Plasma levels of α_1-antitrypsin-derived C-terminal peptides in PiMM and PiZZ COPD patients

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To the Editor:

α_1-Antitrypsin (α_1-AT) is an acute-phase glycoprotein that antagonises the activity of various proteases and performs broad immunomodulatory functions [1, 2]. One of the well-recognised functions of α_1-AT is to protect the lungs against the development of COPD and emphysema. Consequently, people with severe inherited α_1-antitrypsin deficiency (α_1-ATD), and especially smokers, are at a higher risk of developing COPD with emphysema in the third or fourth decades of life [3, 4]. Most clinically recognised α_1-ATD patients carry the Z-allele homozygously (PiZZ, Glu342Lys mutation in *SERPINA1* gene) and have mean serum levels of ~32 mg·dL^{-1}, while individuals with a normal, PiMM genotype have α_1-AT levels of ~130 mg·dL^{-1} [5]. The dominant theory for the pathogenesis of α_1-ATD-related emphysema is an imbalance between proteases and antiproteases towards protease activity [6, 7]. Therefore, in addition to the usual treatment options for COPD and emphysema, patients with α_1-ATD-related emphysema are treated with human plasma-purified pharmaceutical preparations of α_1-AT as an augmentation therapy.

There are reports suggesting that α_1-ATD might arise not only due to inherited mutations of the *SERPINA1* gene but also due to post-translational modifications of α_1-AT causing an "acquired" α_1-ATD. *In vivo*, α_1-AT can undergo oxidation, degradation, complex formation with other substances, self-assembly or other modifications. Some of these may result in "acquired" deficiency of native α_1-AT and in the generation of new molecular forms [8, 9]. For instance, active metalloproteases, like MMP-13, can inactivate α_1-AT by cleavage [10] and generate fragments with novel biological activities [11]. As yet, post-translationally modified forms of α_1-AT and their putative relationship with acquired α_1-ATD have received little attention in COPD and other clinical research areas.

We previously demonstrated that the content of urinary peptides differs between COPD patients with PiMM and PiZZ genotypes [12]. More recent studies found that plasma levels of carboxyl (C)-terminal peptides of α_1-AT are significantly elevated in patients with acute respiratory distress syndrome, severe COVID-19 and bacterial pulmonary sepsis [13–15]. Since peptides of α_1-AT are generated under inflammatory conditions and COPD is characterised by the persistent systemic inflammation [16], we aimed to investigate whether peptides of α_1-AT are present in plasma of COPD patients with PiMM and PiZZ genotypes.

We enrolled 111 clinically stable COPD patients, 67 PiMM and 44 PiZZ, of whom 21 were on intravenous α_1-AT augmentation therapy (*i.v.* α_1-AT) (Prolastin, 60 mg·kg^{-1} body weight). Phenotyping and genotyping were performed to confirm PiMM and PiZZ genotypes. Plasma samples of PiZZ patients on *i.v.* α_1-AT were taken 1 week after therapy prior to the next *i.v.* α_1-AT infusion. All EDTA-treated plasma samples were stored at −80°C until analysis. The PiZZ and PiMM patients were homogeneous regarding age (median (interquartile range) 57 (52–62) *versus* 59 (49–71) years, p=0.161), gender (female/male 16/28 *versus* 32/35, p=0.161) and smoking habits (smoker/non-smoker 7/37 *versus* 20/46, p=0.114). Relative to PiMM, PiZZ patients had mild emphysema and lower gas transfer (mean±SD diffusing capacity of the lung for carbon monoxide (D_{LCO}) 68.5±18.9% (n=28) *versus* 47.1±18.5% predicted (n=39), p<0.001). All participants provided informed consent. The ethics committee of the Institute of Tuberculosis and Lung Diseases (ITLD), Warsaw, Poland, approved the study (KB-23/2019 and KB-79/2020).

