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ORIGINAL ARTICLE
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Circulating desmosine levels do not predict emphysema progression but are associated with cardiovascular risk and mortality in COPD

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ABSTRACT Elastin degradation is a key feature of emphysema and may have a role in the pathogenesis of atherosclerosis associated with chronic obstructive pulmonary disease (COPD). Circulating desmosine is a specific biomarker of elastin degradation. We investigated the association between plasma desmosine (pDES) and emphysema severity/progression, coronary artery calcium score (CACS) and mortality.

pDES was measured in 1177 COPD patients and 110 healthy control subjects from two independent cohorts. Emphysema was assessed on chest computed tomography scans. Aortic arterial stiffness was measured as the aortic–femoral pulse wave velocity.

pDES was elevated in patients with cardiovascular disease ($p<0.005$) and correlated with age (rho=0.39, $p<0.0005$), CACS (rho=0.19, $p<0.0005$) modified Medical Research Council dyspnoea score (rho=0.15, $p<0.0005$), 6-min walking distance (rho=−0.17, $p<0.0005$) and body mass index, airflow obstruction, dyspnoea, exercise capacity index (rho=0.10, $p<0.01$), but not with emphysema, emphysema progression or forced expiratory volume in 1 s decline. pDES predicted all-cause mortality independently of several confounding factors ($p<0.005$). In an independent cohort of 186 patients with COPD and 110 control subjects, pDES levels were higher in COPD patients with cardiovascular disease and correlated with arterial stiffness ($p<0.05$).

In COPD, excess elastin degradation relates to cardiovascular comorbidities, atherosclerosis, arterial stiffness, systemic inflammation and mortality, but not to emphysema or emphysema progression. pDES is a good biomarker of cardiovascular risk and mortality in COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD) is characterised by persistent progressive airflow limitation, and is associated with an enhanced inflammatory response in the lungs to the inhalation of noxious particles and gases [1]. COPD is also frequently complicated by the development of extra-pulmonary comorbidities that have important implications for morbidity and mortality [2], in particular cardiovascular disease (CVD) [3, 4].

We have previously proposed that elastin degradation could potentially contribute to both the pulmonary and extrapulmonary manifestations of COPD [5] and may represent a mechanistic link between emphysema and the increased risk of cardiovascular disease [6]. The destruction of elastin in alveolar walls by proteases, as part of chronic tobacco smoking-induced lung inflammation, is a central feature of the pathogenesis of emphysema [7]. Recent studies have shown that arterial stiffness, a biomarker of cardiovascular risk [8], is increased in COPD patients [6, 9, 10]. Increased arterial stiffness may result from increased elastin degradation and a relative increase in collagen in arterial walls, as occurs with aging [11, 12] and atherosclerosis [13]. Indeed we have shown that increased arterial stiffness in COPD is associated with increased elastin degradation in the skin [14] and with emphysema in COPD patients [15].

Thus, elastin degradation could potentially contribute to both the pulmonary and extrapulmonary manifestations of COPD [5] and may represent a mechanistic link between COPD and the increased risk of cardiovascular disease in this condition [6].

Desmosine, and its isomer iso-desmosine, result from the condensation of four lysine residues in and between elastin proteins after oxidation by lysyl-oxidase and are released when elastin is degraded. These represent ideal biomarkers to monitor elastin degradation since these special crosslinks exist only in mature elastin [16]. The potential of desmosine, usually measured in urine, as a biomarker for pulmonary emphysema has been extensively studied in the past 40 years. However, inconclusive results have hindered its potential utility [16]. With improvements in analytical methods [17–19], we recently demonstrated that plasma total desmosine is elevated in 30–40% of COPD patients [18], results that were confirmed in a larger study [20]. However, the potential of plasma desmosine (pDES) as a biomarker of the severity or progression of emphysema and its role as a marker of cardiovascular comorbidities in COPD remains unclear.

The aim of this study was to explore the relationship of pDES with emphysema, emphysema progression, cardiovascular comorbidities, coronary artery calcium score (CACS), as a surrogate of coronary atherosclerosis, and mortality in a cohort of patients with COPD from the ECLIPSE study (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints). A second independent cohort was used to extend the findings in the ECLIPSE cohort.

