Thorax Online First, published on November 11, 2005 as 10.1136/thx.2005.043471

NEUTROPHILIC INFLAMMATION AND IL-8 LEVELS IN INDUCED SPUTUM OF ALPHA-1-ANTITRYPSIN PiMZ SUBJECTS

MARIO MALERBA. MD; University of Brescia, Internal Medicine.

FABIO RICCIARDOLO MD and PhD. Unit of Pneumology, IRCCS Gaslini Institute, Genova

ALESSANDRO RADAELI MD; University of Brescia, Internal Medicine

CHIARA TORREGIANI MD; University of Brescia, Internal Medicine

LUIGI CERIANI. MD University of Brescia, Internal Medicine

ERICA MORI. MD University of Brescia, Internal Medicine

MARIO BONTEMPELLI. MD; Ospedali Riuniti di Bergamo, Biochemistry Laboratory

CLAUDIO TANTUCCI. Prof. University of Brescia, Internal Medicine

VITTORIO GRASSI. Prof. University of Brescia, Internal Medicine

CORRESPONDANCE:

Dr. Mario Malerba

Department of Internal Medicine
University of Brescia
1° Divisione di Medicina
Spedali Civili
Pzza Spedali Civili 1
25100 Brescia - Italy
Tel: +39 030 3995250
Fax: +39 030 396011
E-Mail: malerba@master.cci.unibs.it

Key words: alpha1-antitrypsin deficiency, induced sputum, airway inflammation, COPD

ABSTRACT

Background: Severe alpha-1-antitrypsin deficiency (AATD), due to homozygosity for the protease inhibitor (Pi) Z allele, is a genetic risk factor for chronic obstructive pulmonary disease (COPD). In a previous study the sputum of severe AATD subjects with airflow obstruction showed a pattern of cellular inflammation similar to COPD patients. It is uncertain whether heterozygotes for the Z allele or intermediate deficiency (PiMZ) have increased risk of developing COPD.

Methods. The study was aimed to investigate the sputum cell counts and the supernatant level of neutrophil chemoattractant IL-8 by sputum induction in 10 non-smoker asymptomatic PiMZ subjects, with normal pulmonary function, in comparison with 10 stable COPD patients and 10 age-matched normal subjects. Data are expressed as mean ± standard deviation.

Results: In sputum the mean number of neutrophils was significantly higher ($p<0.01$) in PiMZ (84.5 ± 22.2 x 10^4/ml), and COPD patients (126,9 ± 18,8 x 10^4/ml) than in matched normal subjects (55.0 ± 8.7 x 10^4/ml). IL-8 levels were increased in Pi MZ (828.5 ± 490.6 ng/ml; median 1003.0 ng/ml; range = 1260 - 100 ng/ml), similarly to those found in COPD patients (882.5 ± 524.3 ng/ml; median = 934.9 ng/ml; range = 1506 - 258 mg/ml), compared to normal subjects (3.5 ± 0.5 ng/mL; median = 3.5 ng/ml; range = 4.5 - 2.5 ng/ml). A significant positive correlation between IL-8 supernatant concentration and neutrophils count in PiMZ subjects was found ($p=0.036$; $r=0.66$). An inverse correlation between the percentage of neutrophils and FEV1 (% predicted) in COPD patients was observed ($p=0.04$; $r=-0.43$).

Conclusions: These findings indicate that PiMZ subjects without airflow obstruction may have an IL-8 related neutrophilic inflammation in the airways, similar to stable COPD patients, suggesting an increased risk of developing pulmonary alterations.

