Upsala J Med Sci 99: 339–346, 1994

7.3 Reference Intervals for α_1-Antitrypsin

Erik Lund[1], Ole Blaabjerg[2], Mogens Blom[3], Per Hyltoft Petersen[2], Jens Rahbek Nørgaard[2], Adam Uldall[4], Hanne Gry[5], Ivan Brandslund[1]

1. Department of Clinical Chemistry, Vejle County Hospital, DK-7100 Vejle, Denmark
2. Department of Clinical Chemistry, Odense University Hospital, DK-5000 Odense C, Denmark.
3. Department of Clinical Chemistry, Hjørring Sygehus, DK-9800 Hjørring, Denmark.
4. Department of Clinical Chemistry, KAS Herlev, DK-2730 Herlev, Denmark.
5. Medi-lab, Adelgade 5-7, DK-1304 Copenhagen, Denmark.

The plasma protein α_1-Antitrypsin (α_1-Proteinase Inhibitor) has many phenotypes, which have a major influence on the concentrations as measured in serum. The most common phenotype in Denmark is the MM-type, less common are the two heterozygote types MS and MZ, and with low frequency the heterozygote SZ and the homozygotes SS and ZZ. As heterozygotes have lower plasma concentrations, we decided to perform the phenotyping of the reference individuals in order to estimate separate reference intervals for type MM and the two heterozygote types MS and MZ.

It has recently been proposed, that if relevant reference intervals for the different phenotypes existed, and certain quality goals could be met (1), quantification of α_1-Antitrypsin could be used to rule out the disease-causing ZZ and SZ phenotypes - and also to identify all possible patients heterozygous for the Z-allele. It may also be reasonable to discontinue the acceptance of MZ as a normal variant, as recent publications points to this phenotype as predisposing to lung disease under certain conditions (3). Hence, only the MM's should determine the 'normal' reference interval.

Analytical Methods

Identification of genetically determined variants for α_1-Antitrypsin was done by phenotyping the α_1-Antitrypsin protein in serum by isoelectric focusing. The procedure described by Görg *et al.* (2) on Immobilize® DryPlates premade by LKB, Sweden (No 80-1128-29, pH 4.2-4.9) was used. In brief, gels were reswelled in 25 % w/v glycerol (applc. note 345, Pharmacia), serum was reduced with dithiothreitol, blocked with iodoacetamide, and 15 µL applied to the gel (4). The electrophoresis was run at 500 V, 2mA for 18 hours, the last 30 minutes at 2500 V, 2mA, at 12 °C on the

LKB Multiphor electrofocusing unit supplied with the LKB 2197 power supply. The proteins were passively transferred by blotting to Immobilon^{TM}-P membranes (Millipore Corp., USA) and the blot-papers stained in Coomassie Brilliant blue R250, (Merck, FRG). This procedure was developed to facilitate storage, filing and reading of the results. The dried paper blots were interpreted by three persons, independently. This was done using known variants (MM, MS, MZ, SS, and ZZ) as references.

Measurements of all samples were performed at the same time as the other eight proteins (cf. Section 7.2) on a Cobas Fara (Roche) according to the method recommendations from DAKO with antisera from DAKO using the Nordic calibrator with values traceable to BCR 470.