METHODS

Study population and ethics

991 stable patients with COPD from the ECLIPSE study [21], and 186 patients with COPD and 110 age, sex and smoking-matched controls from a second independent cohort (The Association of Lung Function and Cardiovascular Risk study [22]), were studied.

All subjects were aged >40 years and of European descent with a smoking history of ⩾10 pack–years. Patients with COPD in both cohorts were current or ex-smokers (⩾10 pack-years), with baseline post-bronchodilator forced expiratory volume in 1 s (FEV_1) 80% of predicted and FEV_1/forced vital capacity (FVC) <0.7 and were studied when clinically stable.

Ethics committees of all participating institutions approved the study and written informed consent was obtained from all subjects.

Circulating inflammatory biomarkers

In blood samples from the ECLIPSE cohort, inflammatory markers were measured in serum or plasma as previously described [23, 24].

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This study is registered at www.clinicaltrials.gove with identifier number NCT00292552.

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pDES measurements

Total pDES concentration was measured using a modified assay of a validated isotope dilution of liquid chromatography (LC) with mass spectrometry (MS) LC-MS/MS method [17] at year 1 and year 2 in the ECLIPSE cohort and at baseline in the Nottingham cohort.

Computed tomography

In the ECLIPSE study, subjects underwent a low-dose chest computed tomography (CT) scan (GE Healthcare or Siemens Healthcare, Erlangen, Germany) at baseline, year 1 and 3. All CT scans were analysed at a central laboratory using Pulmonary Workstation 2.0 software (VIDA Diagnostics, Coralville, IA. USA) [25].

Emphysema was measured as the percentage of low attenuation areas (%LAA) <−950 Hounsfield units in the whole lung or the 15th percentile of the frequency histogram of lung density values when the progression of emphysema was assessed, as previously described [26]. Emphysema was considered to be present if %LAA was >10% [27]. The %LAA was also assessed as a continuous variable.

Coronary artery calcium score

CACS was assessed on CT-lung images in the ECLIPSE cohort with a low spatial frequency algorithm as previously described [27] with images analysed using the Agatston scoring method [28].

Arterial stiffness

Arterial stiffness was measured in the Nottingham cohort as the carotid-femoral pulse wave velocity (aortic pulse wave velocity (PWV)) using Vicorder (Skidmore Medical, Bristol, UK) in triplicate and the average recorded [29].

Statistical analysis

Data are expressed as mean±SD. pDES levels between paired samples were assessed using Wilcoxon test. Comparisons between groups were conducted using ANOVA with Student-Newman–Keuls as a *post hoc* test or the Kruskall–Wallis equivalent with Dunn's test as a *post hoc* test for non-normally distributed variables. ANCOVA was used to control for potential confounders. Chi-squared tests were used to compare frequencies. Correlations were calculated as Pearson's correlation coefficient or Spearman's correlation coefficient for non-normally distributed variables. Logistic regression was conducted to describe the effect of several covariates on death as an event (as the dependent variable).

A Cox proportional hazards model was constructed to compare mortality between subject groups. Analyses were conducted using the SAS Version 9.3 (SAS Institute Inc, Cary, NC, USA). Benjamini–Hochberg false discovery rate method was used to adjust the multiple hypothesis tests. (see online data supplement for more details).

RESULTS

Investigation of elastin degradation in the ECLIPSE cohort

Of the 2746 subjects enrolled in the ECLIPSE study, 1000 COPD patients were included in the study. This cohort of patients was selected to cover for the whole spectrum of severity in lung function, lung function decline and emphysema progression (between baseline and year 3). A total of 991 blood samples from year 1 were available for analysis. From these, 813 patients with COPD had CT scans available for analysis after the exclusion of scans with poor image quality [27]. The demographic details of the study population are shown in table 1 and a comparison between the patients in the ECLIPSE cohort not included in the study and the present cohort is shown in table S1. The two populations were similar in age, sex, body composition, and smoking history. There were statistically significant differences in lung function and 6-min walking distance (6MWD); however, these differences were very small and considered to be clinically irrelevant.