INTRODUCTION

Alpha-1-antitrypsin (AAT) is a serine protease inhibitor that protects the lung from the proteolitic action of proteases, such as neutrophil elastase. Severe alpha-1-antitrypsin deficiency (AATD), a genetic disorder due to homozygosity for the protease inhibitor (Pi) Z allele (PiZZ) and characterized by low AAT serum concentrations, is recognized as a risk factor for chronic obstructive pulmonary disease (COPD) with the typical pathologic findings of premature panacinar emphysema dependent on the protease - antiprotease imbalance.[1] Heterozygotes for the Z allele (most commonly PiMZ) have lower serum levels of AAT than normal individuals (PiMM),[2] but the role of intermediate deficiency PiMZ of developing COPD remains uncertain.[3] Sputum induction is a direct, non-invasive and reproducible method to assess airway inflammation.[4][5] Previous studies have demonstrated a predominance of neutrophils and related chemokines such as interleukin-8 (IL-8) and leukotriene B-4 (LTB-4) in the induced sputum of patients with COPD.[6][7] Airways neutrophilic inflammation plays a fundamental role in the pathogenesis and progression of COPD. Stockley et al. have shown in the sputum of severe AATD subjects with airflow obstruction a pattern of airway inflammation similar to that of COPD patients.[8] In addition, Rouhani et al observed an increased lung neutrophil burden related to pro-inflammatory cytokines such as IL-8 and IL-6 in broncho-alveolar lavage (BAL) fluid of AATD subjects with mild functional lung impairment.[9] The increased neutrophil recruitment is thought to contribute further to the development of the rapidly progressive lung deterioration observed in AATD subjects. The aim of our study was to assess by sputum induction the cellular pattern and IL-8 levels in the airways of non-smoker asymptomatic PiMZ subjects with normal lung function in comparison with COPD patients and healthy control subjects.

METHODS

Study Population

AAT deficiency

We studied 10 consecutive non-smoker and asymptomatic subjects (5 male and 5 female, mean age ± SD = 47.8 ± 9.7 years) heterozygotes for AATD (PiMZ) (mean AAT blood level ± SD = 108 mg/dL ± 31.7) included in the Italian Registry and attending our Regional Reference Center of Brescia (Department of Internal Medicine) in an ambulatory setting. Diagnosis of AAT deficiency was confirmed by phenotyping. At the time of inclusion all the subjects were in a stable condition and had been free from respiratory exacerbations for at least four weeks. A complete medical history was obtained for each subject. No subject had present or past history of exposure to any occupational factor and of passive smoking. All the subjects studied had normal lung function. Before the study other concomitant pulmonary diseases (such as bronchiectasis) were ruled out in PiMZ subjects by chest-x-ray and high resolution CT scan.

COPD

We enrolled 10 patients with stable COPD (6 male and 4 female, mean age ± SD = 65.7 ± 13.3 years) with PiMM (mean AAT blood level ± SD = 140.3 mg/dL ± 10.2). Diagnosis of COPD was made according to the following standard criteria:[10] age > 40 years, current or previous history of smoke (≥ 10 pack-years), history of chronic symptoms (cough with sputum production for at least 3 months in at least 2 consecutive years) and presence of airflow obstruction (forced expiratory volume in one second / forced vital capacity (FEV1/FVC< 70%). At the time of the study all the patients were in a stable condition and free from acute exacerbation for at least 4 weeks. No patient was on systemic

corticosteroids or had received antibiotics within the month preceding the study. Treatment with inhaled corticosteroids was withdrawn at least 4 weeks before entering the study.

Controls

The control group consisted of 10 healthy subjects, age-matched to PiMZ subjects, (4 males and 6 females, mean age ± SD = 46.1± 21.7 years) known to be PiMM (mean AAT blood level ± SD = 162.5 ± 18.5 mg/dL) with checked normal pulmonary function tests. They were excluded if they had a history of respiratory disease or experienced atopy.

Demographic and functional characteristics of the AAT deficiency subjects, COPD patients and controls are reported in Table 1.

The study protocol was approved by the local Ethics Board and all subjects gave their written informed consent to participate to the study, which was conducted in accordance with the Helsinki Declaration.

