Sensitivity of alpha-1 antitrypsin level for inherited deficiency detection in COPD patients

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ABSTRACT

Background and objective. *Alpha-1 antitrypsin deficiency is an underdiagnosed condition in patients with chronic obstructive pulmonary disease. Diagnosis of this genetic condition is confirmed by genetic verification of pathology, but for screening purposes quantitative methods can be useful. The aim of our study was to evaluate sensitivity and specificity of quantitative methods for alpha-1 antitrypsin deficiency detection.* ***Methods.*** *Serum alpha-1 antitrypsin concentrations from patients (n=1167) with chronic obstructive pulmonary disease, defined according to the GOLD criteria, were analysed by nephelometry, alpha-1 antitrypsin genotype was determined by means of isoelectric-focusing.* ***Results.*** *Eight severe alpha-1 antitrypsin deficiency genotypes in homozygous type (ZZ) and 40 in heterozygous genotype (-Z) were identified. Calculated sensitivity of quantitative alpha-1 antitrypsin measurement by nephelometry for heterozygous PI*Z allele is 45% and for homozygous ZZ genotype is 88%. Specificity of quantitative alpha-1 antitrypsin deficiency determining analysis is 99%.* ***Conclusions.*** *A case detection program of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods is specific, but due to limited sensitivity should be used only in screening programs.*

Keywords: *Chronic obstructive pulmonary disease, alpha-1 antitrypsin, sensitivity*

REZUMAT

Sensibilitatea nivelului de alfa-1 antitripsină în detectarea deficienţei moştenite la pacienţii cu BPOC

Cadru general şi obiective. *Deficienţa de alfa-1 antitripsină este o condiţie subdiagnosticată la pacienţi cu bronhopneumopatie cronică obstructivă. Diagnosticul de certitudine este confirmarea mutaţiei genetice, însă pentru screening, măsurarea cantitativă a nivelului alfa-1 antitripsinei poate fi utilă. Scopul acestui studiu a fost evaluarea sensibilităţii şi specificităţii metodelor cantitative de detectare a deficienţei alfa-1 antitripsinei.* ***Metode.*** *Concentraţia serică a alfa-1 antitripsinei de la pacienţi cu bronhopenumopatie cronică obstructivă (n=1167), definită conform criteriilor GOLD, a fost determinată prin nefelometrie, iar genotipul alfa-1 antitripsinei a fost determinat prin isoelectric-focusing.* ***Rezultate.*** *Au fost identificate 8 deficienţe severe de alfa-1 antitripsină la genotipul homozigot (ZZ) şi 40 la genotipul heterozigot (-Z). Sensibilitatea calculată a determinării cantitative a alfa-1 antitripsinei prin nefelometrie pentru alela heterozigotă PI*Z a fost de 45% şi pentru genotipul homozigot ZZ a fost 88%. Specificitatea determinării cantitative a deficienţei de alfa-1 antitripsină a fost de 99%.* ***Concluzii.*** *Un program de detectare a cazurilor de deficienţă de alfa-1 antitripsină prin metode cantitative derulat în rândul pacienţilor cu bronhopneumopatie cronică obstructivă ar fi specific, însă din cauza sensibilităţii reduse se recomandă doar în programe de screening.*

Cuvinte-cheie: *bronhopneumopatie cronică obstructivă, alfa-1 antitripsină, sensibilitate*

Introduction

Alpha-1 antitrypsin (AAT) is characterised by abnormally reduced serum AAT concentration, which, in homozygote form, carries a high risk of developing early pulmonary emphysema or chronic obstructive pulmonary disease (COPD) [1]. AAT is a circulating serine proteinase inhibitor (PI) secreted by the liver, which permeates most body tissues where it acts as an inhibitor of a range of proteolytic enzymes. The most of the pathology related to AAT deficiency is linked to the PI*Z allele, and in clinical practice, 96% of AAT deficiency patients have a homozygous genotype ZZ [2]. Less severe AAT deficiency is caused by heterozygous genotype: -Z.

AAT deficiency is an underdiagnosed condition worldwide [2]. Recent guidelines from both the World Health Organization and the American Thoracic Society/European Respiratory Society [2] recommend the establishment of screening programs for the detection of AAT deficiency in patients with COPD [3], because the detection of coexisting AAT deficiency could lead to family screening, appropriate management (including lifestyle changes such as quitting smoking and replacement therapy in selected cases), and specific counselling for these patients and families [4]. AAT deficiency can be suspected by quantitative serum analysis, however only detection of gene mutation confirms exact diagnosis. The aim of our study was to evaluate sensitivity of quantitative method, that is usually used for screening of AAT deficiency.