pDES and patient characteristics

pDES levels in the ECLIPSE cohort correlated positively and significantly (univariate analysis) with age (rho=0.39, p<0.0005), modified Medical Research Council dyspnoea score (mMRC) (rho=0.15, p<0.0005), body mass index, airflow obstruction, dyspnoea, exercise capacity (BODE) index (rho=0.10, p<0.01), hospitalisations (rho=0.08, p<0.05), pack-years (rho=0.07, p<0.05) and the CACS score (rho=0.19, p<0.0005). pDES correlated negatively with FVC (rho=−0.09, p<0.05), 6MWD (rho=−0.17, p<0.0005) and arterial oxygen saturation measured by pulse oximetry (S_{pO_2}) (rho=−0.1, p<0.05). Significant positive correlations were found between pDES levels and inflammatory biomarkers: fibrinogen (rho=0.12, p<0.001), interleukin (IL)-6 (rho=0.15, p<0.0005), IL-8 (rho=0.11, p<0.005), chemokine (C-C motif) ligand (CCL)-18 (rho=0.13, p<0.0005) and surfactant protein D (SP-D) (rho=0.10, p<0.01). No difference in pDES levels was observed between sex. Most of these correlations (except age) are considered weak, albeit significant correlations (as a result of large sample size).

TABLE 1 Characteristics of the study group

	ECLIPSE COPD	Nottingham COPD	Nottingham controls	ECLIPSE *versus* Nottingham COPD p-value	COPD *versus* Nottingham controls p-value
Subjects n	991	186	110		
Men	633 (64)	116 (62)	67 (61)	NS	
Hypertension	41	31	27		
Angina	11	18	7		
Heart attack	10				
Heart failure	6				
Stroke	4				
Age years	63±7.2	68±7.8	65±9.8	<0.0005	NS
BMI kg·m^{-2}	26.8±5.8	27.2±5.6	27.7±4.6	NS	NS
Smoker# pack-years	47.4±26.0	44.2±25.7	29.4±18.6	NS	<0.0001
mMRC	2.6±1.0	2.9±1.1	1.4±0.6	<0.01	<0.005
FEV_1 L	1.4±0.5	1.5±0.6	2.8±0.7	<0.05	<0.0001
FEV_1 % pred	50.5±15.2	57.9±18.5	99.9±13.8	<0.0005	<0.0001
FVC L	3.1±0.9	3.1±0.9	3.8±1.0	NS	<0.0001
FVC % pred	89.2±19.7	93.8±19.9	109.1±17.3	<0.0005	<0.0001
FEV_1/FVC	0.46±0.1	0.49±0.13	0.74±0.07	<0.005	<0.0001
S_{pO_2}%	94.7±2.8	94.6±2.4	95.5±8.4	NS	<0.0001
6MWD m	384±118.9				
6MWD % pred	59.5±18.0				
BODE	2.9±2.0				

Data are presented as n (%), % or mean±SD, unless otherwise stated. ECLIPSE: Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study; COPD: chronic obstructive pulmonary disease; BMI: Body mass index; #: cumulative history of smoking; mMRC: modified Medical Research Council dyspnoea score; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; S_{pO_2}: oxygen saturation measured by pulse oximetry; 6MWD: 6-min walking distance; BODE: BMI, airflow obstruction, dyspnoea, exercise capacity index; NS: nonsifgnificant. Benjamini–Hochberg correction was applied to prevent α-error accumulation.

Subjects were divided into quartiles of pDES levels. The highest pDES quartile had significantly higher values for age, mMRC dyspnoea score, number of hospitalisations recorded in the 3 years of the study, fibrinogen, IL-6, CCL-18, SP-D and lower values of 6MWD (table S2).