Measurements

AAT assay and phenotyping

AAT serum concentrations were measured in all the subjects studied by nephelometry method (normal serum level of AAT range from 90 to 200 mg/dl).[11] AAT phenotyping was performed by isoelectric focusing.[12]

Pulmonary function tests

Lung function was measured in accordance with the American Thoracic Society (ATS) standard procedure.[13] The patients underwent spirometry to measure FEV1, FVC and FEV1/FVC ratio. The lung diffusion capacity for carbon monoxide (DLCO) was assessed by means of the single breath method (PF/DX system MCG, MN USA) with patients in the sitting position.

Sputum induction procedure

Inhalation procedure: after baseline FEV1 and FVC measurements, subjects were pre-treated with salbutamol given by inhalation (200 µg by metered-dose inhaler) and 10 minutes later inhaled hypertonic (4%) sterile saline nebulized solution for 3 periods of 5 minutes at most by an ultrasonic nebulizer (DeVilbiss nebulizes Ultraneb 2000).The subjects were instructed to cough sputum into containers. If any symptoms occurred nebulization was discontinued.

Sputum processing

The collected sputum samples were examined within 2 hours. Sputum plugs originating from lower respiratory tract were chosen, they were separated from the collected samples and then weighted. Dithiotreitol (DTT; Sputolysin; Calbiochem Corp., San Diego, CA) was freshly prepared in a 1:10 dilution with distilled water and then it was added to the plugs in a volume (in microliters) equal to four times the weight of the selected plugs (in milligrams). The test tube containing DTT and sputum was homogenized, it was left in a thermostat at 37° C for 20 minutes and then it was diluted with a volume of phosphate buffered saline (PBS) equal to the DTT. The suspension was filtered through a 48µm gauze and it was centrifuged at 1000 rpm for 5 minutes. The supernatant was removed and then frozen at –70°C for later analysis. The cell pellet was resuspended in PBS in a volume equal to DTT volume added at the beginning of the test, it was spun in a cytocentrifuge (PK120R ALC International) and then stained using Diff-Quik. Cells count was determined with a Burkers chamber hemocytometer, only samples with a cell viability >50% and <20% squamous cell contamination were considered adequate. The differential cell count was expressed as the percentage of the total nonsquamous cells (%NSC) and as the number of cell x 10^4/ml.

Sputum biochemistry

IL-8 was measured in sputum supernatant by enzyme-linked immunosorbent assay method using commercially available kits (Bender Med-Systems). This assay has demonstrated to function normally in the presence of DTT.

Statistical analysis

Statistical analysis was performed with the SPSS software package (SPSS; Chicago, IL). A p-value < 0.05 was considered as significant. All values are expressed as mean ± standard deviation (S.D.) Comparison of groups was performed by analysis of variance testing (ANOVA). Unpaired t-tests were used when appropriate. Categorical data were compared using the Fisher exact test, and Kruskal-Wallis test or Mann-Whitney test for unpaired data (when appropriate).

RESULTS

Clinical findings

The demographic and pulmonary functional characteristics of the patients studied are reported in table 1.

Table 1: Demographic and functional characteristics and AAT blood levels of the subjects studied.

	PiMZ	COPD	CONTROL SUBJECTS	p
Number of subjects	10	10	10	
M/F	5/5	6/4	4/6	0.67*
Age (years)	47.8 ± 9.7	65.7 ± 13.3 †	46.1 ± 21.7	0.019
AAT (mg/dl)	108 ± 31.7‡	140.3 ± 10.2	162.5 ± 18.5	0.002
FEV1 (% pred.)	112.9 ± 12.2	60.8 ± 23.2 §	107.6 ± 15.1	0,000
FEV1/FVC (% pred)	88.5 ± 6.7	58.3 ± 13.6 §	87.7 ± 8.2	0,000
DLCO (% pred)	103.5 ± 4.0	96.9 ± 12.6	104.9 ± 5.6	0.09
Smoking history:				
Smokers	0	5	0	0.000
Ex-smokers	0	5	0	*
Never smokers	10	0	10	

Data are expressed as mean ± standard deviation

* performed by χ^2

† vs PiMZ p=0.38; vs Controls p=0.047 (ANOVA with Bonferroni correction)