Methods

Sample sources and subjects selection

The study design was approved by the Regional Ethics Committee. 1167 patients with COPD were offered to participate in the study at the Department of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, and gave their informed consent.

Only patients who met the GOLD [3] requested spirometric criteria for COPD – 1) ratio of post-bronchodilator forced expiratory volume in one second (FEV1) to forced vital capacity (FVC) less than 0.7 and 2) FEV1 less than 80% of the predicted value – were included in the study. After an appropriate physical examination, data on the symptoms of the patient

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and the diagnosis of COPD were also collected. Smoking history was calculated in pack-years as the product of tobacco use (in years) and the average number of cigarettes smoked per day/20 (years x cig. per day/20).

Sample collection and evaluation

Blood samples were drawn in serum tubes, clotted at room temperature for 30-60 minutes and centrifuged for 15 minutes at 4000 rpm. Then, serum samples were immediately frozen at -70°C for further assay. The serum concentrations of AAT were determined by means of nephelometry, using commercially available kits (Dade Behring Marburg GmbH, Germany) according to the manufacturer's instructions. Presence of PI*Z allele was checked by using enzyme-linked immunosorbent assay (ELISA) kits (Euro-Diagnostica/Wieslab, Sweden) [5]; qualitative method by prepared standard guidelines of product. AAT phenotyping was carried out by means of isoelectric-focusing (LKB Multiphor II and LKB Macrodrive [5] Constant Power Supply, Amarcham Pharmacia Biotech, Piscataway, NJ, USA), as previously described [6].

Calculations and statistical analyses

Descriptive statistics were used to tabulate the primary cohort database. Quantitative variables were expressed as means with standard deviations (SD). Differences of quantitative data were assessed Kruskal-Wallis H test. A P value of less than 0.05 was considered significant. Statistical analysis was performed with the SPSS 14.0 program. Statistical sensitivity and specificity were calculated by binary classification test:

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

Results

The genotypes and the corresponding AAT concentration of 1167 COPD patients are shown in Table I. As expected, significant differences in AAT serum concentrations between groups were found (P=0.01). The ZZ group showed a significant lower AAT serum concentration (43mg/dL). Nearly 83% of the patients were current (57.6%) or former (25.2%) smokers of 22.1 ±12.2 pack-years, and 17.2% never smoked.

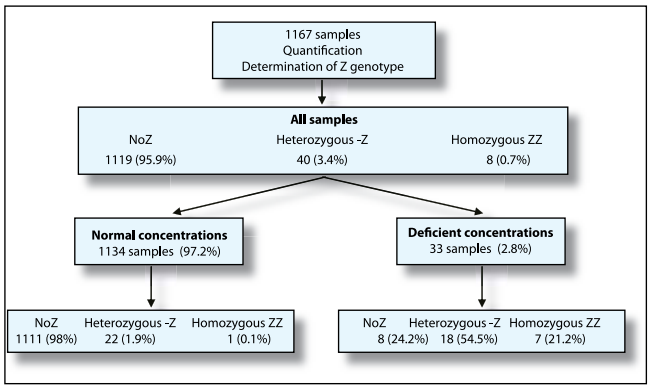
All 1167 samples were processed both for the quantification of AAT and determination of PI*Z deficiency allele. Concentrations above the cut-off point established as normal were detected in 1134 samples (97.2%) and 33 samples (2.8%) presented low concentration (Figure 1). Among the individuals with normal concentrations, the PI*Z allele was not detected in 1111 (98%) and was detected in 23 (2%) patients (Figure 1).

Calculated sensitivity of quantitative AAT measurement by nephelometry for heterozygous PI*Z allele was 45% and for homozygous ZZ genotype, 88%. Specificity of quantitative AAT deficiency determining method was 99%.

Discussion

Although the diagnosis of AAT deficiency is relatively simple, population studies have indicated that this disease is underdiagnosed and a delay in diagnosis is very common up till now [7]. The quantifying of AAT concentration is very important for identifying individuals with congenital AAT deficiency in screening purposes. However, our study showed that quantitative method's sensitivity for heterozygous PI*Z mutation is only 45% and for homozygous ZZ genotype is 88%. The most striking finding was that even a severe homozygous ZZ deficiency was found in one subject with normal AAT concentration. We speculate that it could be because of bronchial damage due to terminal stage of disease. This COPD patient had very low FEV1 value (15% predicted), although general inflammatory markers were not elevated (CRP 3mg/l). It shows that the presence of false negative results didn't allow all samples with normal concentrations to be qualified as non-deficient.

