-E)	**0.23-0.39**		
Albumin *	M (>50) W (>50) W (+E)	**36.6-48.2**	M (<50) W (-E)	**39.6-51.1**		
Orosomucoid α_1-Acid Glycoprotein	M (all) W (>50)	**0.54-1.17**	W (-E)	**0.45-1.08**	W (+E)	**0.40-0.95**
α_1-Antitrypsin ** α_1-Trypsin Inhibitor	M (all) W (>50) W (-E)	**(0.97-1.68)**	W (+E)	**(1.29-2.23)**	Type MS Type MZ	**(0.85-1.32)** **(0.60-0.99)**
Haptoglobin ***	M (>50) W (>50)	**D: 0.47-2.05** **F: 0.32-1.90**	M (<50) W (-E) W (+E)	**D: 0.35-1.85** **F: 0.20-1.70**		
Transferrin	M (all) W (>50) W (-E)	**1.94-3.26**	W (+E)	**2.25-3.85**		
IgA **** Immunoglobulin A	M (>50) W (>50)	**0.70-4.30**	M (<50) W (-E) W (+E)	**0.70-3.65**		
IgG Immunoglobulin G	M (all) W (>50)	**6.1-14.9**	W (-E) W (+E)	**6.9-15.7**		
IgM Immunoglobulin M	M (all) W (>50)	**0.39-2.08**	W (-E) W (+E)	**0.55-2.30**		

M (all) = all men,
M (>50) = men over 50 years,
M (<50) = men under 50 years,
W (>50) = women over 50 years,
W (-E) = women under 50 years, not using estrogens,
W (+E) = women under 50 years, using estrogens,

* The reference intervals for S-Albumin include an analytic CV_A ~ 3.4 %.
** Reference intervals for Type MM and for MS and MZ (without estrogen group).
The results for S-α_1-Antitrypsin are only valid when antisera from DAKO are used.
See text and chapters 5 and 6.
*** D = Danes, F = Finns.
Slightly lower S-Haptoglobin-values in the Finnish population, approx. 0.15 g/L.
**** Non-parametric estimates.

All individuals sitting at least 15 min before blood sampling in arm-vein.

Use of different units (grams or moles) together with minor differences in presentation may, however, give a heterogeneous impression. So, even if the same material with the same method for estimation of the reference intervals have been used the list will be different in each of the Nordic countries. This situation was not foreseen at any time during the projects, and it was a great surprise to us. The table with reference intervals for the nine plasma proteins are the results estimated by the project group - with values traceable to CRM 470.

Discussion

There are several week points in the reference intervals produced.

Analytical problems

Concerning S-α_1-Antitrypsin a problem with the transfer of values from CRM 470 to the Nordic calibrator exists (cf. chapter 5). Therefore, at present we can only guarantee the reference intervals for this protein when DAKO antisera are used. For the other eight proteins there should be no problems as long as the patient samples are not turbid. Turbid samples for nephelometry should be ultracentrifuged.

Estimation of the reference intervals

The division of reference intervals into two groups for the eight proteins (three for S-Orosomucoid) is more or less obvious. In more questionable cases it has been our decision and we are responsible for the conclusion in each case. In all the cases with convincing log-Gaussian distributions the division seems to be reasonable, but for S-Haptoglobin and S-IgA the non-log-Gaussian distributions point to problems indicating that the division is not optimal.

Are the reference intervals common ?

The question whether the reference intervals are common for the Nordic countries - except from S-Haptoglobin and S-IgA can only be answered by a new and extended investigation with a special design for the clarification of the weaker points in this work.

Are hospital employees and their relatives representative ?

This question cannot be answered by this investigation, but it should be examined in another project.

The present situation where each laboratory has its own reference interval for each quantity is not justified by the analytical methods in use. Even with such a performance due to the analytical method, the procedure cannot be characterized as professional, as there are no biological reasons justifying different reference intervals except from ethnic differences and environmental conditions - and this has not been taken into consideration when laboratory-individual reference intervals were established.

There are good reasons for establishing common reference intervals in the Nordic countries - and even with the incomplete attempt described here for the nine plasma proteins - the use of common reference intervals is the only way to strive for. The analytical possibilities based on common calibration and specific methods are the logical consequence for the plasma proteins. It is in any case better than to maintain the situation of laboratory-individual reference intervals.

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