‡ vs COPD p=0.046; vs Controls p=0.002 (ANOVA with Bonferroni correction)

§ vs PiMZ and Controls p=0.000 (ANOVA with Bonferroni correction)

The COPD patients were significantly older (p=0.019) whereas PiMZ subjects and controls were age-matched. Plasma AAT levels were lower in PiMZ than in the other groups (p=0.002; vs COPD p=0.046; vs controls p=0.002). COPD patients showed similar levels of serum AAT concentration compared to the healthy control subjects (p=0.69).
The PiMZ and healthy control subjects did not show airflow obstruction whilst COPD and had FEV1 (% predicted) and FEV1/FVC (%) values significantly lower in comparison with other two groups (both p=0.000). No significant difference of DLCO values were observed among the groups (p=0.09; COPD vs PiMZ p=0.26; COPD vs controls p=0.12). (Table 1)

Sputum induction findings

Sputum induction results are listed in table 2.

Table 2: Sputum induction findings.

	PiMZ	COPD	CONTROL SUBJECTS	p
Number of subjects	10	10	10	
Neutrophils (%NSC) n x 10^4/ml	69.3 ± 18.2 84.5 ± 22.2	76.3 ± 9.9 126.9 ± 18.8	45.1 ± 7.2 * 55.0 ± 8.7 *	0.000 0.000
Macrophages (%NSC) n x 10^4/ml	26.8 ± 9.4 32.7 ± 11.5	18.5 ± 11.3 30.1 ± 21.4	50.5 ± 7.6 * 61.6 ± 9.2 *	0.000 0.000
Eosinophils (%NSC) n x 10^4/ml	0.6 ± 1.0 0.7 ± 1.2	2.4 ± 2.9 3.8 ± 5.5	1.2 ± 1.3 1.4 ± 1.5	0.103 0.083
Lymphocytes (%NSC) n x 10^4/ml	3.1 ± 1.1 3.7 ± 1.3	2.6 ± 2.2 3.9 ± 4.1	2.4 ± 1.8 2.9 ± 2.1	0.971 0.95
IL-8 (ng/mL) Median Range	828.5 ± 490.6 1003.0 1260 - 100	882.5 ± 524.3 934.9 1506 - 258	3.5 ± 0.5 * 3.5 4.5 - 2.5	0.000

Data are expressed as mean ± standard deviation
* p=0.000 vs PiMZ and COPD (ANOVA with Bonferroni correction)

The mean percentage of total cells and epithelial cells did not differ significantly (p=0.106 and p=0.116) among the groups. The mean neutrophil count was higher in PiMZ subjects (84.5 ± 22.2 x 10^4/ml; 69,3 ± 18.2 %NSC) and in COPD patients (126,9 ± 18,8 x 10^4/ml; 76.3 ± 9.9 %NSC) than in healthy control subjects (55.0 ± 8.7 x 10^4/ml ; 45.1 ± 7.2 %NSC) (p=0.000). In contrast, the mean number of macrophages was lower in PiMZ (32.7 ± 11.3 x 10^4/ml; 26.8 ± 9.4 %NSC) and in COPD (30.1 ± 21.4 x 10^4/ml; 18.5 ± 11.3 %NSC) than in healthy control subjects (61.6 ± 9.2 x 10^4/ml; 50.5 ± 7.6 %NSC) (p=0.000). Lastly

lymphocyte population was similar among the three studied groups (p=0.971). The PiMZ subjects and COPD patients showed an inverted macrophages / neutrophils ratio as compared to normal subjects.[14] In sputum supernatant, the values of IL-8 measured in COPD patients (882.5 ± 524.3 ng/ml ; median = 934.9 ng/ml; range = 1506 - 258 mg/ml), in PiMZ (828.5 ± 490.6 ng/ml; median 1003.0 ng/ml; range = 1260 - 100 ng/ml) subjects were similar (p= 0.88) but higher (p=0.000) than IL-8 levels in healthy control subjects (3.5 ± 0.5 ng/mL; median = 3.5 ng/ml; range = 4.5 - 2.5 ng/ml).

