

Predictive value of alpha-1 antitrypsin level for Z mutation detection in chronic obstructive pulmonary disease

Danielius Serapinas^{1, 2*}

¹ *Lithuanian Health Science University, Department of Pulmonology and Immunology, Eivenių st. 2, LT-50009 Kaunas, Lithuania*

² *Mykolas Romeris University, Ateities st. 20, LT-08303 Vilnius, Lithuania*

Alpha1-antitrypsin (AAT) deficiency is an under-diagnosed condition in patients with chronic obstructive pulmonary disease (COPD). The aim of our study was to evaluate predictive value of quantitative methods of alpha-1 antitrypsin for Z mutation detection in patients with chronic obstructive pulmonary disease. Ninety-one AAT deficiency genotypes (40 MZ, 39 MS, 1 SS, 3 SZ and 8 ZZ) were analysed. Calculated sensitivity of quantitative alpha-1 antitrypsin measurement by nephelometry for heterozygous PI*Z allele was 45% and for homozygous ZZ genotype – 88%. Specificity of quantitative alpha-1 antitrypsin analysis for heterozygous deficiency was 98% and for homozygous deficiency – 100%. Thus sensitivity of quantitative alpha-1 antitrypsin analysis is higher than specificity for both – heterozygous and homozygous deficiency.

Key words: chronic obstructive pulmonary disease, alpha-1 antitrypsin, prediction value, quality of diagnostics

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a prevalent and costly disease characterized by progressive airflow limitation and related to an abnormal inflammatory response of the lung to long-term tobacco smoking or gases in-

halation (Global Initiative..., 2006). A mixture of bronchiolitis and lung emphysema causes the airflow limitation. The best documented genetic risk factor is severe hereditary deficiency of alpha-1 antitrypsin (AAT), a genetic disorder most commonly seen in individuals of Northern, Western and Central Europe origin (Blanco et al., 2006; De Serres et al., 2006). AAT is

* Corresponding author. E-mail: dserapinas@gmail.com

a 52 kDa alpha-1-glycoprotein produced mainly by hepatocytes and secreted into the blood, where it acts as a circulating serine protease inhibitor. AAT permeates most body tissues where it acts as a broad-spectrum anti-inflammatory and connective tissue repairer molecule, which modulates most inflammatory reactions occurring in the human body (American Thoracic Society..., 2003). About 100 genetic variant phenotypes (PI) of AAT have been identified to date. Severe AAT deficiency predisposes to the development of several diseases, mainly COPD (Popławska et al., 2013) in adults and several liver diseases (Şımşek et al., 2012) in children and adults. Most of the pathology related to AAT deficiency is linked to the Z allele, and in clinical practice 96% of AAT deficiency patients have a ZZ genotype. The remaining four percent belong mostly to SZ, MZ, and other rare deficiency or null genotypes (European Respiratory..., 2003; Popławska et al., 2013). AAT deficiency is an under-diagnosed condition worldwide. Recent guidelines from both the World Health Organization and the American Thoracic Society / European Respiratory Society recommend the establishment of screening programs for the detection of alpha-1 antitrypsin deficiency in patients with COPD, because detection of coexisting AAT deficiency in COPD patients could lead to family screening, appropriate management (including replacement therapy in selected cases), and specific counselling for these patients and families (Global Initiative..., 2006).

AAT deficiency can be suspected by quantitative serum analysis, however, only detection of gene mutation confirms the exact diagnosis. The aim of our study was to evaluate sensitivity of quantitative method which is usually used for screening of AAT deficiency.

MATERIALS AND METHODS

Sample analysis

Serum alpha-1 antitrypsin concentration from patients (n = 1 167) with chronic obstructive pulmonary disease, defined according to the GOLD criteria, was analysed by nephelometry, alpha-1 antitrypsin genotype was determined by means of isoelectric-focusing.