Changes in pDES in stable COPD over a 1-year period and characteristics of patients with persistent elastin degradation

From the 991 patients assessed at year 1, 981 samples were available for pDES assessment 1 year later. The levels at the two visits were significantly correlated (r=0.37, p<0.0001). There were no significant differences between pDES levels measured at year 1 and year 2 (p=0.75), and mean differences (bias) between both assessments was 0.0019 ng·mL^{-1} showing a good stability of pDES in repeated assessments (figure S1). We have also shown in a previous report a small short-term intra-subject variability of pDES over 2 weeks [18]. This allowed us to use a nominal cut-off pDES of 0.35 ng·mL^{-1} calculated from the mean 2.575×SD (to achieve the 99% confidence level) derived from healthy volunteers in a previous study [18]. We found that approximately 50% of patients in the ECLIPSE cohort had abnormal elastin degradation at year 1, which (65%) continued to have high elastin degradation activity 1 year later. In the other 50% of patients that had low initial pDES levels, most (63%) continued to have low pDES levels 1 year later. To investigate potential relationships between persistent elastin degradation and patient characteristics, patients were divided into four groups according to the nominal cut-off for pDES; pDESLL (normal levels of pDES at both visits (persistently low), n=312), pDESLH (normal levels of pDES at year 1 but high levels at year 2, n=180), pDESHL (high levels of pDES at year 1 and normal levels at year 2, n=173), and pDESHH (high levels of pDES at both time points (persistently high), n=316).

pDESHH patients were older, had lower FEV_1, S_{pO_2} and 6MWD and higher mMRC, number of years smoked, BODE index, number of hospitalisations recorded in the first 3 years of the study, fibrinogen, IL-6, CCL-18 and circulating neutrophils in comparison to pDESLL (table 2).

The relationship between pDES and emphysema and FEV_1 severity and progression

No differences in pDES were seen between patients with and without emphysema on a CT scan (p=0.68) and no significant correlations were found between pDES and emphysema (%LAA) (rho=0.07, p-value was

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TABLE 2 Characteristics of the study group

	pDES$_{LL}$	pDES$_{LH}$	pDES$_{HL}$	pDES$_{HH}$	p-value
Subjects	312 (32)	180 (18)	173 (18)	316 (32)	NS
Current/former smoker n	41/59	36/64	36/64	34/66	
Age years	59.7±7.4	62.4±6.7	63.0±6.6	66.7±5.7#	<0.0005
FEV$_1$ L	1.4±0.5	1.3±0.5	1.3±0.4	1.2±0.4#	<0.05
S_{pO_2} %	95.2±2.7	94.7±3.1	94.5±2.8	94.5±2.6#	<0.005
mMRC	1.4±1.0	1.6±1.0	1.6±1.0	1.8±1.1#	<0.0005
Smoker pack-years	44.0±24.3	49.6±29.8	48.7±20.9	48.1±27.9	<0.01
Years smoked	37.7±8.8	39±8.8	39.3±10.1	41.0±10.7#<0.01	<0.0005
Hospitalisations	0.55±1.5	0.63±1.3	0.71±1.6	0.79±1.6#	<0.01
6MWD m	413.8±120.6	382.5±121.0	386.8±110.5	354.7±115.4#	<0.0005
BODE index	2.5±2.0	3.0±1.9	2.8±2.0	3.2±2.0#	<0.0005
%LAA	14.7±10.9	17.7±11.6	17.0±11.5	17.2±11.7	<0.05
Fibrinogen mg·dL^{-1}	437.7±94.4	463.8±97.9	447.4±93.0	474.7±104.9#	<0.0005
IL-6 pg·mL^{-1}	2.8±11.1	4.2±12.6	4.4±15.5	6.7±41.6#	<0.0005
IL-8 pg·mL^{-1}	14.2±37.8	14.6±33.3	12.4±18.0	14.1±36.5#	<0.005
CCL-18 pg·mL^{-1}	104.7±38.8	111.1±41.0	109.9±44.6	121.1±47.8*	<0.0005
Lymphocytes	2.0±0.7	2.1±0.6	2.1±0.7	1.9±0.6#	<0.0005
Lymphocytes %	26.9±7.6	26.5±7.5	27.5±8.2	24.4±7.4#	<0.0005
Neutrophils	5.0±1.7	5.3±2.0	4.9±1.7	5.3±1.9	<0.05
Neutrophils %	63.7±8.4	64.0±8.3	63.0±8.6	66.0±8.4#	<0.005
SP-D pg·mL^{-1}	131.7±65.5	139.1±95.3	139.6±69.4	144.7±80.7	NS
CACS	267.9±500.5	470.9±831.3	373.6±577.1	651.7±967.8	<0.0001
Cardiovascular history	58 (25.1)	40 (17.3)	37 (16.0)	96 (41.6)	<0.005
Hypertension	92 (24.2)	77 (20.3)	59 (15.5)	152 (40.0)	<0.0001
Angina	32 (31.1)	13 (12.6)	18 (17.5)	40 (38.8)	NS
Heart Attack	21 (22.8)	14 (15.2)	16 (17.4)	41 (44.6)	<0.05
Heart Failure	11 (21.6)	7 (13.7)	13 (25.5)	20 (39.2)	NS

