EXTENDED ABSTRACT

Comparison of telomere length and skin auto fluorescence as markers of ageing in chronic obstructive pulmonary disease

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Abstract

Accelerated ageing has been proposed as a pathological mechanism of various chronic diseases, including chronic obstructive pulmonary disease (COPD). This concept has almost exclusively been approached by analyses of individual markers. We investigated if COPD is associated with accelerated ageing comparing telomere length and skin auto fluorescence as markers of the ageing process.

Lung function, leukocyte telomere length, skin auto fluorescence, and markers of systemic inflammation were determined in the ICE-Age study which includes 160 COPD patients, 82 smoking and 38 never smoking controls.

Skin auto fluorescence was higher and telomere length was shorter in the COPD patients compared to both control groups. Significant associations with age, pack years smoked and inflammatory parameters were found for both skin auto fluorescence and telomere length. However, no significant relation between both ageing markers was observed. Furthermore, only telomere length and not skin auto fluorescence was found to be independently associated with lung function.

The present study supports the hypothesis that COPD is a syndrome of accelerated biological ageing. Lung function impairment in COPD is more closely related to telomere attrition compared to increased skin auto fluorescence.

Introduction

Chronic obstructive pulmonary disease (COPD) is primarily a lung disease characterized by a chronic inflammatory response in the lungs to noxious particles or gases. It is recognized that COPD in addition is hallmarked by multiple systemic effects, including systemic inflammation[1]. Accelerated ageing has been proposed as a pathological mechanism in COPD[2], as features of the pulmonary pathology are also a characteristic of normal aging of the lungs, albeit to a much lesser extent. Moreover, an accelerated rate of lung function decline with age driven by smoking is seen in a proportion of susceptible individuals[3]. Systemically, skin wrinkling is, independent of ageing, associated with COPD and emphysema[4]. And ageing is, like COPD albeit to a lesser extent, associated with enhanced systemic inflammation and oxidative stress. Lastly, COPD preferentially affects elderly individuals, with those >65y having a higher disease rate than younger age groups, independent of pack years of smoking. We recently examined the concept of COPD as a syndrome of accelerated ageing by assessing a panel of markers representing various interconnected molecular aspects of the ageing process and found that only telomere attrition was consistently associated with lung function[5].

Advanced glycation end products (AGEs) form stable inter- and intramolecular cross-links on long-lived proteins such as skin collagen[6]. A subset of AGEs, including pentosidine, is furthermore characterized by a yellow-brown fluorescent color and can be estimated noninvasively by determining skin auto fluorescence[7]. This accumulation of AGEs on skin collagen correlates with age and can be regarded as a biological marker of age. We previously reported increased skin auto fluorescence in COPD[8]. Since the concept of accelerated ageing in COPD has almost exclusively been approached by analyses of individual markers, we compared telomere length, the most widely accepted marker of biological ageing, to skin auto fluorescence in COPD patients, smoking and non-smoking controls, to evaluate which systemic marker and underlying processes is most closely related to lung function impairment, and whether they are interrelated.

Material and Methods

Study design

160 clinically stable patients with COPD and 120 healthy elderly, matched for sex and age with the COPD patients were enrolled. Inclusion criteria for the COPD patients were diagnosis of COPD according to the GOLD guidelines[9], no respiratory tract infection or exacerbation of the disease for <4 weeks before the study. Inclusion criteria for the control group included absence of objectively diagnosed COPD, untreated diabetes mellitus type II, severe and untreated osteoporosis, severe renal failure or heart failure. Both males and females, age-range from 45 to 75y, were included. Exclusion criteria for both COPD patients and healthy subjects consisted of any type of carcinogenic pathology <5y before study participation, chronic use of oral corticosteroids >10mg/day, investigator's uncertainty about the willingness or ability of the subject to comply with the protocol requirements, participation in any other study involving investigational or marketed products concomitantly or <4 weeks prior to entry into the study. All participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki and good clinical practice guidelines, and was approved by the ethics and review board of the Maastricht University Medical Center+, Maastricht, the Netherlands (MEC 10-3-033).

Blood collection and markers of systemic inflammation

Fasting blood samples were collected as previously described[5]. Interleukin 6 (IL-6) and Interleukin 8 (IL-8) were measured in EDTA plasma using validated immunoassays (R&D Systems, Inc., Minneapolis, MN,

USA). High sensitivity CRP was measured in EDTA plasma using an auto-analyzer (ABX Pentra 400, HORIBA ABXS.A.S, France) and fibrinogen was assessed in citrate plasma using a coagulation analyzer (Sysmex CA-7000; Dade-Behring, Lieder Bach, Germany).