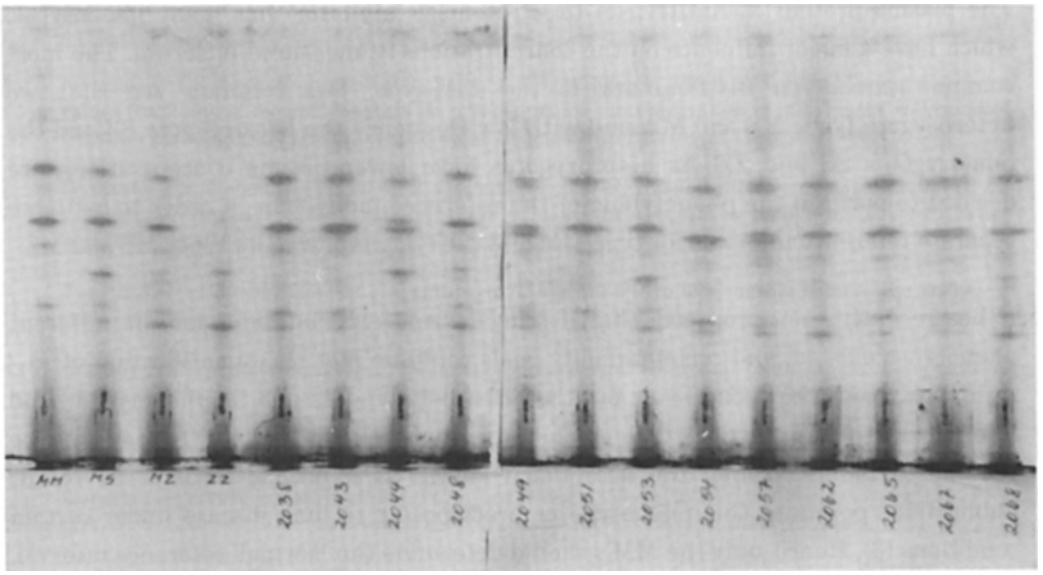

Fig. 7.3.1. *Illustration of the electrophoretic technique used for phenotyping the individuals for estimating of reference intervals for the plasma protein α_1-Antitrypsin.*

Distribution of phenotypes

Among the 516 individuals accepted as reference population, seven was not phenotyped because of lack of sample or lost sample identification. The distribution of phenotypes in the remaining 509 individuals is shown in Table 7.3.1.

Table 7.3.1 α_1-Antitrypsin Phenotypes

	MM	MS	MZ	SS	SZ	ZZ	Other	Total
Men	181	9	5	0	0	0	5	200
Women								
not using estrogens	175	18	8	0	1	1	2	205
using estrogens	92	6	6	0	0	0	0	104
Total	448	33	19	0	1	1	7	509

Biological Variation and Reference Intervals

The biological variations are illustrated in Fig. 7.3.2 with mean values and 90 % confidence intervals for each subgroup (cf. chapter 7.2) using log-concentrations as ordinate and age-groups as abscissa. The women using estrogens are separated from the remaining groups with approx. 30 % higher values, whereas, the rest are disclosing an astounding constancy.

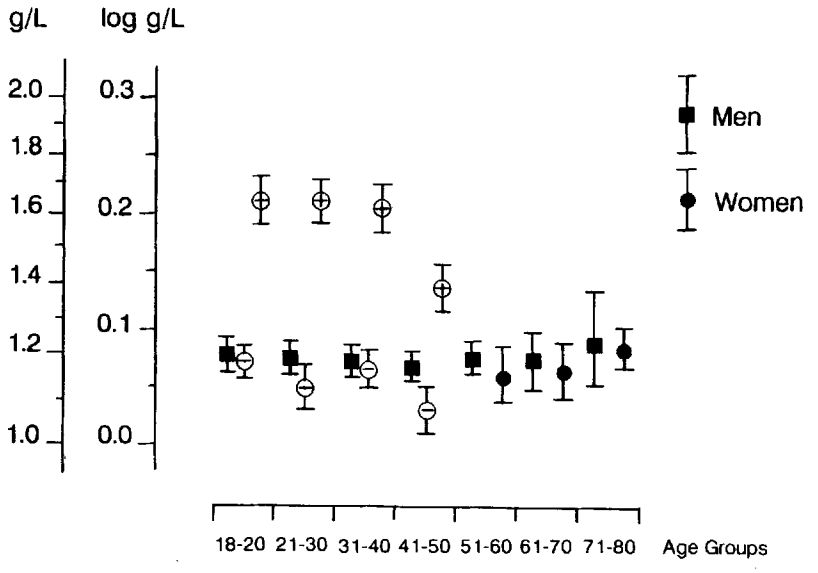

Fig. 7.3.2. *Mean concentrations with 90 % CI for each subgroup shown on a log-scale in relation to age for α_1-Antitrypsin.*