Calculations and statistical analyses

Descriptive statistics was used to tabulate the primary cohort database. Quantitative variables were expressed as means with standard deviations (SD). Differences of quantitative data were assessed by 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:

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

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

Table. Main characteristics of COPD patients with different AAT genotypes

Genotypes	N	%	Age (years)	Male / female
MM	1 076	92.2	64 ± 10	775 (72) / 301 (28)
MS	39	3.3	64 ± 8	25 (64) / 14 (36)
MZ	40	3.4	67 ± 11	27 (67) / 13 (33)
SS	1	0.1	61	0 (0) / 1 (100)
SZ	3	0.3	63 ± 3	2 (67) / 1 (33)
ZZ	8	0.7	54 ± 11*	5 (62) / 3 (38)
Total	1 167	100	64 ± 12	834 (71) / 333 (29)

Data are presented as n (%) or mean ± SD; * *p* < 0.05 ZZ genotype comparing with MM, MZ and MS genotypes

RESULTS

As it is shown in Table, the cohort consisted of 1 167 patients with COPD.

Genotypes distribution (number and percentage) was as follows: 1 076 (92.2%) MM, 40 (3.4%) MZ, 39 (3.3%) MS, 1 (0.1%) SS, 3 (0.3%) SZ and 8 (0.7%) ZZ. The ZZ patients were younger (54 ± 11 years, $p < 0.05$) compared to the MM, MS, MZ patients. The majority of the studied COPD patients were men (72%). Such high number of men with COPD in Lithuania could be explained by smoking habits. According to the Finbalt Health Monitor Programme, the number of male smokers in the general Lithuanian population is 6.9 times higher than the number of female smokers (Grabauskas et al., 2002). All 1 167 samples

were processed both for the quantification of AAT and determination of PI*Z deficiency allele. The mean AAT serum concentration was 1.58 ± 0.43 g/l. As it was expected, we found significant differences in serum AAT concentrations comparing groups ($p < 0.05$). The ZZ group showed a significantly lower serum AAT concentration (0.40 ± 0.34 g/l) as compared to other genotype groups (Fig. 1).

Concentrations above the cut-off point established as normal were detected in 1 134 samples (97.2%) and 33 samples (2.8%) presented low concentration (Fig. 2). Among the individuals with normal concentrations the PI*Z allele was not detected in 1 111 (98.0%) and was detected in 23 (2%) patients (Fig. 2).

Calculated sensitivity of quantitative AAT measurement by nephelometry for hetero-

