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6.1.1.4 Is a Low Serum Concentration of α1-Antitrypsin Associated with an Increased Susceptibility for Byssinosis in Cotton Mill Workers? Considerations Regarding Analytical Quality Requirements and Economical Consequences

Ivan Brandslund,[1] Erik D. Lund[1] and Torben Sigsgaard[2]

[1]*Department of Clinical Chemistry, Vejle County Hospital, DK-7100 Vejle,* [2]*Institute of Environmental and Occupational Medicine, Aarhus University, DK-8000, Aarhus, Denmark*

ABSTRACT

We have previously demonstrated an association between development of the cotton lung disease byssinosis and endotoxin concentrations in the work environment. Endotoxin has been shown to exert its effects through granulocyte activation and hence release of elastase and other proteases at the bronchoalveolar surface. α1-Antitrypsin is a protease inhibitor, and hence, α1-Antitrypsin concentrations in the blood and then on the alveolar surface might be important for the protection against endotoxin effects.

Airborne endotoxin concentrations in the work place and S-α1-Antitrypsin (a1A) was measured in 226 workers in cotton mills in Vejle and of these 206 were further phenotyped.

The following models were considered:

Model 1. The S-a1A concentration is determining the risk for development of byssinosis.The lower the concentration, the higher the risk.

Model 2. The degree of exposure to endotoxin is determining. The higher the airborne concentration and the longer time working in that, the higher is the risk.

Model 3. The phenotype of a1-A is determining. Only MS and/or MZ phenotypes represent a risk disposition.

The goals for analytical quality for a1-A measurements were estimated in the two relevant models. The specifications are:

Regarding model 1: analytical coefficient of variation CV_A <3% and analytical bias - 1 μmol/L $< B_A <$ + 1μmol/L.

Model 2: a1-A is not of significant importance and specifications cannot be evaluated.
Regarding model 3: There is a direct relationship between cutt-off point and analytical performance, e.g. an imprecision of S_A 3 μmol/L and cutt-off of 38 μmol/L will allow for a B_A of -1 μmol/L.

CLINICAL SITUATION

The association between emphysema of the lung and a1-A deficiency was established by Eriksson (1). Studies on the association between intermediate deficiency and lung disease have not been conclusive.

In a previous study of cotton workers the airborne concentration of respirable endotoxin was found to be associated with the cotton lung disease byssinosis (2). We expected the underlying mechanism to be inflammation, and as we knew that some of the mediators released by granulocytes during inflammation is inhibited by a1-A, we planned this study to evaluate a possible connection between low serum concentrations of a1-A and the prevalence of byssinosis among cotton workers in the two cotton mills in Denmark, both located in Vejle.

We found a rise in prevalence of byssinosis with a1-A below 35 μmol/L , 5 of 18 (28%) against 25 of 208 (12%) with a1-A above 35 μmol/L ($p \leq 0.10$, Fisher's exact test). In a multiple logistic regression model controlling for confounding effects of endotoxin,tobacco, sex and age, the odds ratio for byssinosis was significantly raised for values below 35 μmol/L to 5.0 (95% c.i. 1.4-17). The low a1-A almost exclusively belonged to the MZ -phenotype, and the odds ratio for byssinosis determined by MZ accordingly was 5.8 (95% c.i. 1.1-30). The findings also show, that low a1-A is diagnostic for the MZ phenotype.

CHARACTERISTICS OF THE METHODS
QUANTIFICATION OF S-a1-ANTITRYPSIN

Serum a1-A was determined by turbidometry using a Roche Cobas Fara centrifugal analyzer with antibodies (code no Q363) and procedure (application note PJS/880406) from Dakopatts, (Copenhagen). The 95% reference interval in 100 healthy persons, unselected according to Pi-phenotypes, was 22-56 μmol/ L. As calibrator was used N-

Protein Standard Serum 2.19 g/l = 40.56 μmol/l, from Behringwerke AG, FRG (Lot no, 067635). As a control for low values Seronorm Human (Nycomed, Norway 26.0 μmol/L) was chosen. The total CV% registered around the designated value of 26.0 μmol/L was 3.1%. The measured value based on 15 determinations was 25.0 μmol/L, bias hence -1 μmol/L (-4%).

