Upsala J Med Sci 99: 271–276, 1994

5.5 Evaluation of Target Values for α_1-Antitrypsin

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In section 5.4 a discrepancy was disclosed for α_1-Antitrypsin regarding the transfer of concentration values from the IFCC reference preparation (CRM 470) to The Nordic Calibrator between the nephelometric method (BNA, Behringwerke) and the turbidimetric methods (three Cobas Fara, Roche, and one Hitachi 911). This difference, about 20 %, could not be neglected and further investigation was therefore needed.

Differences in the Measurement Procedures

The main difference in the measurement procedures is the analytical principle, nephelometric and turbidimetric for the BNA and the other instruments, respectively. Both calibrators (CRM 470 and The Nordic Calibrator) are, however, measured in the same runs (in several dilutions and in replicates) so, the difference must be related to the structure of α_1-Antitrypsin in the two calibrators.

According to the IFCC-protocol for value transfer antisera from Behringwerke are to be used for the BNA-measurements and antisera from DAKO for the other instruments.

The possible reasons for the difference are

* Analytical principle
* Antisera
* Structure of α_1-Antitrypsin in the two calibrators

Therefore, a comparison to fresh human sera was decided with a design where both calibrators were measured according to the two analytical principles using both antisera.

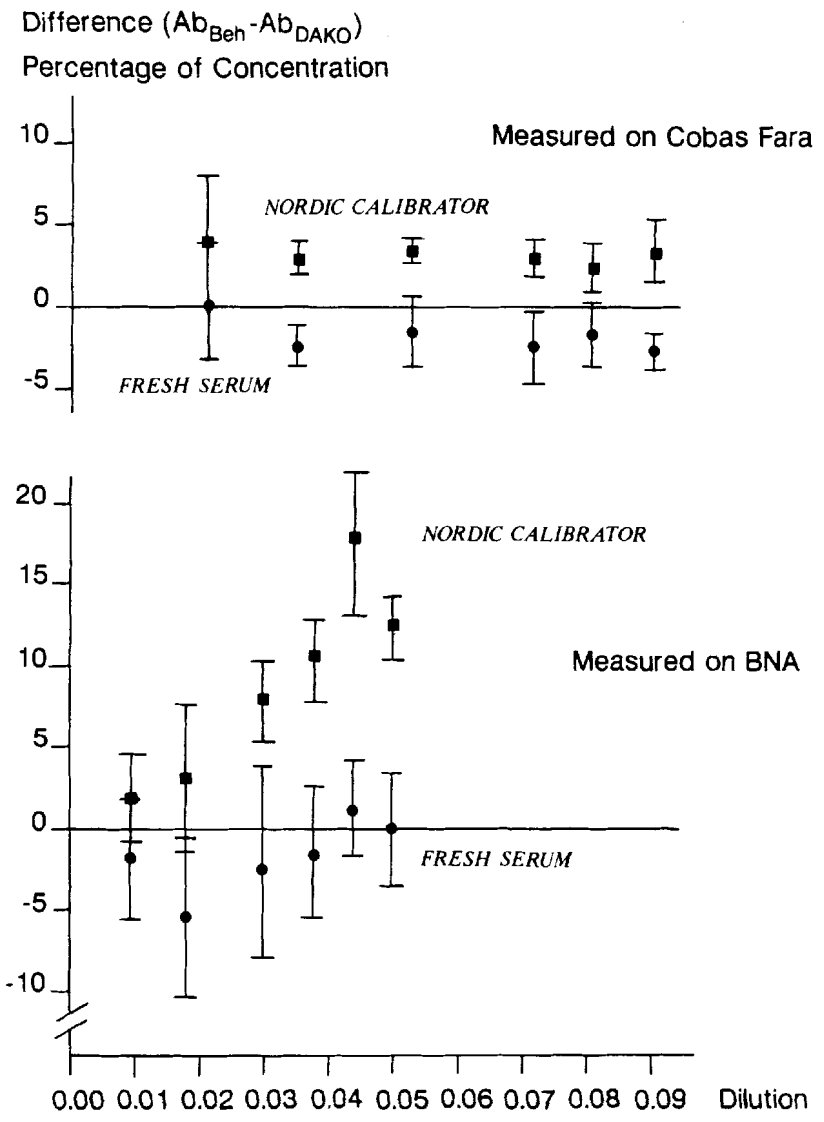

Fig. 5.5.1 *Differences in concentrations of α_1-Antitrypsin measured with antisera from Behringwerke and from DAKO {Δ = conc(Beh) - conc(DAKO)} of dilutions of a fresh human serum (•) and of The Nordic Calibrator (■), using CRM 470 as primary calibrator, plotted as function of dilution. 90 % confidence intervals are indicated.*
Upper: *Measurements on Cobas Fara*
Lower: *Measurements on BNA*

Materials and Design of Investigation

Calibrators: CRM 470: Collected from February to September 1990, lyophilized in June 1991.
The Nordic Protein Calibrator: Collected from August to December 1987, ultracentrifuged from February to September 1989, alliquoted in November 1989, stored at - 80 °C.