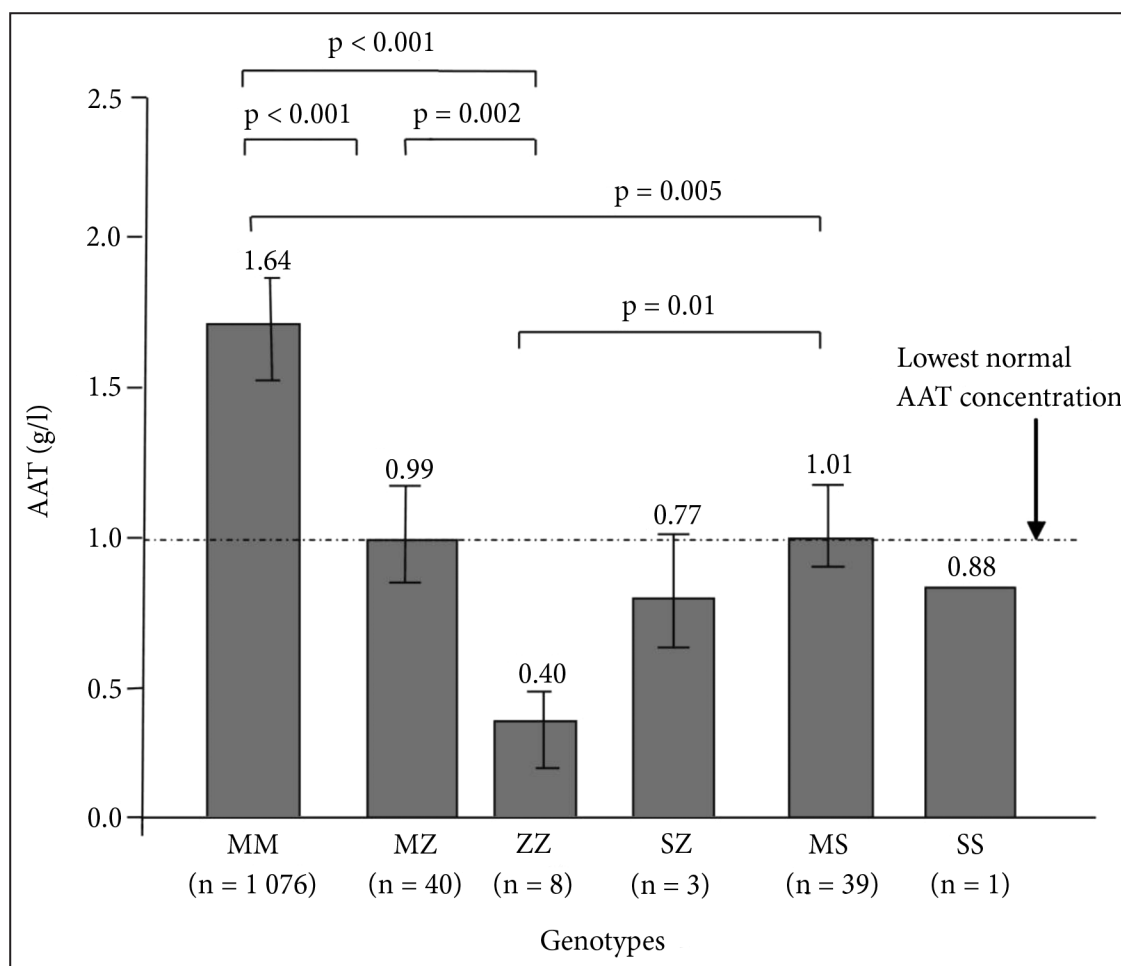


Fig. 1. Serum AAT concentration in COPD patients with different AAT genotypes. Data are presented as mean \pm SD; AAT – alpha-1 antitrypsin