The high control was HK87 from the Danish Society for Clinical Chemistry with a national consensus value of 36.68 μmol/L. The total CV% was 3.2% and the measured value based on 15 determinations was 38.0 μmol/L, "bias" from consensus value then +1.3μmol/L (+3.6%).

Phenotyping of genetic variants of a1-Antitrypsin

Identification of genetically determined variants for a1-A was done by phenotyping the protein in serum by isoelectric focusing on immobiline dry plates premade by LKB, Sweden (no 1824-420, pH 4.2-4.9).

Evaluation of quality specifications

Model 1. The a-1A concentration is determining for the development of byssinosis.

This model presumes, that there is an insignificant difference in exposure to the harmful and byssinosis - causing agent, endotoxin, from the raw cotton balls.

Figure 1 shows the distribution of the 226 workers' S-a1-A concentrations. It is seen, that none are below the lower 95% reference interval of 22 μmol/L, this value being based on measurements on healthy persons unselected according to phenotypes. It is further seen, that a large proportion is above the reference interval of 56 μmol/L. This could be in response to the exposition to endotoxin.

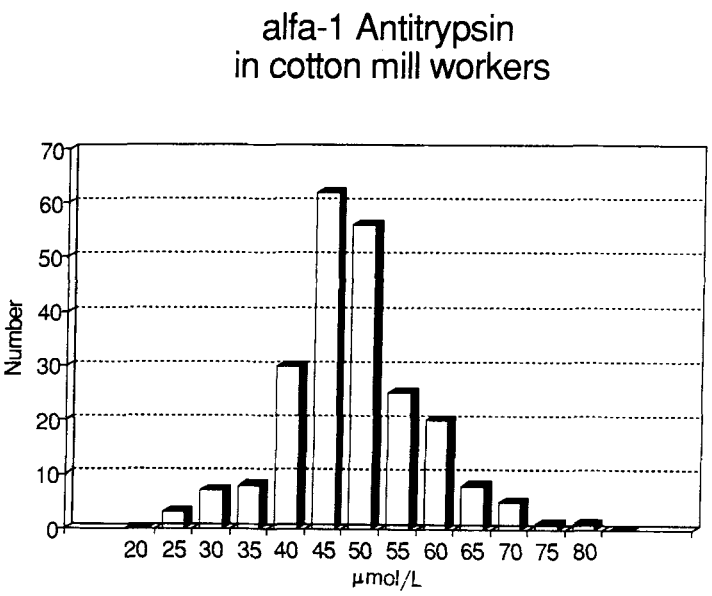

Figure 1. The distribution of alfa 1-Antitrypsin concentrations in 226 cotton mill workers. The 95% reference interval in a normal population, unselected according to Pi- phenotype, was 22 - 56 µmol/L.

Figure 2 shows the observed prevalence of byssinosis according to a1-A concentration. Five of 18 with a a1-A below 35 µmol/L have byssinosis, with a possible higher percentage the lower the a1-A, against 25 of 208 above 35 µmol/L ($p \leq 0.10$, Fisher´s exact test).

Further, in a logistic regression model on the SPSS program, the reference odds risk of 1 was defined in the group exposed to 0 - 9 ng/m3 of endotoxin. In the group with a1-A below 35 µmol/L the odds ratio was increased to 5 (95 % c.i. 1.4 - 18). This consolidates the validity of the finding demonstrated in fig. 2.

This means, that model 1 is correct, and hence, the quality of S-a1-A quantifications is important.