Data are presented as n (%) or mean±SD or unless otherwise stated. pDES: plasma desmosine; pDES$_{LL}$: normal pDES levels at both visits (persistently low); pDES$_{LH}$: normal pDES levels at year 1 but high levels at year 2; pDES$_{HL}$: high levels of pDES at year 1 and normal levels at year 2; pDES$_{HH}$: high levels of pDES at both time points (persistently high); FEV$_1$: forced expiratory volume in 1 s; S_{pO_2}: arterial oxygen saturation measured by pulse oximetry; mMRC: modified Medical Research Council dyspnoea score; hospitalisations: number of hospitalisations recorded in the first 3 years of the study; 6MWD: 6-min walking distance; BODE: body mass index, airflow obstruction, dyspnoea, exercise capacity index; %LAA: per cent low attenuation areas; IL: interleukin; CCL-18: chemokine (C-C motif) ligand-18; SP-D: surfactant protein D; CACS: coronary artery calcification score; NS; nonsignificant. Comparisons among groups were done using Wilcoxon test and Dunn test as a *post hoc* test. Benjamini–Hochberg correction was applied to prevent α-error accumulation. #: statistical difference between pDES$_{LL}$ and pDES$_{HH}$.

nonsignificant), emphysema progression (change in PD15) (rho=0.02, p-value was nonsignificant), FEV$_1$ (rho=−0.05, p-value was nonsignificant), or FEV$_1$ decline (rho=−0.01, p-value was nonsignificant), There were no differences in %LAA between the different pDES quartiles (p=0.27) or between pDES$_{LL}$ and pDES$_{HH}$ patients (Kruskal–Wallis p=0.01, Dunn *post hoc* test: significance only between pDES$_{LL}$ and pDES$_{LH}$).

pDES and cardiovascular comorbidity

A self-reported history of CVD was present in 25% of patients (table 1). pDES was higher in patients with a history of CVD compared to those without CVD (p<0.005) and specifically in patients with hypertension (p<0.0005), heart attack (p<0.05) and heart failure (p<0.05) (figure S2).

We further investigated the relationship between aortic calcification, a surrogate for atherosclerotic burden, and elastin degradation. CACS was significantly higher in the highest pDES quartile compared with all other quartiles at both year 1 and 2 (p<0.0005) (figure S3). After correcting for %LAA, age, sex, cumulative smoking history (pack-year history), years smoked and inflammation, the significance remains for year 2 results, suggesting the correlation is confounded with these variables. When grouping based on pDES levels at both visits. pDES$_{HH}$ patients had a higher Agatston score in comparison with pDES$_{LL}$, pDES$_{LH}$ and pDES$_{HL}$ patients (p<0.0005) (figure 1a) and remained significant after correcting for the mentioned confounders.