Date of investigation: 25.-26. August 1994.

Fresh human sera: Ten fresh sera from healthy volunteers.
A fresh serum 'pool', from one healthy volunteer, type MM.

Instruments: BNA Nephelometer (Behringwerke) and Cobas Fara (Roche).

Design: Both calibrators and the ten fresh sera and the pool were analysed on both instruments and with both antisera in a total of four runs within the same day.
The two calibrators and one fresh serum were determined in six dilutions according to the IFCC-protocol (i.e. that the weight of each dilution step was controlled), and CRM 470 was used as the primary calibrator. The fresh pool and the rest of the sera were determined in one dilution. The four runs gave four independent calibration curves.

Blank reaction: An independant control run with a non-immune gammaglobulin preparation (DAKO) was performed on both instruments.

Correction: In the BNA-measurements the results for the lowest dilution deviated from the remaining dilutions and these values were omitted.

Results

In Fig. 5.5.1 the effect of antisera on measurements of the concentrations of α_1-Antitrypsin in a fresh human serum and The Nordic Calibrator (using CRM 470 as the primary calibrator) are shown as function of the dilution for the two instruments. The ordinate is the difference between concentrations measured by the two antisera in percentage. For the Cobas Fara (upper figure), the effect of antiserum is small. The effect on the two materials, however, is negative (approx. - 2 %) for the fresh pool and

positive (approx. 3 %) for The Nordic Calibrator. The results from measurements on the BNA-instrument (lower figure) show more pronounced differences between the two materials. Whereas, the effect of antiserum is negligible (but variable) for the fresh serum, the effect on The Nordic Calibrator is increasing from negligible to approx. 15 %.

For the fresh sera and the fresh pool the results of measurements of α_1-Antitrypsin (from one dilution) are illustrated in Fig. 5.5.2. Here, results from both instruments are shown in one figure. On the Cobas Fara the differences are distributed about zero, and on the BNA the differences are from - 1 to +5 % (mean of approx. 3.5 %).