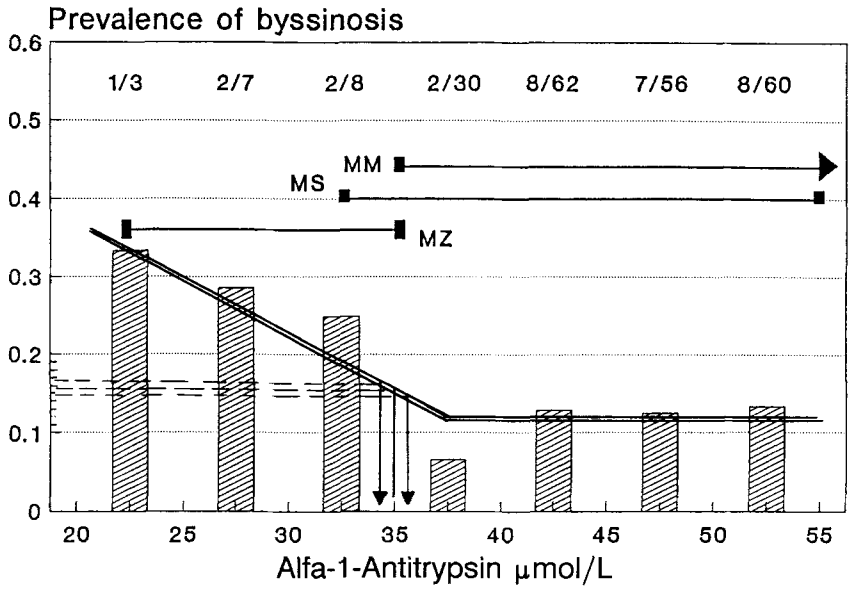

Figure 2. Observed prevalence of byssinosis according to a1-Antitrypsin concentration, and phenotype according to a1-A. Numbers state observed absolute number of workers. Double line is expected absolute risk of byssinosis. Arrows indicate allowable bias provided a risk assessment accuracy of +/- 1% is acceptable.

It is seen in fig. 2, that if it is desirable to detect an absolute risk above the general level ("normal" risk level) of 12 % with a margin of +/- 1%, then a bias of about -1 µmol/L will be acceptable, as it will falsely increase the risk prognosis with 1%. Reversely, a bias of +1 µmol/L will falsely reduce the risk prognosis with about 1%. Above 40 µmol/L the bias becomes less and less important.

The observed bias in the critical concentration area was +1.3 µmol/L, which is less than optimal, as it will decrease the predicted absolute risk with about 1.3 to 1.5%. As the average absolute risk is 13.3%, this corresponds to an underestimation of the relative risk with about 11%.

It has been shown, that the intraindividuel biological variation (CV_{Bw}) of S-a1-A is from 6 to 9% (3). Hence, a CV_A of 3% is sufficient, as the effect compared to the influence from CV_{Bw} is negligable (less than 12% of the total CV).

The observed CV in this investigation thus fulfils the demand for quality.

Model 2. The degree of exposure to endotoxin is determining.

In the multiple logistic regression model, using the SPSS package, the reference odds of 1 was defined in the group exposed to 0-9 ng of endotoxin per m^3. According to this model, the odds ratio for the groups exposed to 10-99 ng/m^3 and to above 100 ng/m^3 was respectively increased to 6.0 (95% c.i. 1.3-28) and 20.9 (95% c.i. 3.3-131). But a1-A is not determining in this evaluation model, where the influence of uncertainty of endotoxin quantification should be investigated instead. It can be concluded, that both high endotoxin concentrations and low a1-A concentration is determining for development of byssinosis. If a person both have a a1-A of below 35 μmol/L and work in an area with an endotoxin concentration of more the 100 ng/m^3, his total risk expressed in odds ratio is $5 \cdot 20 = 100$.

Model 3. The phenotype of a1-A (MZ and MS) is determining.