A significant positive correlation between IL-8 supernatant concentration and neutrophils number in the induced sputum of PiMZ subjects was found (p= 0.036; r= 0.66 Figure 1). There was a weak, but significant, negative correlation between the mean percentage of neutrophils and FEV1 (% predicted) in COPD patients (p=0.04; r=-0.43), that was not found in asymptomatic nonsmoker PiMZ subjects (p=0.55, r=0.05).

DISCUSSION

This study, for the first time, demonstrates that in asymptomatic non-smoker PiMZ subjects without airflow obstruction the number of neutrophils in induced sputum and the level of IL-8 in sputum supernatant were higher than in healthy control subjects and similar to COPD patients. Furthermore, we showed that the count of neutrophils is related to the levels of IL-8 in the sputum of the PiMZ subjects suggesting a role for IL-8 in the recruitment of neutrophils or IL-8 release by activated neutrophils in the airways of PiMZ asymptomatic subjects.

An increased number of neutrophils with a high concentration of cytokines involved in neutrophilic chemotaxis has been previously found in induced sputum of stable COPD patients[15][16][17][18] and COPD with severe AATD.[19] Moreover, using spontaneous sputum and BAL an increase of both airway neutrophil burden and soluble neutrophilic chemoactractants IL-8 and LTB-4 was also shown in AATD subjects with airflow obstruction.[9][20][21] In the present study, we demonstrated an increased number of neutrophils and a decreased number of macrophages, similar to that observed in stable COPD patients, in asymptomatic non-smoker PiMZ subjects without airflow obstruction by induced sputum. These data suggest that the elevated number of neutrophils might play a potential deteriorating role on lung function promoting a cascade of inflammatory events in the airways of asymptomatic PiMZ subjects, due to an impaired equilibrium of the balance between proteases and anti-proteases.

According to our data, the lung neutrophil burden in PiMZ subjects was correlated with high levels of IL-8 in sputum supernatant as previously observed in PiZZ AATD subjects with COPD [9][21]. A possible explanation for the source of neutrophil infiltration and IL-8 in PiMZ subjects is the presence in the airways of free elastase. High concentration of IL-8 in the sputum supernatant observed in PiMZ subjects without lung impairment may be induced either by increased neutrophilic recruitment itself or by the secretion from bronchial epithelium and alveolar macrophages as a direct effect of uninhibited free elastase. IL-8 may also be produced in airway epithelial cells, in order to induce chemotaxis and activation of neutrophils and of eosinophils,[22][23] after exposure with different stimuli such as viral and/or bacterial infection.[24] However our PiMZ subjects were asymptomatic and we may clinically rule out the potential role of respiratory viral or bacterial infections in influencing the present results.

Furthermore, Lomas et al. have recently demonstrated the formation of AAT polymers within the lungs in subjects with AATD due to the Z mutation.[25] This polymerization not only inactivates AAT but also converts the molecule into a powerful proinflammatory chemoattractant agent for human neutrophils.[26] These polymers can form spontaneously

within the lungs of subjects with Z -AATD related emphysema (PiZZ), that have increased release of LTB-4 secreted from alveolar macrophages and high levels of LTB-4 and IL-8 in induced sputum compared with subjects with normal AAT levels.[21] It is possible that the chemotactic properties of polymeric AAT may provide a further explanation for the excessive neutrophil burden found also in the lung of PiMZ subjects before the development of airflow obstruction, and theoretically Z polymer formation might play a role in the pathogenesis of COPD in some MZ heterozygotes. This has been recently proposed as a potential mechanism of airway inflammation in PiMZ subjects[27], although it has not been proven, further studies assessing the presence of AAT polymers in the lung of PiMZ subjects could confirm this hypothesis. Basing on these results, we can speculate that in AATD the process leading to lung destruction and emphysema might be related not only to the destruction of connective tissue due to uninhibited free elastase, but also to the greater degree of persistent neutrophilic inflammation in the airways and lung parenchyma of MZ heterozygotes.