Patients were divided into commonly defined CACS groups [27], low (<100 Agatston units (AU)), intermediate (101–400 AU), high (401–1000 AU) or very high (>1000 AU) CACS. Patients with very high

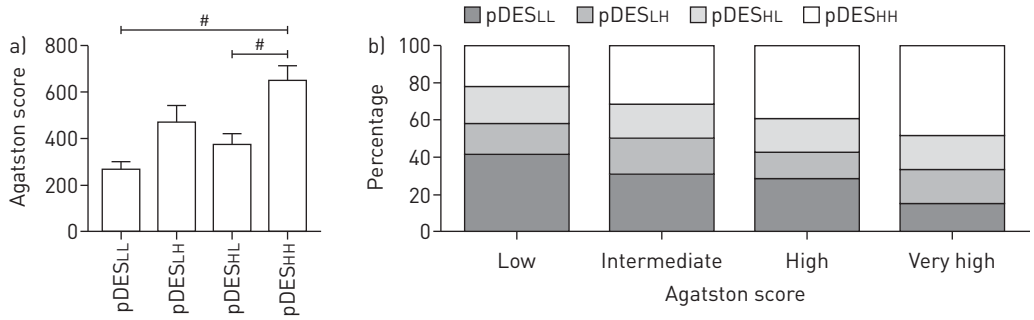

FIGURE 1 Relationship between plasma desmosine (pDES) and coronary artery calcium score (CACS). a) Shows levels of CACS in the four different pDES groups after correcting for per cent low attenuation area (%LAA), age, cumulative smoking history (pack-years) and years smoked. b) Shows patient's distribution in the different CACS categories (low, intermediate, high and very high) according to their pDES at year 1 and year 2. pDESLL: normal levels of pDES at both visits (persistently low); pDESLH: normal levels of pDES at year 1 but high levels at year 2; pDESHL: high levels of pDES at year 1 and normal levels at year 2; pDESHH: high levels of pDES at both time points (persistently high). Chi-squared p<0.0001; #: p<0.0005.

Agatston score at baseline were more likely to have high pDES levels in the subsequent 2 years (pDESHH) (figure 1b), showing that persistently high pDES levels associate with very high Agatston score.

pDES and mortality

pDES levels were higher in patients who died during the 3-year follow-up period (p<0.001) than in those who survived. Patients who died during the 3-year follow-up period had also higher values for age, fibrinogen, IL-6 and CCL18.

In a logistic regression with death as the dependent variable and pDES, age, sex, smoking history, mMRC, hospitalisations, inflammation, CACS and cardiovascular comorbidities as independent variables, only pDES was significantly associated with mortality (p<0.005). For a 0.1 $ng{\cdot}mL^{-1}$ of change in pDES the odds ratio is 1.31 (95% CI 1.12–1.54). Given the other variables in the model are held constant, the odds of death increased by 31% for each 0.1 $ng{\cdot}mL^{-1}$ increase in pDES. A Cox proportional hazards model for patients with COPD and pDES quartiles adjusted for age, sex, smoking history, mMRC, hospitalisations, inflammation, CACS and cardiovascular comorbidities showed that patients in the highest pDES quartile had a significantly lower probability of survival (p<0.05) (figure 2).

Patients with persistently high pDES (pDESHH) had a higher risk of dying during the 3-year follow-up than the other pDES groups; however, this difference is likely to be driven by age and smoking history as the significance disappeared when adjusted for age, sex and smoking history.

The relationship between pDES and arterial stiffness in the Nottingham cohort

To further evaluate the relationship between pDES and cardiovascular comorbidities and risk in COPD and to eliminate the potential influence of kidney function on pDES levels, an independent cohort

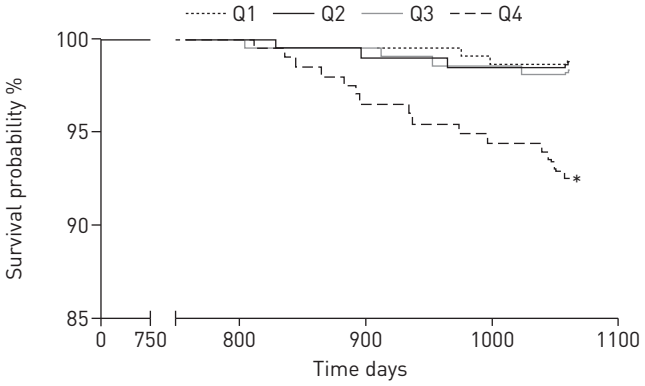

FIGURE 2 Survival probability in relationship to plasma desmosine (pDES). Cox proportional hazard model for patients with chronic obstructive pulmonary disease (COPD) and pDES quartiles adjusted for age, sex, smoking history, hospitalisations, inflammation and coronary artery calcium score. A similar result was observed in an unadjusted Cox model (data not shown). *: p<0.05.