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Plasma levels of α_1-antitrypsin-derived C-terminal peptides might be valid as novel biomarkers to predict and/or characterise exacerbations in PiMM and PiZZ COPD patients, or to reflect the efficiency of augmentation therapy in PiZZ patients https://bit.ly/3rNJeLd

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Plasma levels of α_1-AT and high-sensitivity C-reactive protein (hs-CRP) were determined by nephelometry (IMMAGE 800 Protein Chemistry Analyzer; Beckman Coulter Inc., Brea, CA, USA) at the Department of Genetics and Clinical Immunology, ITLD. The lower detection limit was 10 mg·dL^{-1} for α_1-AT and 0.02 mg·dL^{-1} for hs-CRP. Plasma levels of C-terminal peptides of α_1-AT differing in the number of amino acids (C22, C36, C37, C39, C40, C42, C43, C44 and C45) were determined by an improved version of the previously published liquid chromatography–tandem mass spectrometry method, validated according to US Food and Drug Administration criteria [17]. The concentrations of peptides were determined in relation to the respective internal standards ($C22^{IS}$ for C22; $C37^{IS}$ for C36 and C37; and $C42^{IS}$ for C40, C42, C43, C44 and C45) (0.8 µM each; sb-PEPTIDE, Saint Egrève, France) and $1/x^2$ weighted quadratic regression using separate calibration curves for each peptide. Data acquisition and processing was performed with Analyst Software (version 1.6.2 and 1.7.1). One common single-nucleotide polymorphism (SNP) within M α_1-AT alleles (M3 allele; Asp376, rs1303) affects the mass of C-terminal peptides. Therefore, we applied a parallel quantification of C-terminal peptides from M-alleles with and without this polymorphism. The concentrations of wild type (wt) and SNP variants were first determined in the most abundant peptide, C42. Then, single values of wt and SNP measurements were summarised to give a final concentration for each peptide in carriers of M-alleles. Values of peptides in PiMM patient plasma, which were below lower limit of quantification (LLOQ) (0.025 µM for C36 and C42, and 0.01 µM for other peptides), were imputed (six values of C36, 11 of C37, three of C40 and two of C42) using the imputeLCMD (version 2.1) package of R Statistical Software (version 4.1.0, R Core Team 2021).

As expected, plasma α_1-AT levels were lower in PiZZ than in PiMM patients whereas PiZZ patients on *i.v.* α_1-AT had higher α_1-AT levels those off *i.v.* α_1-AT (figure 1a). Plasma hs-CRP levels varied in between 0.2 (0.1–0.9) mg·dL^{-1} for PiMM, 0.5 (0.1–0.9) mg·dL^{-1} for PiZZ off *i.v.* α_1-AT and 0.3 (0.2–0.5) mg·dL^{-1} for PiZZ on *i.v.* α_1-AT. In entire cohort, we found no correlation between α_1-AT and hs-CRP levels (Spearman's rank correlation). In PiZZ patients off *i.v.* α_1-AT, plasma levels of all analysed peptides were below the LLOQ. However, C36, C37 C40, and C42 peptides were measurable in PiMM and in PiZZ patients on *i.v.* α_1-AT. As shown in figure 1b, in PiMM and PiZZ on *i.v.* α_1-AT, levels of C36 and C42 peptides were higher than those of C37 or C40. Moreover, the level of C36 and C42 peptides in PiMM (C36 0.068 (0.041–0.096) µM and C42 0.082 (0.062–0.105) µM were found to be about twice those in PiZZ on *i.v.* α_1-AT (C36 0.035 (0.029–0.051) µM and C42 0.042 (0.034–0.050) µM) with $p=0.0008$ for C36 and $p<0.0001$ for C42 (Mann–Whitney test). Positive correlations were found between α_1-AT and C36 or C42 levels in 102 patients; unfortunately, nine samples were not available for the peptide analysis (figure 1c and d).

There was no relationship between C36 and C42 levels and patient age, gender or spirometry tests (Mann–Whitney tests were used for age and spirometry tests, and Fisher's exact test was employed for categorical variables; data not shown). Among 60 patients for whom paired data were available, a weak positive correlation was found between C42 and D_{LCO} % predicted (Spearman's test: r=0.38, p=0.02).

Taken together, we provide evidence that C-terminal peptides of α_1-AT, notably C36 and C42, are present in plasma of stable PiMM but not in PiZZ COPD patients off *i.v.* α_1-AT. Since these peptides occur in plasma of PiZZ patients on *i.v.* α_1-AT and strongly correlate with α_1-AT levels, it is reasonable to assume that the peptides originate from α_1-AT protein cleavage rather than from previously suggested alternative transcripts of the *SERPINA1* gene [18]. Confirming this, PiZZ patients off *i.v.* α_1-AT had very low plasma levels of α_1-AT relative to PiMM patients (28±9 mg·dL^{-1} (n=23) *versus* 152±26 mg·dL^{-1} (n=67), respectively; $p<0.001$) (figure 1a) and therefore, peptide levels in these patients are undetectable. However, we cannot exclude that proteolytic cleavage of misfolded Z α_1-AT generates the measured peptides and/or hydrophobic C-terminal peptides are hidden within the polymeric structures of circulating Z α_1-AT.