It is noteworthy that PiMZ subjects were asymptomatic despite the presence of neutrophils infiltration similar to that of COPD patients. This could be explained by the fact that PiMZ subjects were non-smoker and also that were younger than COPD patients. In COPD patients there was a significant inverse correlation between the percentage of neutrophils and FEV1 (% predicted). In line with other reports, this finding indicates that in COPD the severity of airflow obstruction seems to be influenced by the amount of chronic neutrophilic recruitment.[28] Conversely, in non-smoker asymptomatic PiMZ subjects the neutrophil burden was not related to FEV1 (% predicted), suggesting an early and low-grade persistent inflammatory process which could accelerate the lung destruction in the presence of severe AATD or smoking. Our healthy control subjects were age-matched with PiMZ subjects, but not with COPD patients. We know that the cellular pattern in sputum is age-related[29] and on the basis of this concept we strongly point out the long-term clinical relevance of the early inflammatory status in the airways of PiMZ subjects.

We included COPD patients as reference group for sputum neutrophilia in order to identify similarity in airway inflammation between PiMZ subjects without airway obstruction and COPD patients with irreversible airflow limitation. On the other hand, we decided to rule out in this study Pi ZZ patients with airflow limitation because in these subjects the airway neutrophilic inflammation has been already demonstrated both in BAL fluid [9] and in sputum analysis.[21] Although there is a substantial debate within the scientific community as to whether the heterozygous condition of AATD is associated with an elevated risk for COPD, we showed that in a group of PiMZ AATD subjects without airflow obstruction a neutrophilic airway inflammation is present. A limitation of this study might be the lack of dosage of free elastase in the airways of PiMZ subjects. We believe, however, that present data represent a novel and relevant contribution to better understand the biology of the lung of PiMZ subjects. Further, larger studies are needed evaluating also other important parameters as elastase to make clear the natural history of PiMZ subjects.

In conclusion, our results indicate that asymptomatic non-smoking PiMZ subjects without airflow obstruction exhibit a significant number of neutrophils in the induced sputum comparable to that observed in COPD patients, reflecting an early and persistent low-grade of airway inflammation due to neutrophilic recruitment, likely sustained by chemotactic factors. Such findings, together with the deficiency of anti-protease activity, might explain the possibility of progression towards the obstructive lung disease observed in some PiMZ subjects.