Distributions of the two MM-groups, i.e. women using estrogens (n = 92) and the remaining group (n = 356) are clearly straight lines indicating log-Gaussian distributions with the lines close to be parallel. The two heterogeneous groups (n = 13 for MZ and n = 27 for type MS) consist of fewer individuals, so the question of whether they are log-Gaussian can not be answered. Both distributions, however, show lower values with MZ approx. 60 % and MS approx. 80 % of the MM-values.

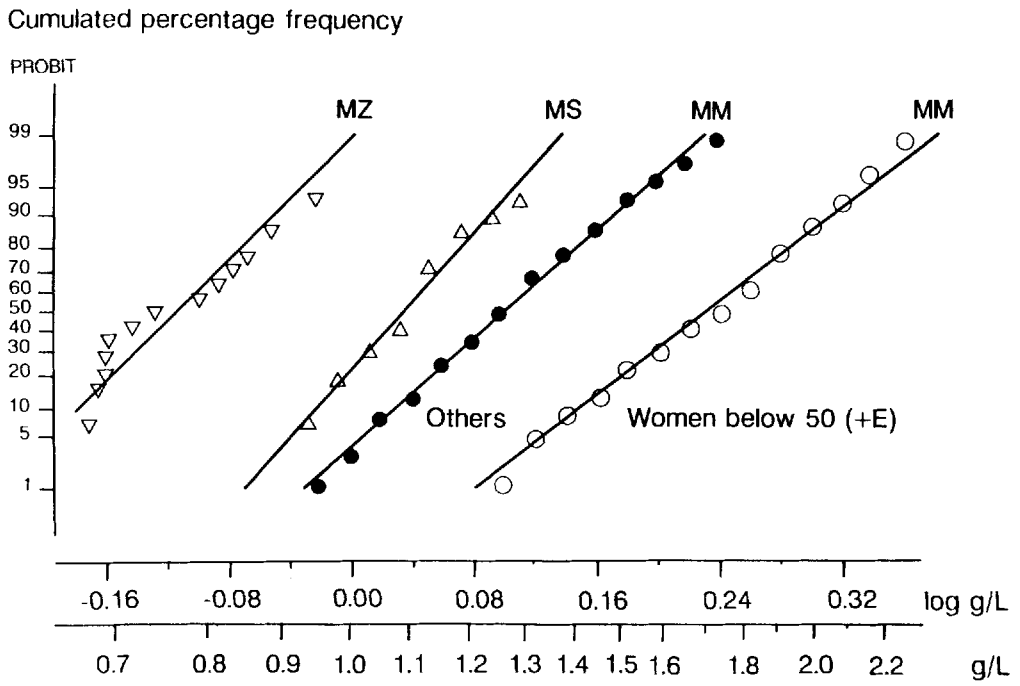

Fig. 7.3.3. *Probit-plot with log-abscissa showing α_1-Antitrypsin distributions for the phenotypes MZ, MS, and MM, where the MM-group is separated into women using estrogens and the remaining group.*

As for S-Transferrin (chapter 7.1 and 7.2) the group of women using estrogens is separated into 'high dose group' and 'low dose group' indicating negligible effect for the 'low dose group' as illustrated in Fig. 7.3.4.

The reference interval for women below 50 years of age using estrogens (type MM) is 1.29 to 2.23 g/L and for the remaining group (type MM) 0.97 to 1.68 g/L, whereas, for type MS it is 0.85 to 1.32 g/L and for type MZ 0.60 to 0.99 g/L (both without women using estrogens).