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(M. John and C.E. Bolton, unpublished data) of 186 COPD patients and 110 age-sex- and smoking -matched controls (table 1) was studied. COPD patients in this cohort had a similar sex distribution, body mass index (BMI), pack-years history and S_{PO_2} but were slightly older, had a slightly higher FEV_1 and worse mMRC compared with the ECLIPSE cohort. pDES levels were not significantly different between the two COPD cohorts and so was the proportion of patients expressing abnormal levels of pDES (54% for the Nottingham cohort *versus* 50% for the ECLIPSE cohort).

pDES and PWV

Patients with COPD in the Nottingham cohort had significantly higher mean±SD pDES (0.38±0.16 ng·mL^{-1}) than the controls (0.30±0.15 ng·mL^{-1}, p<0.0001) and higher PWV (10.3±2.1 m·s^{-1}) than the controls (9.6±1.9 m·s^{-1}, p<0.005).

In COPD patients, pDES correlated positively with age (r=0.38, p<0.0001) and PWV (r=0.15, p<0.05) and negatively with FEV_1 (r=−0.19, p<0.01). pDES was also higher in patients with COPD and a history of ischaemic heart disease (IHD)(0.43±0.18 ng·mL^{-1}) compared to those without IHD (0.37±0.15 ng·mL^{-1}; p=0.05).

pDES levels and renal function

There was no significant correlation between pDES and creatinine levels (rho=0.09, p-value was nonsignificant) or estimated glomerular filtration rate (eGFR) (rho=−0.14, p-value was nonsignificant) nor any differences in creatinine levels (p=0.59) eGFR (p=0.16) between the different pDES quartiles or between patients with normal and abnormal levels of pDES (creatinine levels p=0.25, eGFR p=0.18) or between COPD patients and controls (creatinine levels p=0.89, eGFR p=0.65).

DISCUSSION

In the largest study of its kind, to date, we have shown that elevated pDES levels relate to cardiovascular comorbidities, aortic stiffness, and mortality in patients with COPD, but not to emphysema, emphysema progression, as assessed by CT scans, or FEV_1 decline. The association with coronary artery disease was particularly significant in patients with persistently elevated levels of pDES. We also confirmed that patients with COPD had higher pDES compared with age- and sex-matched controls.

These observations suggest that pDES is predominantly a reflection of elastin degradation in vascular tissue, potentially caused by aberrant inflammation in vascular tissues, contributing to worse cardiovascular outcomes and mortality. This notion is supported by results in a second independent cohort where pDES was related to cardiovascular comorbidities and aortic PWV as a measure of arterial stiffness, suggesting that increased arterial stiffness may also result from systemic elastin degradation in the arterial walls [14].

In contrast, the lack of association between pDES levels and emphysema progression or lung function decline, as assessed by CT scan, suggests that pDES is not a good biomarker of lung elastin degradation.

Our study contrasts with previous studies that have shown associations between lung function [20] or measurements of emphysema [18, 20] and desmosine levels. These differences may be explained by differences in the patient populations studied and differences in the measurements of emphysema (or its surrogate) between studies. We have used measurement of emphysema on CT scans as a direct assessment of emphysema rather than surrogates, such as the diffusing capacity for carbon monoxide. Moreover, we selected our population based on emphysema, lung function, emphysema progression and lung function decline. This has enabled us to be in the best position to assess the relationship between these parameters and pDES.