****

REFERENCES

1. Aboussuan LS, Stoller JK: New developments in alpha 1-antitrypsin deficiency. Am J Respir Crit Care Med 1999;20(4):301-10.
2. American Thoracic Society. Guidelines for the approach to the patient with severe hereditary alpha-1-antitrypsin deficiency. Am Rev Respir Dis 1989;40(5):494-7.
3. Hersh CP, Dhal M, Ly NP, et al. Chronic obstructive pulmonary disease in alpha1-antitrypsin PIMZ heterozygotes: a meta -analysis. Thorax 2004; 59:843-9.
4. Beeh KM, Beier J, Kornmann O, et al. Long-term repeatability of induced sputum cells and inflammatory markers in stable,moderately severe COPD.Chest 2003;123:778-783.
5. Fahy JV, Boushey HA, Lazarus SC, et al. NHLBI Asthma Clinical Research Network. Safety and reproducibility of sputum induction in asthmatic subjects in a multicenter study. AM J Resp Crit Care Med 2001;163:1470-5.
6. Pesci A, Majori M, Cuomo A, et al. Neutrophils infiltrating bronchial epithelium in chronic obstructive pulmonary disease. Respir Med 1998;92:863-870.
7. Tsoumakidou M, Tzanakis N, Siafakas NM. Induced sputum in the investigation of airway inflammation of COPD. Respir Med 2003;97(8):863-71.
8. Stockley RA, Hill AT, Hill SL, et al. Bronchial inflammation: its relationship to colonizing microbial load and alpha(1)-antitrypsin deficiency. Chest 2000;117:291S-3S.
9. Rouhani F, Paone G, Smith NK, et al. Lung neutrophil burden correlates with increased pro-inflammatory cytokines and decreased lung function in individuals with alpha 1-antitrypsin deficiency. Chest, 2000;117:250S-1S
10. Pearson MG on behalf of The COPD Guidelines Group of the Standards Care Commitee of the BTS: BTS guidelines for the management of chronic obstructive pulmonary disease. Thorax 1997; 52 (s5): S1-28.
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. Massi G, Marano G, Patalano F, et al. Silver-stained phenotyping alpha-1-antitrypsin in dried blood and serum specimens. Clin. Chem. 1984;30(10):1674-1676.
13. Clausen JL, Coates AL, Quanjer PH. Measurement of lung volumes in humans: review and recommendations from an ATS/ERS workshop. Eur. Respir. J. 1997;10(6):1205-6.
14. Spanevello A, Confalonieri M, Sulotto F, et al. Induced sputum cellularity. Reference values and distribution in normal volunteers. Am J Respir Crit Care Med 2000;162:1172-4.
15. Keatings VM, Collins PD, Scott DM, et al. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med 1996;153:530-534.
16. Keatings V, Barnes P. Comparison of inflammatory cytokines in chronic obstructive pulmonary disease, asthma and controls. Eur Respir Rev 1997;7:146-150.
17. Beeh KM, Kornmann O, Buhl R,et al. Neutrophil chemotactic activity of sputum from patients with COPD:role of interleukin8 and leukotrieneB4.Chest 2003;123:1240-1247.

18. Stockley RA. Neutrophils and the pathogenesis of COPD. Chest 2002;121:151S -155S.
19. Hill AT, Bayley DL, Campbell EJ, et al. Airways inflammation in chronic bronchitis: the effects of smoking and alpha1-antitrypsin deficiency. Eur Respir J 2000;15:886-890.
20. Hubbard RC, Fells G, Gadek J, et al. Neutrophil accumulation in the lung in alpha 1-antitrypsin deficiency. Spontaneous release of leukotriene B4 by alveolar macrophage. J Clin Inv 1991;88:891-897.
21. Woolhouse IS, Bayley DL, Stockley RA. Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of alpha(1)-antitrypsin deficiency and the role of leukotriene B(4) and interleukin 8. Thorax 2002;57:709-714.
22. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. J Clin Invest 1989;84(4):1045-9.
23. Nakamura H, Yoshimura K, Jaffe HA, et al. Interleukin-8 gene expression in human bronchial epithelial cells. J Biol Chem. 1991;266(29):19611-7.
24. Wedzicha JA, Donaldson GC. Exacerbations of chronic obstructive pulmonary disease. Respir Care. 2003;48(12):1204-13.
25. Elliott PR, Bilton D, Lomas DA. Lung polymers in Z alpha1-antitrypsin deficiency-related emphysema. Am J Respir Cell Mol Biol. 1998;18(5):670-4.
26. Parmar JS, Mahadeva R, Reed BJ, et al. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. Am J Respir Cell Mol Biol. 2002;26(6):723-30.
27. Mahadeva R, Atkinson C, Li Z, et al. Polymers of Z alpha1-atitripsin co-localize with neutrophils in emphysematous alveoli and are chemotactic in vivo. Am J Pathol 2005; 166: 377-86.
28. Peleman RA, Rytila PH, Kips JC, et al. The cellular composition of induced sputum in COPD. Respir Med 2003;97(8):863-71.
29. Thomas RA, Green RH, Brightling CE, et al. The influence of age on induced sputum differential cell counts in normal subjects. Chest. 2004;126(6):1811-4.