The risk/prevalence of byssinosis was defined as 1 in the MM-group with an endotoxin exposure of 0-9 ng/m^3. The data were fed to the SPSS multiple logistic regression program. The odds ratio's significantly differing from the defined average of 1 were for the MZ phenotype factor 5.8 (95% c.i. 1.1-30), but not for MS (0,1-5,5). Further, endotoxin exposure of 10-99 ng/m^3 gave an odds ratio of factor 5.7 (95% c.i. 1.2-27) and above 100 ng/m^3 a factor of 19.8 (95% c.i. 3.1-128).

It can be concluded, that quantitatively in importance, the environmental concentration of endotoxin is the crucial factor, but also here, like in model 1 and 2, we find a significant contribution from low a1-A's, caused by having the MZ-phenotype.

This finding makes it important to investigate the relationship between the MZ phenotypes and a1-A concentrations. As can be seen from the table below, all MZ's have a1-A's below 37 μmol/L (some of the 9 below 37 μmol/L, but untyped, are probably MZ´s)

α1-A	>37μmol/L	<37μmol/L
MZ phenotype	0	8
MS and MM phenotype	196	2
Untyped	11	9

If the phenotype is the determining factor the problem is changed to identifying Z heterozygotes. Thus, quantification of a1-A is superfluous, unless it is so laborious or

expensive to phenotype on the protein level or genotype on the DNA level, that a preliminary determination of a1-A concentration is rational. It is seen that the discriminator should be 37 μmol/L to identify all MZ heterozygotes with a low fraction of MM and MS types. This would facilitate and reduce the price of a subsequent phenotyping considerably, and, providing the necessary analytical requirements could be met by most laboratories, allow decentralized screening for MZ heterozygote with standard methods anywhere.

Supposing that the highest MZ´s have a1-A concentrations of 37 μmol/L, that the CVi is 6% (2.2 μmol/L), then the cutt-off point should be adjusted to 37 + (1.8 * 2.2) = 39.8 μmol/L to identify all MZ persons with a certainty of 90%.

Supposing an increasing analytical variation, from the ideal Sa of 0 μmol/L to 1, 2, 3 and even 4 μmol/L, it will be necessary to increase the cutt-off value further to be 90% sure still to be able to identify this MZ-person with the highest a1-A value of 37 μmol/L (Fig. 3)

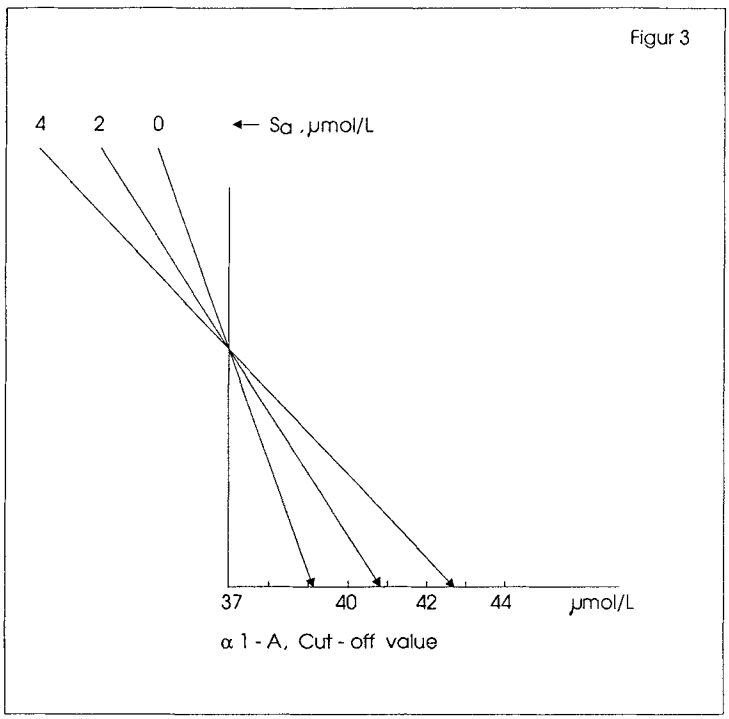

Figure 3. Increase in analytical imprecision necessitates increase in cut-off value to be sure still to be able to identify an MZ type with a concentration of 37 μmol/L with 90 % confidence.