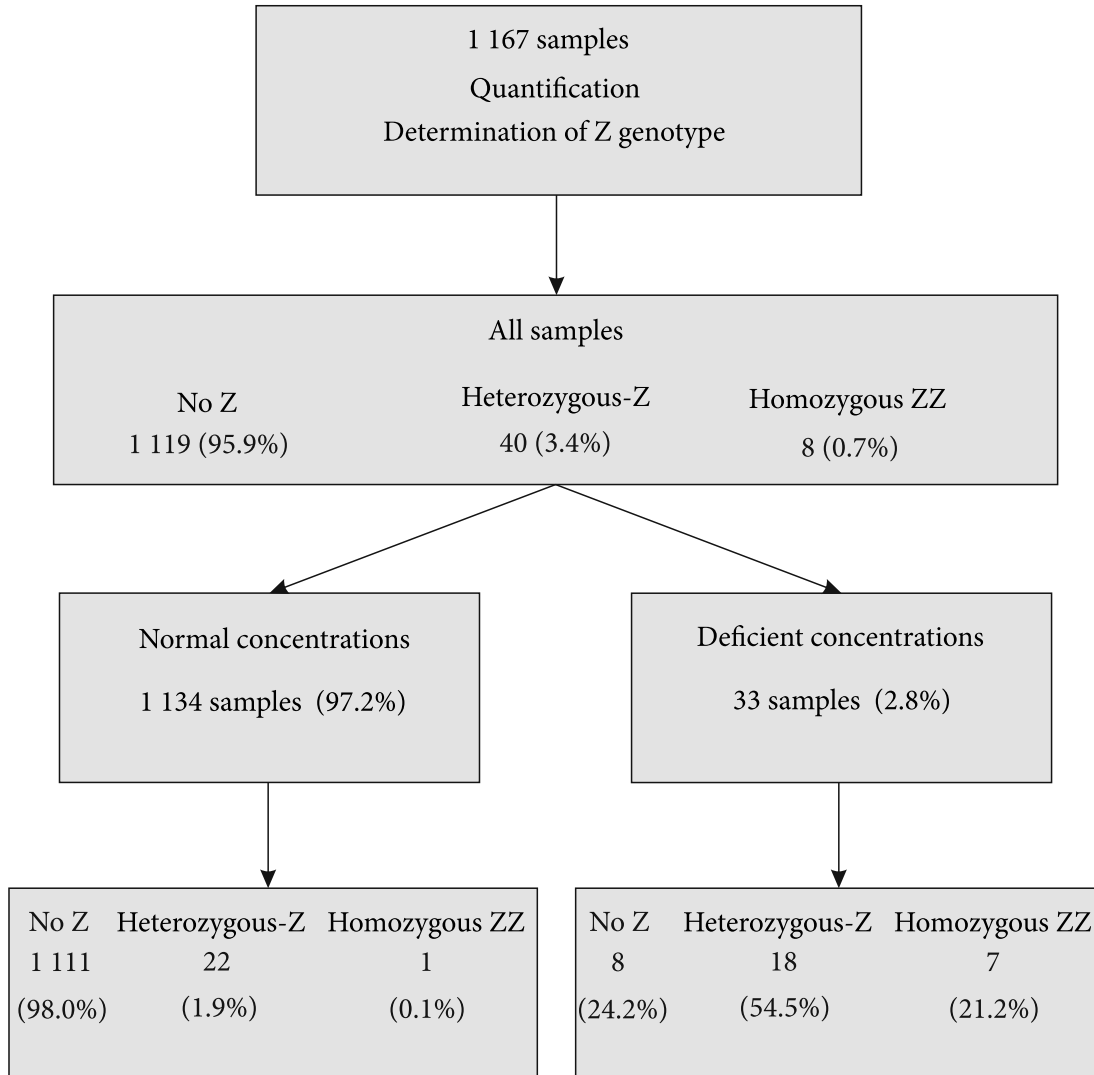


Fig. 2. Alpha-1 antitrypsin concentration and genotype. Data are presented as n (%). Deficient concentrations defined as those equivalent to concentrations < 1 g/L

zygous PI*Z allele was 45% and for homozygous ZZ genotype – 88%. Specificity of quantitative alpha-1 antitrypsin analysis for heterozygous deficiency (PI*Z allele) was 98% and for homozygous deficiency – 100%.

DISCUSSION

The diagnosis of AAT deficiency is relatively simple, however population studies have indicated that this disease is under-diagnosed and a delay in diagnosis is very common up till now (Global Initiative..., 2006; Garcia-Rio et al., 2010). The present study comparing

the ZZ group against all remaining 5 genotypes groups shows: a lower AAT serum concentration (0.40 g/L, significant difference of $p < 0.05$), thus in Lithuania alpha-1 antitrypsin deficiency ZZ almost always is detected during scientific projects but not during common clinical practice. 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 – 8%. 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 3 mg/l). It shows that the presence of false negative results did not allow all samples with normal concentrations to be qualified as non-deficient.

In heterozygous state a 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 cessation of smoking. Even in healthy individuals, positive associations between active smoking and AAT levels have been reported before (Senn et al., 2008). The quantity of AAT that diffuses passively from the blood to the lung increases during an inflammatory process which takes place in COPD (Grabauskas et al., 2002). This may indicate an 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 (Stockley et al., 2009). Increase of AAT level in smokers and ex-smokers reflects 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 (Langereis et al., 2011; Serapinas et al., 2011) 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 (Senn et al., 2008; Serapinas et al., 2011; Şimşek et al., 2013; Stockley et al., 2009). Our study detected 8 (0.7%) cases of homozygous severe AAT deficiency from 1 167 COPD patients. For example, similar case-detection program of 2 137 COPD patients in Spain revealed 7 cases of ZZ deficiency (0.37%) (De la Roza et al., 2005). In their study the authors used a dried blood spot on filter paper since it is the 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 (in total 4 cases) sensitivity was 100%. In another program undertaken in Italy the detection rate for ZZ was 6.4% (Luisetti et al., 1999). 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 2 272 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 (Bals et al., 2007). Our results are close to the data received from the study carried out in Denmark: frequency of AAT deficiency in COPD patients was ZZ 0.8% (Dahl et al., 2002).

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 programme of alpha-1 antitrypsin deficiency in patients with chronic obstructive pulmonary disease using quantitative methods could be used only in screening programs and the exact diagnosis

must be confirmed by determining AAT genotype.