We also found that commercial α_1-AT preparations contain small amounts of C36 and C42 peptides. Based on the analyses of three different lots of Prolastin preparations (2.5 mg·mL^{-1}), peptide concentrations ranged from 0.152 to 0.445 µM for C36 and values just above the LLOQ (0.026 µM) for C42. Hence, in PiZZ patients on *i.v.* α_1-AT, small amounts of α_1-AT peptides can be delivered with therapy. In our case, plasma samples of PiZZ on *i.v.* α_1-AT were obtained 1 week after administration of *i.v.* α_1-AT. Therefore, we assume that peptides in these patients arise from endogenous cleavage of administered α_1-AT rather than from the α_1-AT preparation *per se*. Unfortunately, nothing is known about peptide pharmacodynamic and pharmacokinetic properties, and we hope our pilot study will encourage further investigations in this field.

The interest is high in circulating peptides as diagnostic and prognostic markers for a variety of diseases. Peptides of α_1-AT have been identified in human urine, bronchoalveolar lavage fluid, gingival crevicular

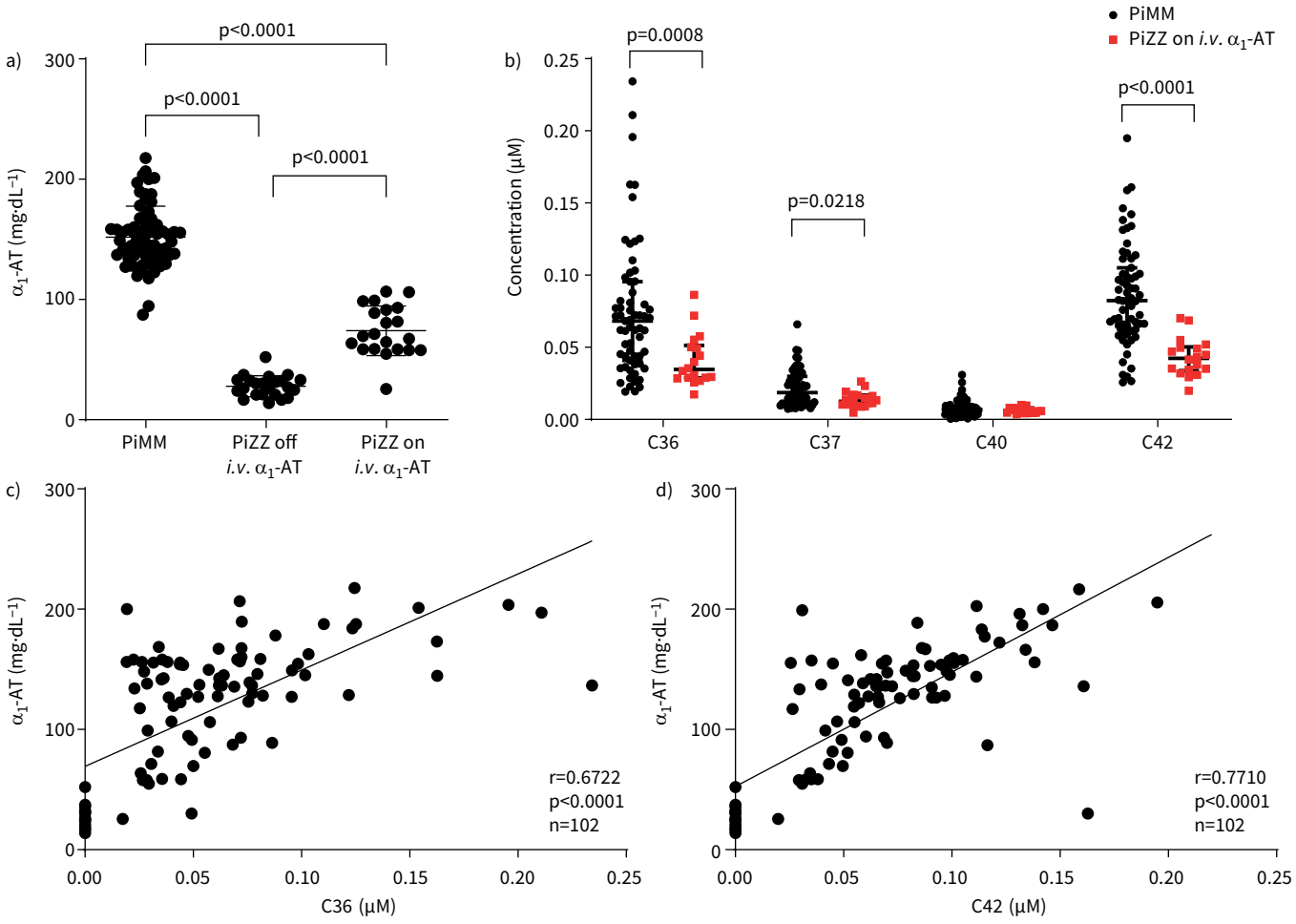

FIGURE 1 Plasma levels of α_1-antitrypsin (α_1-AT) and C-terminal α_1-AT peptides in PiMM and PiZZ COPD patients. a) Plasma levels of α_1-AT in PiMM patients (n=67), and PiZZ paitents off (n=23) and on intravenous α_1-AT (n=21). Data were calculated by using one-way ANOVA. Values passed Shapiro–Wilk normality test and are presented as mean±SD. b) Peptide levels in PiMM and PiZZ COPD patients on *i.v.* α_1-AT. Peptide concentrations below the lower limit of quantification (LLOQ) were imputed or set to zero. Values failed Shapiro–Wilk normality test and are presented as median (interquartile range). Peptide levels in PiMM and PiZZ on *i.v.* α_1-AT were calculated using Mann–Whitney test. In PiZZ patients off *i.v.* α_1-AT, plasma levels of all analysed peptides were below the LLOQ. Correlations between α_1-AT and c) C36 and d) C42 levels. Correlations were calculated using Pearson's test; n indicates the number of available data pairs. The correlation factor r is given. For statistical analysis and data presentation, Prism (version 9.1.2, GraphPad Software) was used. A p-value <0.05 indicates significance.