Figure 1. Alpha-1 antitrypsin concentration and genotype Data are presented as n (%). Deficient concentrations defined as those equivalent to concentrations < 100mg/dL



In heterozygous state, quantitative test may not allow detection of some individuals, possibly due to pathophysiological inflammatory processes. Even smoking in COPD patients may be associated with higher AAT and CRP production in the liver of COPD patients and mechanisms connected with systemic inflammation which continues even after smoking cessation. Even in healthy individuals, positive associations between active smoking and AAT levels have been reported before [8]. The quantity of AAT that diffuses passively from the blood to the lung increases during an inflammatory process, which takes place in COPD [9]. This may indicate increased requirement of AAT to meet the needs of overcoming the release of various enzymes from neutrophilic cells in the lungs, but its protective function may be overrun by the high concentration of proteases [10]. Increase AAT level in smokers and ex-smokers reflect the dual role of AAT as a respiratory disease biomarker. The net impact of AAT on lung function seems to be a result of context-dependent (i.e. AAT genotype) and contrasting protective and inflammatory effects in respiratory tract. On the one hand, elevated serum AAT can reflect a beneficial shift in the protease-antiprotease balance, the centre piece of the pathophysiological pathway mediating the effect of severe congenital AAT deficiency on COPD. On the other hand, elevated serum AAT can also reflect low-grade inflammatory processes in the lung [11] it is hypothesized COPD risk factor.

In European countries, AAT deficiency detection programs have been carried out by using different methodologies. Case control studies demonstrated an increase in the prevalence of PI*Z heterozygotes in patients with COPD compared with the control group when using genotyping methods [12-15]. Our study detected 8 (0.7%) cases of homozygous severe AAT deficiency from 1167 COPD patients. For example, similar the

Table I. Demographic characteristics, spirometric values, AAT genotypes and serum concentrations from 1167 COPD patients

AAT genotypes	N	Sex % M	Age (SD)	FEV1% pred.	AAT serum concentration
NoZ (- -)	1119	84	66 (10.4)	46 (15.8)	164.7 (39.8)
Heterozygous Z (-Z)	40	65	67 (11.2)	51.5 (14.5)	99 (14)
Homozygous Z (ZZ)	8	62	54 (11.3)	38.5 (18.1)	43 (12.4)
TOTAL	1167	82	62 (10.3)	46.5 (15.9)	158 (43.6)

Data are presented as mean or %, unless otherwise stated. N: Number; AAT: Alpha1-antitrypsin; %: percent; SD: Standard deviation; M: Males; FEV1: forced expiratory volume in one second expressed in percent of the predicted normal value; AAT serum concentrations measured by nephelometry, values expressed in mg/dL.

case-detection program of 2137 COPD patients in Spain revealed 7 cases of ZZ deficiency (0.37%) [12]. In their study, authors used dried blood spot on filter paper since it's a method used in screening of other genetic diseases. They found similar sensitivity for detection of heterozygous PI*Z mutation – 60%. And for homozygous ZZ genotype (total 4 cases) sensitivity was 100%. In another program undertaken in Italy, the detection rate for ZZ was 6.4% [13]. The higher rate of ZZ cases in that study could be due to the design of the study, because only COPD patients with clinical suspicion or familial AAT deficiency were selected and to all patients genotyping was performed. In another recent study performed in Germany in 2272 samples, making pre-screening by determining the AAT serum levels by the submitting physicians, the detection rate in the selected cases with low AAT serum concentrations significantly increased, and 335 patients with severe AAT deficiency, including 16 individuals with rare genotypes, were identified from the studied subjects [15]. Our results are close to the data received from the study carried in Denmark: frequency of AAT deficiency in COPD patients was ZZ 0.8% [16].

The results of the present study support the general concept of targeted screening for AAT deficiency with adequate laboratory methods in European countries with PI*Z high frequency and large population of COPD patients with highest diagnostic value - AAT genotyping. A case detection program of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods could be used only in screening programs and exact diagnosis must be confirmed by determining AAT genotype.

In conclusion, when designing a case detection program, both the protocol of sample processing and the inclusion criteria for the candidates should be taken into account, since both factors have a decisive influence on the performance of the program.

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