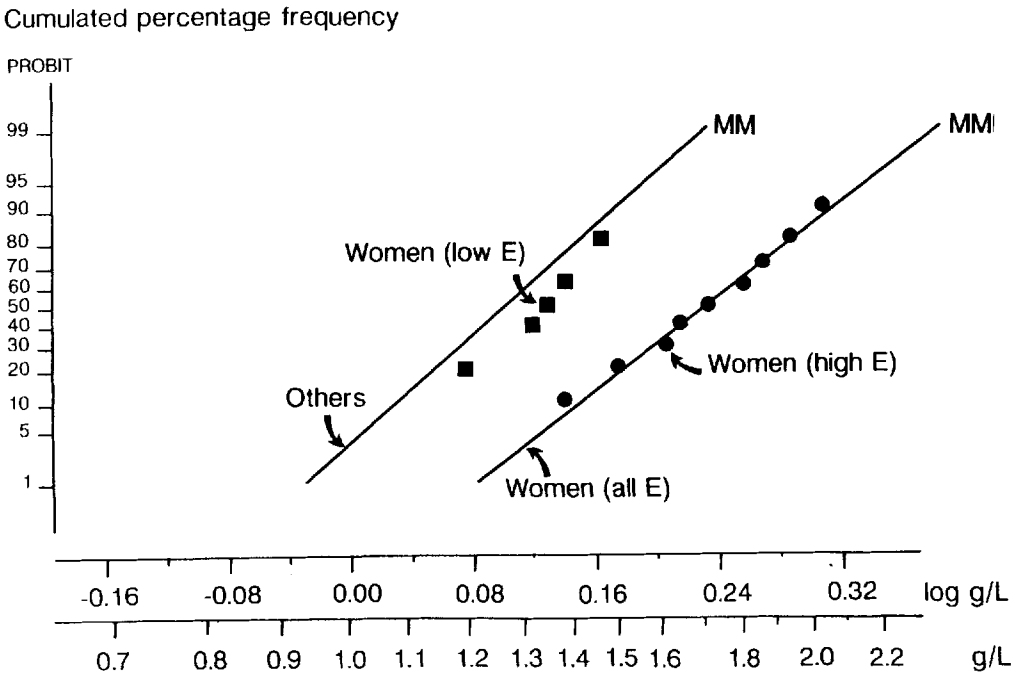

Fig. 7.3.4. *Distributions of α_1-Antitrypsin for women using high and low dose of estrogens.*

Results for the excluded individuals according to the rule out criteria

As described for the other eight proteins 37 individuals were ruled out due to high Erythrocyte Sedimentation Rate, high S-CRP or the presence of M-Components. Details of the groups as for S-Transferrin (cf. sections 7.1 and 7.2).

The distributions are shown in Fig. 7.3.5 on a probit-scale, for type MM only (women below 50 years of age using estrogens and the remaining group). There is a clear increase in all S-α_1-Antitrypsin values for individuals with elevated S-CRP. For M-Components and elevated Sedimentation Rate there is a tendency towards higher values with broader distributions.

Discussion

The limitations for evaluation of S-α_1-Antitrypsin are the same as for the other eight proteins (cf. chapter 7.2) but, in addition the separation into phenotypes MM, MS, and MZ (and others) reduces the number of individuals in the subgroups. Therefore, the reference intervals for MS and MZ are to be considered more as guidelines than *exact.*