fluid, spleen and bile, and nipple aspiration fluids [17]. For example, the C36 peptide of α_1-AT (typical cleavage product of serine proteases) has been reported as a regulator of bile acid synthesis in a rat model [19] and as a pro-inflammatory activator of human monocytes [11]; the C42 peptide of α_1-AT (generated by metalloprotease cleavage) was suggested as a putative biomarker of sepsis [14] and acute respiratory distress syndrome severity [15]. Other peptides were proposed as biomarkers for glomerular kidney diseases, pulmonary fibrosis, gingivitis and carotid artery stenosis [17]. Whether peptides of α_1-AT *per se* or in relation to α_1-AT protein can be clinically useful to characterise chronic systemic inflammation [20], exacerbation severity and/or effects of therapeutics in COPD patients, remains to be answered.

Our data provide further evidence that *in vivo* cleavage of α_1-AT results in a generation of specific profiles and levels of peptides. This post-translational modification may not only lead to acquired deficiency of α_1-AT but also to the generation of byproducts with novel biological activities. We believe that a ratio between α_1-AT and the peptides generated after α_1-AT cleavage may help us better understand protease/antiprotease imbalance mechanisms in PiZZ and PiMM COPD.

Friedemann R. Börner[1], Urszula Lechowicz[2], Sabine Wrenger[3], Beatriz Martinez-Delgado[4], Beata Olejnicka[3], Tobias Welte[3], Joanna Chorostowska-Wynimko[2], Michael Kiehntopf[1,5] and Sabina Janciauskiene[2,3,5]

[1]Institute of Clinical Chemistry and Laboratory Diagnostics, Jena University Hospital, Jena, Germany. [2]Department of Genetics and Clinical Immunology, National Institute of Tuberculosis and Lung Diseases Warsaw, Poland, Poland. [3]Department of Respiratory Medicine and Infectious Diseases, Hannover Medical School, Biomedical Research in Endstage and Obstructive Lung Disease Hannover BREATH, German Center for Lung Research DZL, Hannover, Germany. [4]Molecular Genetics Unit, Institute of Rare Diseases Research (IIER), CIBER of Rare Diseases (CIBERER), Institute of Health Carlos III (ISCIII), Madrid, Spain. [5]These authors contributed equally and share last authorship.

Corresponding author: Sabina Janciauskiene (sabinajanciauskiene@gmail.com)

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