We suspect that the relative size of cardiovascular tissues compared to the lung may be one explanation for these results. The average adult lung weighs about 1.3 kg of which 28% is elastin protein [30], compared to 4.9 kg of the cardiovascular system (based on 7% of body weight of a 70 kg adult), of which up to 50% of its dry weight consists of elastin [31]. Therefore, the contribution from the cardiovascular system to circulating desmosine may be greater than the lungs. Sputum desmosine, which is increased in COPD patients [32], may be a more sensitive marker of lung elastin degradation. The lack of relationship between pDES and emphysema, as assessed by CT scan, may also relate to diminished lung elastin content in patients with emphysema who have less lung tissue. In addition, processes that affect lung density other than emphysema could contribute to masking a relationship with desmosine. Finally, another possible source of pDES could be from elastin degradation in the skin since we have already shown evidence of increased elastin degradation in the skin of patients with COPD compared to matched controls [14].

As the products of elastin degradation, elastokines, can actively participate in the progression of atherosclerosis by accelerating low-density lipoprotein oxidation and calcification of the vascular wall [33], it is also possible that the association observed was indirect or a combination of direct and indirect effects.

Vascular elastin degradation has previously been shown to occur in several conditions such as atherosclerosis [33], aortic aneurysms [34], hypertension [35] and chronic kidney disease [36]. However, evidence of the role of pDES as a prognostic biomarker is scarce and inconsistent. Noticeably, increased elastin degradation as assessed by elastin–derived peptides was associated with increased arterial stiffness and with all-cause mortality in chronic kidney disease [36]. In contrast, the EVA study [37] showed that a decrease in serum elastin peptide levels was associated with risk factors for atherosclerosis-related diseases. Our results in a cohort of COPD patients are consistent with the former. While the discrepancy between these studies is unexplained, we suspect that differences in analytical methods, and the study population, are the likely causes. Matrix metalloproteinase (MMP)-2 and cathepsin-S were implicated as key enzymes for the vascular elastin degradation in chronic kidney [36] and lungs [38] diseases, but have never been investigated in the vascular bed in COPD patients. Interestingly, we have shown increased MMP-2 and MMP-9 gene expression associated with increased elastin degradation in the skin of patients with COPD [14].

Our results suggest that elevated elastin degradation is persistent in a subgroup of COPD patients (*e.g.* ~30% in the ECLIPSE cohort). This subgroup appears to be older with worse mMRC, BODE index, 6MWD, S_{pO_2} and CACS, as well as exhibiting high levels of inflammatory biomarkers. Since pDES levels did not correlate with FEV_1 decline in COPD and excess elastin degradation can occur at an early state of the disease, timely identification of this subgroup of patients may offer an attractive strategy for therapeutic and/or lifestyle intervention to improve clinical outcomes.

Our results indicate that pDES is a predictor of all-cause mortality in COPD patients. Several other biochemical biomarkers are also able to predict mortality in COPD patients including fibronectin to C-reactive protein ratio [39] and other inflammatory biomarkers [40]. However, in a logistic regression analysis only pDES was significantly related to death after correcting for several variables including inflammatory markers. pDES may, therefore, be a good biomarker to identify COPD patients at-risk of death and cardiovascular comorbidity.

As the kidney is the major route for desmosine excretion [41], pDES levels could potentially be affected by renal function. We found no difference in renal function between COPD patients and controls in the Nottingham cohort, nor any significant relationship between renal function and pDES. Thus we believe that our observations are not confounded by differences in renal function.

Study limitations

Our study has some limitations. The two cohorts included in this study do not have the same measurements nor did the second cohort have repeated sampling. Therefore, we were not able to validate either the lack of relationship between pDES and emphysema or the relationship between prospective changes in pDES and outcomes, nor the positive correlation with CACS. However, this was not the main reason for the inclusion of this second cohort, which was to determine if there was a relationship between pDES and arterial stiffness. It also allowed us to confirm the finding of the relationship between pDES and CVD, and to exclude potential effect of renal function. The confirmation of an association between pDES and cardiovascular risk assessed in a totally independent cohort and with a different marker of cardiovascular risk is, therefore, a strength of this study. Unfortunately, due to the low prevalence of cardiovascular disease in the control group in the Nottingham cohort, it was not possible to explore the relationship between pDES and CVD in the healthy subjects.

Conclusions

Our study shows that pDES in patients with COPD is a useful marker of cardiovascular risk and all-cause mortality and may reflect a mechanistic link between COPD and increased cardiovascular risk.

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