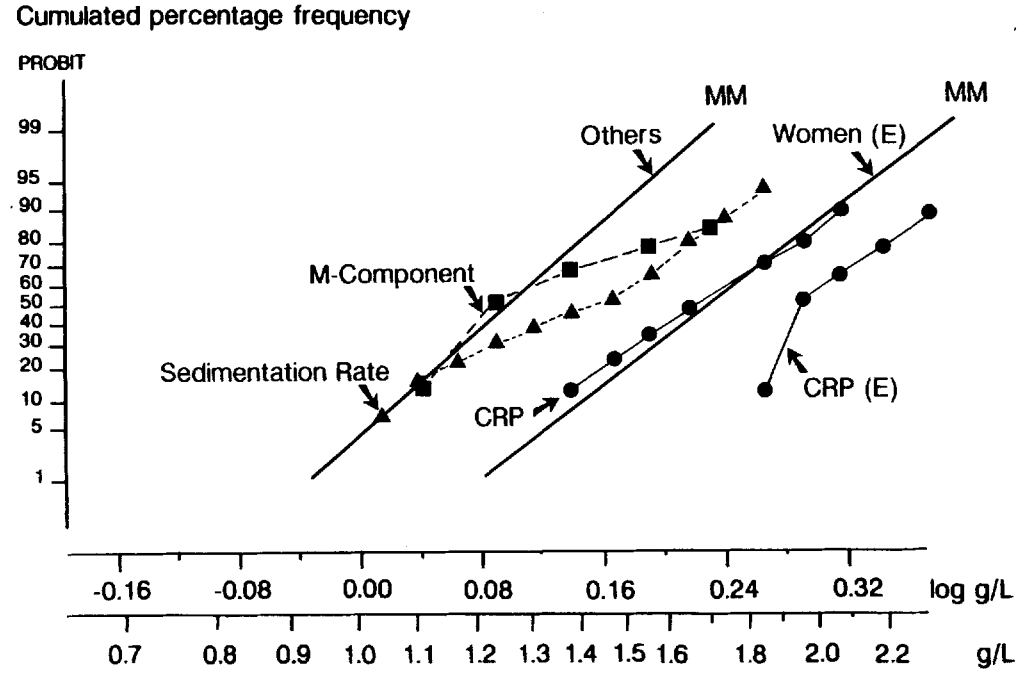

Fig. 7.3.5. *Probit-plot with log-abscissa showing α_1-Antitrypsin distributions for the phenotype MM, the ruled out individuals.*

Due to the difficulties in the traceability of the values to CRM 470 as described in chapters 5 and 6, there is an additional problem for S-α_1-Antitrypsin, as the results are dependent on the analytical principles and the antisera used. Therefore, the reference intervals are valid for turbidimetric methods and with the use of antisera from DAKO, only. For other methods and other antisera, the traceability has to be documented (section 5.5).

Acknowledgements

We want to thank all the persons who have contributed to this work, volunteers who gave blood to the investigation and the 20 Danish laboratories involved in collecting the blood samples and doing analytical work. We are specially in debt of gratitude to Yrsa Eriksen and Gudrun Andersen, who performed the isoelectric focusing, and to Inger Nørgaard, who performed the protein determinations and the agarose electrophoreses for estimation of M-Components, and to Ane Richter, who performed the registrations and calculations of the many results. Further we are grateful to Søren Blirup and Per Just Svendsen, who performed the measurements of S-CRP and for the donation of antisera used in all the determinations delivered by DAKO .

References

1. Brandslund I, Lund ED, Sigsgaard T. Is a low serum concentration of α_1-Antitrypsin associated with an increased susceptibility for byssinosis in cotton mill workers? *Upsala J Med Sci* 1993;98:299-310.

2. Görg A, Postel W, Weser J, Weidinger S, Patutschnick W, Cleve H. Isoelectrick focusing in immobilized pH gradients for the determination of the genetic Pi (α_1-antitrypsin) variants. *Electrophoresis* 1983;4:153-7.

3. Sigsgaard T, Brandslund I, Rasmussen JB, Lund ED, Varming H. Low normal α_1-Antitrypsin serum concentrations and MZ-phenotype are associated with byssinosis and familial allergy in cotton mill workers. *Pharmacogenetics* 1994;4:135-41.

4. Thymann M. Distribution of Alpha-1-antitrypsin (Pi) phenotypes in Denmark determined by separator isoelectric focusing in agarose gel. *Hum Hered* 1986;36:19-23.