Now it is possible to combine the bias and CV (4) of a given method to determine, which discriminator value should be used (Fig. 4), provided:

1. all MZ's should be identified, the MZ with the highest value with a certainty of 90%
2. the highest possible value of any MZ is 37 μmol/L
3. the biological intraindividual variation CVi is 6% (2.2 μmol/L).

Figure 4 , however, only deals with the safety of correct classification, not with the economical aspects of using a1-A quantification instead of phenotyping of all persons, omitting preliminary quantification.

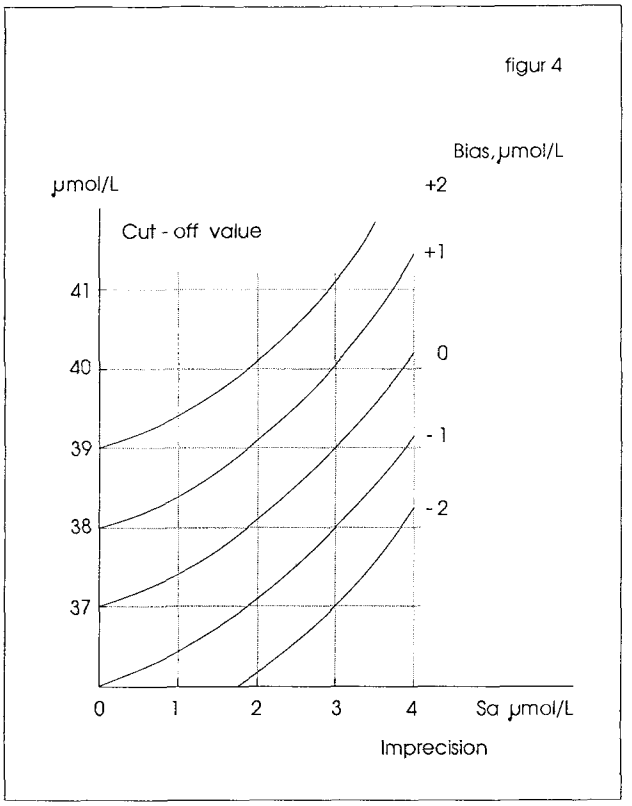

Figure 4. The figure shows the effect of combined changes in analytical bias and imprecision on selection of cut-off point to ensure identification of the highest MZ of 37 μmol/L with 90% confidence.

It is evident from fig. 4, that the lower the analytical quality (high imprecision and bias), the higher should the cut-off point be set, and this increases the number of misclassifications (MM and MS included as possible MZ and then phenotyped). On the

other hand, the lower the cut-off point, the higher will the number of lost MZs be. Depending on the consequences, this could be a very expensive way to try to reduce analytical costs.

Based on the present investigation and data the economical consequences of choosing different cut-off points can be illustrated. On the assumption, that the price of a quantification is 50 DKR , the price of a phenotype determination 170 DKR and the price of a missed MZ is, say 5,000 DKR(just as an example!), figure 5 can be constructed.