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

Received 20 February 2013

Accepted 16 December 2013

References

1. American Thoracic Society / European Respiratory Society Statement: standards for the diagnosis and management of individuals with alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med* 2003; 168: 818–900.
2. Bals R, Koczulla R, Kotke V et al. Identification of individuals with alpha-1-antitrypsin deficiency by a targeted screening program. *Respir Med* 2007; 101: 1708–1714.
3. Blanco I, de Serres FJ, Fernandez-Bustillo E et al. Estimated numbers and prevalence of PI*S and PI*Z alleles of alpha1-antitrypsin deficiency in European countries. *Eur Respir J* 2006; 27: 77–84.
4. Dahl M, Tybjaerg-Hansen A, Lange P et al. Change in lung function and morbidity from chronic obstructive pulmonary disease in alpha1-antitrypsin MZ heterozygotes: A longitudinal study of the general population. *Ann Intern Med* 2002; 136: 270–279.
5. De la Roza C, Rodríguez-Frías F, Lara B et al. Results of a case-detection programme for alpha1-antitrypsin deficiency in COPD patients. *Eur Respir J* 2005; 26: 616–622.
6. De Serres FJ, Blanco I, Fernandez-Bustillo E. Estimating the risk for alpha-1 antitrypsin deficiency among COPD patients: evidence supporting targeted screening. *COPD* 2006; 3: 133–9.
7. Garcia-Rio F, Miravittles M, Soriano JB et al. Systemic inflammation in chronic obstructive pulmonary disease: a population-based study. *Respir Res* 2010; 25: 63.
8. Global Initiative for Chronic Obstructive Lung Disease (GOLD). 2006. Global strategy for the diagnosis, management, and prevention of COPD, Executive Summary. Date last accessed: August 2012.
9. Grabauskas V, Klumbiene J, Petkeviciene J, Dregval L, Kinderyte G, Šackute A ir kt. Suaugusiųjų Lietuvos žmonių gyvenamosios tyrimas. 2002. Kauno medicinos universitetas, Nacionalinis visuomenės sveikatos institutas, Suomija.
10. Langereis JD, Schweizer RC, Lammers JW et al. A unique protein profile of peripheral neutrophils from COPD patients does not reflect cytokine-induced protein profiles of neutrophils *in vitro*. *BMC Pulm Med* 2011 Sep 6; 11: 44.
11. Luisetti M, Massi G, Massobrio M et al. A national program for detection of alpha-1 antitrypsin deficiency in Italy. *Respir Med* 1999; 93: 169–172.
12. Popławska B, Janciauskiene S, Chorostowska-Wynimko J. Genetic variants of alpha-1 antitrypsin: classification and clinical implications. *Pneumonol Alergol Pol* 2013; 81: 45–54.
13. Senn O, Russi EW, Schindler C et al. Circulating alpha1-antitrypsin in the general population: determinants and association with lung function. *Respir Res* 2008; 25: 9–35.
14. Serapinas D, Narbekovas A, Juskevicius J, Sakalauskas R. Systemic inflammation in COPD in relation to smoking status. *Multidisc Respir Medic* 2011; 6: 214–219.
15. Şımşek H, Pinar A, Altınbaş A, Alp A, Balaban YH, Büyükaşık Y et al. Alpha-1 antitrypsin deficiency in patients with chronic hepatitis. *Turk J Gastroenterol* 2012; 23(5): 569–73.
16. Stockley RA, Mannino D, Barnes PJ. Burden and pathogenesis of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2009; 6: 524–6.

Danielius Serapinas

**PREDIKCINĖ ALFA-1 ANTITRIPSINO
KIEKIO VERTĖ NUSTATANT Z MUTACIJĄ
LĖTINĖS OBSTRUKCINĖS PLAUČIŲ LIGOS
ATVEJU**

Santrauka

Alfa-1 antitripsino deficitas visame pasaulyje yra nepakankamai diagnozuojama būklė sergant lėtine obstrukcine plaučių liga. Tyrimo tikslas – nustatyti predikcinę kiekybinio alfa-1 antitripsino kiekio vertę išskiriant Z mutaciją sergantiesiems lėtine obstrukcine plaučių liga. Tyrimo metu įvertintas 91 deficito atvejis (40 MZ, 39 MS, 1 SS, 3 SZ ir 8 ZZ). Apskaičiuotas kiekybinio alfa-1 antitripsino nustatymo jautrumas, esant heterozigotinei būsenai (PI*Z aleliui), buvo 45 %, o homozigotinei (ZZ genotipui) – 88 %. Taip pat nustatytas jautrumas esant heterozigotinei būsenai (PI*Z aleliui) – 98 %, o homozigotinei – 100 %. Tyrimas atskleidė, kad nefelometrinio alfa-1 antitripsino kiekį nustatančio metodo jautrumas yra didesnis nei specifiskumas.

Raktažodžiai: lėtinė obstrukcinė plaučių liga, alfa-1 antitripsinas, predikcinė vertė, diagnostikos kokybė