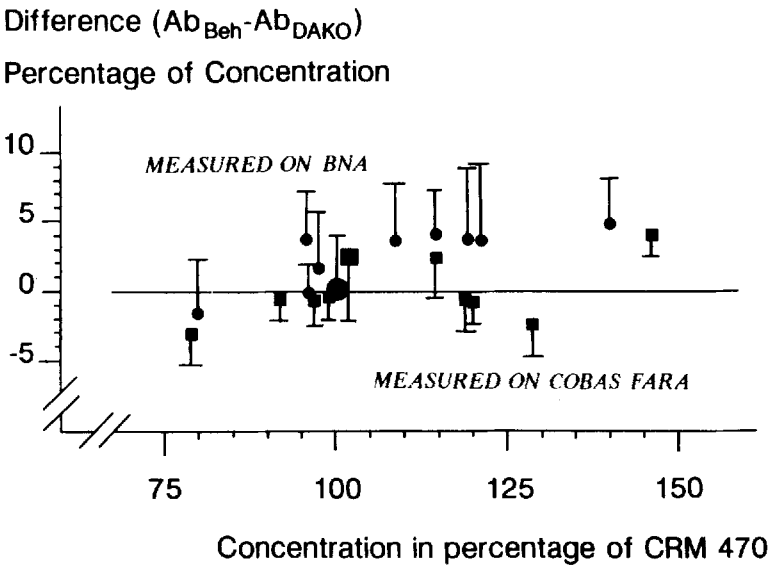

Fig 5.5.2. *Difference of α_1-Antitrypsin in fresh sera measured with antisera from Behringwerke and DAKO {Δ = conc(Behring) - conc(DAKO)} (as percentage of concentration) measured on BNA (•) and on Cobas Fara (■), using CRM 470 as primary calibrator, and plotted as function of concentration. 90 % confidence intervals are indicated.*

Discussion

Evaluation of the results may be a little complicated, but it is clear that the 'signals' from measurements of α_1-Antitrypsin in CRM 470 and the fresh sera and pool are very close in all the combinations with maximum deviations of 5 %. This means that the

structure of the protein in CRM 470 is very close to the genuine as judged from the antibodies in the two antisera. Minor differences can, however, be seen as measurements on the BNA of fresh sera results in a difference of approx. 3.5 % when the two antibodies are used.

Regarding The Nordic Calibrator, the deviations from the fresh sera is approx. five percent on the Cobas Fara and variable (up to 15 percent) on the BNA. The changes in structure of the protein, therefore, seem to be more pronounced or of an other type, which is disclosed by the two antisera, and to a greater extent by the nephelometric analytical principle.

The changes in α_1-Antitrypsin structure is not seen in the electrophoretic mobility as demonstrated in section 5.3, or in loss of protein, as the concentration in the calibrator is close to the mean value of the reference interval (even the distribution is log-Gaussian). The changes seem to be related to some steps in the preparation procedure as a hint was given by preparation of a new calibrator, where the serum pool by accident was filtered after the ultracentrifugation. In this preparation the effect is doubled, with deviations from fresh sera from zero up to approx. 30 percent (results not shown).

It has not been investigated whether commercial calibrators have the same problems, but evaluations of a series of these (cf. section 5.3) may indicate serious problems for α_1-Antitrypsin in these preparations. With the description of the production of CRM 470 (cf. sections 5.1 and 5.2 and ref. 1), however, the means for producing reliable commercial calibrators are given.

The described deviations have some impact on the estimated reference intervals for S-α_1-Antitrypsin:

* The reference intervals (cf. chapter 7) are correct for turbidimetric methods using The Nordic Calibrator and antisera from DAKO
* In direct relation to CRM 470, i.e. used as calibrator for the actual measurements, the reference intervals are valid as well
* For measurements on BNA with antisera from Behringwerke, and by use of The Nordic Calibrator, the concentration value of S-α_1-Antitrypsin for the calibrator should be approx. 15 % lower
* For all other calibrators the validity should be tested in direct relation to CRM 470 and fresh human sera.

These results disclose problems with α_1-Antitrypsin in The Nordic Calibrator and in the majority of commercial calibrators as well, resulting in restrictions for the use of the common reference intervals.

Conclusions

Due to the problems with the structure of α_1-Antitrypsin in The Nordic Calibrator the estimated reference intervals for this protein are only valid by use of The Nordic Calibrator, or by direct investigation of the secondary (commercial) calibrator in relation to CRM 470 and a number of fresh human sera.

Acknowledgements

We want to thank Marianne Käehne for her skilful analytical work.

References

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