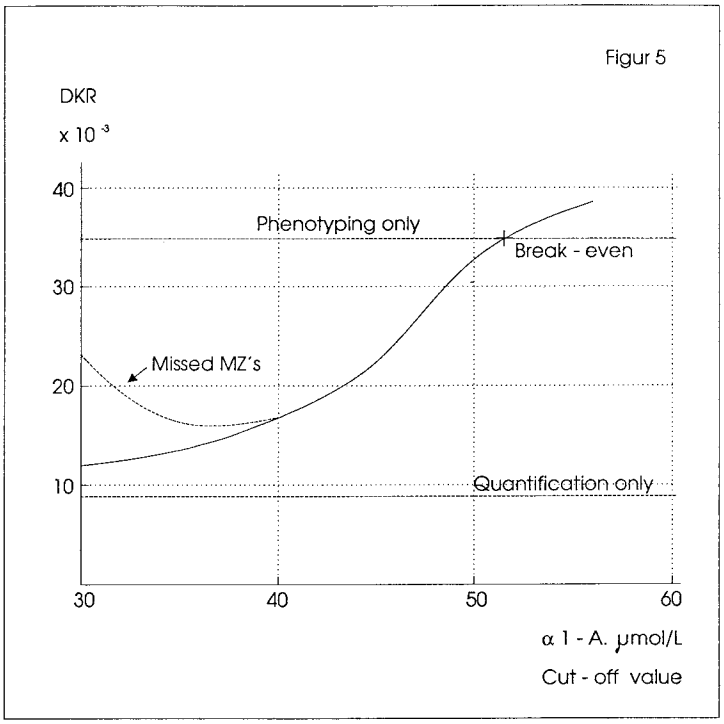

Figure 5. Economical consequences of different cut-off values for identification of MZ phenotypes, as determined by the analytical quality of the method used for a1-Antitrypsin quantification. A method allowing cut-off between 37 and 42 μmol/L is most cost-effective, provided the data are as found or assumed.

It is seen, that under these assumptions and conditions, there is an economical advantage of doing quantifications under analytical quality conditions requiring adjustment of chosen cut-off point up to around 53 μmol/L. It can be seen from fig 4, that even the worst method will be sufficient in quality to be usable.On the other hand, a method with analytical quality specifications allowing cut-off setting between 37 and 42 μmol/L will provide both optimal safety and economy. Intermediate calculations are not shown, but

are straightforward. They can be repeated for other investigations under different conditions.

Design of control system

Based on the present investigation, the following suggestions are put forward.

The internal quality control system should include genuine materials with assigned values of around 25, 35 and 45 μmol/L to cover the important area.

An external system should employ a material with an assigned value near the important value of around 35 μmol/L and ideally also around 25 μmol/L and 45 μmol/L to control for linearity.

DISCUSSION

Goals and need for quality in a1-A quantification in human serum is dependent on the purpose for quantification.

a1-A should not be used as a marker for acute phase reactions any more.This leaves a1-A quantification as a tool for detection of a1-A deficiencies.

Only the connection between ZZ, SZ (and SS?) phenotypes and the development of lung disease has been documented firmly enough to justify actions taken on part of an individual human being.

This means, that the only justifiable reason for determing a1-A in a patient at present is the suspicion of a deficiency,as the correlation between low normal a1-A, as seen in MZ phenotypes, and lung disease, have not been confirmed by repetition in a larger scale, than in this investigation.

So, the clinical need is at present, that a quantification should be able to distinguish between the lowest MZ's and phenotypes 00, ZZ, ZS and SS. It has been reported that these types have values between 0 and 20 μmol/L, which would make a value of <22 diagnostic.

To investigate if this is at all possible, a study should be carried out to define reference ranges for ZZ, ZS and SS's, as opposed to MZ's as opposed to MS and MM's. Whether the reference interval should be redefined to contain only MM's and MS's should be discussed. At this time it seems not reasonable to classify MZ's as being "abnormal" or "below the normal reference interval".

CONCLUSION

The necessary quality specifications to be met depend on the purpose of a 1-A quantifications. It should be possible to identify all ZZs, SSs and SZs. Establishment of a suitable cut-off point for this purpose, and hence, quality requirements, have not been accurately defined.

This study defines which cut-off points to choose, depending on the actual analytical quality, to enable identification of persons with increased risk of byssinosis in a cotton dust environment, because of low a1-Antitrypsin (model 1), and because of the MZ phenotype, both observed to be in high-risk groups.

An external quality assurance system should enable any laboratory to transfer data from investigations defining ranges for all known phenotypes. This implies the use of reference materials assigned values from 10 to 60 μmol/L, 20-25 and 33-36 being the areas, where quality demands are most important.

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Correspondence:
Ivan Brandslund
Department of Clinical Chemistry,
Vejle County Hospital,
DK-8000 Aarhus,
Denmark