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Exhaled nitric oxide in patients with α1 antitrypsin (AAT) deficiency

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Epidemiology and pathophysiology of AAT deficiency

Alpha 1 antitrypsin (AAT) deficiency is an inherited disorder characterised by low AAT blood concentrations and predisposes to the early development of pulmonary emphysema, particularly in smokers [1]. The condition was first described in 1963 by LAURELL and ERIKSSON [2]. AAT deficiency is the second most common inherited disease (after cystic fibrosis), with an incidence estimated at between 1:1600 and 1:3500. For the difference between expected and diagnosed cases, it is estimated that at present not more than 20% of patients are identified; thus a large number of AAT deficiency subjects remain hidden, leading to the suggestive label of "iceberg disease" for AAT deficiency.

The disease is caused by an inborn error in the liver's production of AAT. The AAT gene (cr.14 - 14q32.1) is polymorphic and the phenotype (Pi) is determined by two co-dominant alleles inherited from each parent. The alleles are characterised according to AAT production rate and to their position of migration on iso-electric focusing. Normal serum levels of AAT range from 150 to 350 mg/dL and are associated with M allele which exhibits a medium position on iso-electric focusing [3].

The increased risk of developing pulmonary emphysema is associated with alleles coding for AAT blood levels ≤80 mg/dl, the most common being referred to as Z. Other abnormal deficient variants with increased risk are those involving the Null alleles, which are associated with no AAT production, and the S allele, which confers a significant risk only in combination with Z or Null alleles [4]. AAT is the predominant anti-protease in the lung that protects alveoli from the effects of serine proteases by inhibiting the neutrophil elastase. In AAT deficiency, emphysema likely results from an imbalance in the lungs between the elastase burden and the decreased antiprotease activity due to low AAT levels. The induction of emphysema caused by smoking can be explained by the marked increase of neutrophils and macrophages in lung tissue. Thus, in the lungs of subjects with AAT deficiency the elastase activity is increased while the anti-elastase activity is decreased, generating an elastase-anti-elastase imbalance [5]. The degree of lung pathology and the rate of decline in the pulmonary function varies markedly in subjects with AAT deficiency, indicating that other agents play a role in the pathophysiology and clinical presentation of this disease. Smoking habit typically promotes the development of lung emphysema in subjects affected by AAT severe deficiency, but this explains the genesis of lung disease only in some patients [4].

Nitric oxide and AAT deficiency disease

We hypothesised that other factors may influence the pathophysiological process in the presence of AAT deficiency, affecting the individual susceptibility to lung destruction.

Recently, it has been suggested that nitric oxide can play a role in the pathogenesis of different diseases involving chronic damage to the connective tissue [6]. Nitric oxide may regulate vascular and airway tone in the lung influencing various aspects of airway homeostasis [7]. In view of the importance of these functions, we reasoned that abnormalities in nitric oxide may contribute to the pathogenesis of chronic pulmonary diseases.

Polymorphic variants of nitric oxide synthase (NOS) are involved in the pathogenesis of different diseases, such as hypertension and coronary artery disease [6]. In a recent paper, NOVORADOVSKY and co-workers [8] found an association between 2 polymorphic sites of NOS3 gene (constitutive NOS) and severity of lung disease in AAT deficiency subjects (with severe deficit) suggesting that genetic variants of NOS3 may modulate the clinical presentation of AAT deficiency.

On the basis of these considerations we investigated the relationship between lung function and exhaled nitric oxide (eNO) in subjects with AAT deficiency. In our Regional Reference Center in Brescia between 1995 and 2000 we found 114 subjects with AAT deficiency [9, 10] enrolled in the Italian National Register: 24 subjects with severe deficiency (with pathological alleles Z, S, and rare

deficiency alleles) and 90 subjects with intermediate deficiency (Pi MS-MZ).

Among these, we studied a population of 40 subjects with AAT deficiency (21 male and 19 female, mean age 43±14 years). They were divided into 3 groups according to the phenotype of the protease inhibitor (Pi): group 1 (PiMZ-MS: 25), group 2 (PiSZ: 6), group 3 (PiZZ: 9), and a control group of 19 subjects (PiMM). Lung function tests [static, dynamic volumes and lung diffusion capacity (DLCO)], methacholine challenge and eNO analysis (by the single breath chemiluminescence technique) were performed.

The main results showed that Fractional Exhaled nitric oxide concentration (FE_{NO}) was statistically significantly ($p<0.05$) lower in PiZZ group than in the other groups and in normal controls (see table 1), whereas eNO and bronchial hyperresponsiveness in AAT deficiency appear not to be related each other, thus confirming our previous preliminary report [11]. As expected, a worse lung function, in terms of level of FEV1 and DLCO, was associated with ZZ phenotype patients.

Table 1. – Exhaled nitric oxide levels in the study group

		95% Conf Intervals	
	[illegible]	[illegible]	[illegible]
pFE_{NO} (ppb)			
PiMZ/MS	18.7±9.5	17.6	25.8
PiSZ	19.3±9.9	8.8	29.7
PiZZ	12.2±4.2 *+§	8.1	16.3
Controls	18.8±4.5	16.8	20.8
$plFE_{NO}$ (ppb)			
PiMZ/MS	9.5±3.2	5.3	16.9
PiSZ	9.0±5.4	3.3	14.7
PiZZ	4.5±1.4 *+§	3.2	5.4
Controls	9.3±2.8	7.9	10.6

Data expressed as mean±SD.

* p=0.04 versus PiMZ/MS
+ p=0.015 versus PiSZ
§ p=0.0002 versus controls

Legend: pFE_{NO} = Value of exhaled nitric oxide at the peak level in the single breath exhalation curve; $plFE_{NO}$ = Value of exhaled nitric oxide at the plateau level in the single-breath exhalation curve.

Our study confirms that PiZZ subjects are at higher risk of developing chronic lung obstructive disease. Interestingly the lower level of FE_{NO} that we found in PiZZ individuals, may substantiate, from a clinical point of view, what NOVORADOVSKY *et al.* [8] have observed at a genetic level. The reduced nitric oxide production by the airways of PiZZ individuals may be an associated risk factor involved in the susceptibility of AAT deficiency subjects to lung destruction. Therefore, nitric oxide might act as an independent factor in the pathogenesis of the disease and eNO may result as an early marker of lung damage.

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