****

Conflict of interest: none

Figure Legend:
Figure 1: correlation between IL-8 supernatant concentration and number of neutrophils in the induced sputum of PiMZ subjects

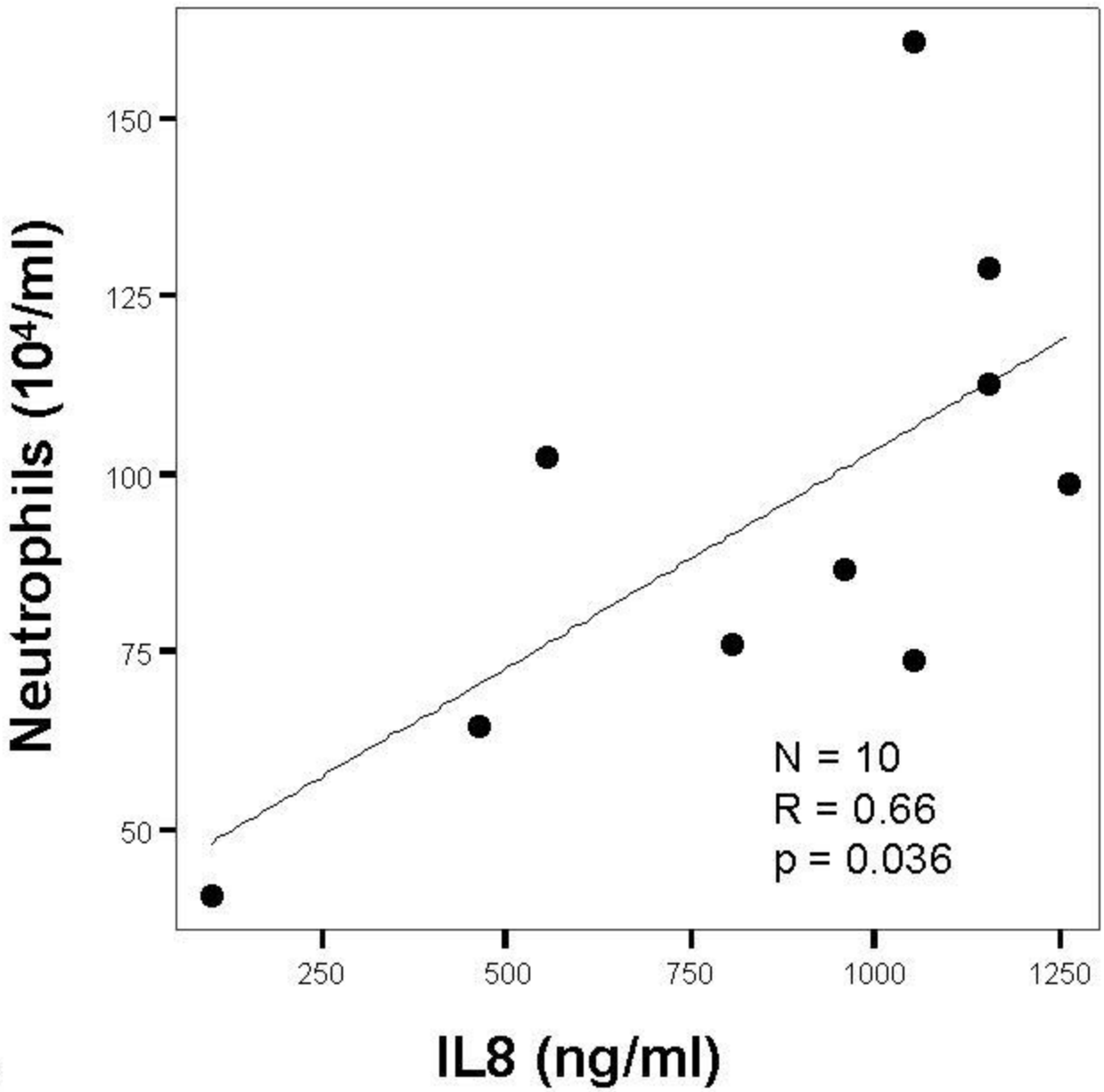

Figure 1