Leukocyte telomere length measurement
Leukocyte telomere length was determined by qPCR in genomic DNA extracted from buffy coats using the QIAamp DNA mini kit (Qiagen) [10].

Skin autofluorescence
Skin auto fluorescence (AFR) was determined by an AGE reader (DiagnOptics Technologies BV, Groningen, the Netherlands) on the volar side of the lower part of the dominant arm in a dark environment. To correct for difference in light absorption, AFR was calculated as the ratio of excitation light (300–420 nm) to emitted light (420–600 nm) and expressed as arbitrary units (AU)[7]. Only AFR with a reflectance >0.1 was used for further investigation.

Statistical analysis
Variables were analysed for normality and expressed as median (interquartile range) if not normally distributed. The analysis of variance (ANOVA) test and post-hoc bonferoni were used to compare continuous variables between the three groups. For non-parametric data, the Man-Whitney test was used. Spearman correlation coefficients were calculated to determine correlating variables for telomere length and AFR, after which they were included in multivariate regression analysis.

Results and discussion

AFR in COPD patients was found to be increased compared to both smoking and never-smoking controls. In line with our previous study, no difference was observed between the smoking and non-smoking control groups[8]. AFR was however positively related to smoking history, i.e. pack years smoked (R=0.41, p<0.01). Significant correlations were furthermore observed with age (R=0.21, p<0.01), lung function (FEV$_1$: R=-0.32, FEV$_1$/FVC: R=-0.33, p<0.01), and the plasma CRP level (R=0.24, p<0.01), but not with other measures of systemic inflammation in the entire study population. In multivariate regression analyses however, none of these variables remained associated with AFR after correction for age, gender and pack years. The lack of association with lung function in patients confirms our previous study[8]. In these COPD patients we reported a shorter telomere length compared to both control groups[5], but here no significant correlation with AFR was determined (R=-0.08, p=0.28). The observations of telomere attrition[5] and increased AFR add to the body of literature that supports the concept of COPD as a syndrome of accelerated biological ageing. Both biological markers of ageing however represent different hallmarks of ageing in separate compartments. Indeed, whereas telomere length is measured in blood leucocytes and represents cellular age, AFR is largely thought to be a reflection of accumulation of AGEs on skin collagens[6]. It remains however unclear what mechanisms underlie these alterations in COPD. There is evidence that smoking increases AFR[11] and in the present study, AFR was indeed correlated with pack years smoked. The lack of effect in the smoking control group compared to never smokers might be attributed to the low pack year history and the higher percentage of ex-smokers compared to the patient group. Although the telomere length was independently associated with lung function in COPD, the main drivers of telomere shortening also remain to be determined.

In conclusion, the degree of lung function impairment in COPD is more closely related to telomere attrition compared to increased AFR. Gaining more insight into the mechanisms responsible for this accelerated attrition could provide new avenues for much needed pharmacological interventions.

Table 1 Subject characteristics and measures of biological ageing.

	COPD	Smoking controls	Non-smoking controls
Number, n	160	82	38
Age, y	62 (7)	62 (6)	59 (8)
Gender, %male	57	50	39
Lung function/smoking history			
FEV$_1$, %pred	49.4 (15.1)	119.1 (15.3)*	118.4 (16.3)*
FEV$_1$/FVC, %	41.2 (11.2)	78.1 (4.3)*	78.7 (4.9)*
Pack years, y	42.5 (30.2-57.5)	14.1 (6.2-25.6)*	0*,&
Smoking status,			
%current/ex/never	36/63/2	15/85/0*	0/0/100*
Inflammation/telomere length			
CRP, mg/mL	3.1 (0.9-7.5)	0.9 (0.6-2.2)*	0.6 (0.3-1.3)*
IL-6, pg/mL	4.8 (2.7-8.6)	2.4 (1.6-4.9)*	2.7 (1.7-5.0)*
IL-8, pg/mL	9.9 (6.1-14.9)	6.8 (4.6-13.1)	6.7 (3.7-9.6) $p<0.06$
Fibrinogen, g/dL	3.1 (2.6-3.6)	2.7 (2.4-3.1)*	2.5 (2.3-2.8)*
Telomere lentgh, kbp	4.4 (4.0-4.7)	4.6 (4.1-5.2)*	4.7 (4.2-5.1)*
AFR, AU	2.69 (2.16-3.05)	2.33 (2.03-2.64)*	2.11 (1.9-2.35)*

*denotes $p<0.01$ vs COPD, #AFR available from 92 patients, 57 smoking and 25 non-smoking control subjects

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