



Epigenetic blood biomarkers of ageing and mortality in COPD

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To the Editor:

COPD is an age-related condition that is linked to cellular senescence [1]. In COPD, contributors to cellular senescence include oxidative stress from environmental factors, such as cigarette smoking and persistent lung inflammation [2]. These factors can also augment replicative senescence, which is characterised by progressive telomere attrition, ultimately leading to cell cycle arrest and death. Patients with COPD have shorter telomeres [3] and faster rates of telomere attrition [4] compared to controls; however, the clinical impact of cellular or replicative senescence in COPD remains uncertain.

Peripheral blood DNA methylation patterns are altered by age, disease and environmental exposures, and may better reflect cellular ageing of an individual and provide prognostic information beyond that of chronological age. Epigenetic “clocks” are statistical models that relate methylation at nominated cytosine-guanine residues to chronological age; advanced methylation age relative to chronological age may be an indicator of accelerated cellular ageing. Several epigenetic “clocks” have been developed for this purpose and have been shown to predict mortality in a variety of different settings [5, 6].

We examined the relationship between mortality and epigenetic measurements of biological and telomeric age in 327 patients with COPD who enrolled in the COPD Rapid Transition Program at two major teaching hospitals in Vancouver, BC, Canada (ClinicalTrials.gov identifier: NCT02050022; University of British Columbia Clinical Research Ethics Board certificate numbers H11-00786 and H13-00790). This cohort has been previously described [7]; 102 stable outpatient participants and 225 participants hospitalised for acute exacerbation of COPD were followed for 1 year post-enrolment, during which vital status was determined. Blood samples were collected at the time of enrolment. Of these participants, 264 survived (155 current and 89 former smokers, mean±SD exposure 41±13 pack years) and 63 died (31 current and 26 former smokers, mean±SD exposure 42±11 pack years) during follow-up.

DNA was extracted from the blood samples as previously described [8] and methylation was quantified using the Illumina HumanMethylation450 microarray for 42 participants and the Illumina Infinium MethylationEPIC BeadChip microarray for the remaining 285 participants. Quality control, batch correction, and normalisation steps have been previously described [8]. Datasets were combined before the normalisation step and 447 506 methylation probes were retained.

The epigenetic clocks based on Horvath’s methods (<https://dnamage.genetics.ucla.edu/new>) entitled DNAmSkinBlood [9] and grim age (DNAmGrimAge) [10] were used to calculate DNA methylation age. DNAmSkinBlood was derived from blood and developed based on the association of DNA methylation with chronological age, while DNAmGrimAge is a composite of DNA methylation associations with age, sex and surrogate blood DNA methylation markers for seven inflammatory proteins related to mortality and pack-years of smoking. Thus, DNAmGrimAge captures epigenetic regulation of both ageing and cellular inflammation [10]. In addition, we calculated the DNA methylation-based estimator of telomere length (DNAmTL) according to Horvath’s methods to estimate replicative senescence [11]. To investigate the overall effect of DNAmSkinBlood, DNAmGrimAge and DNAmTL on 1-year mortality, we used Cox proportional hazards models (“survival” package in R) [12], adjusted for age, sex, smoking status, hospitalisation due to acute COPD exacerbation, corticosteroid use (systemic or inhaled), time of blood collection and cell proportions (CD8⁺ and CD4⁺ T cells, NK cells, B cells, monocytes, and granulocytes). Significant effects were defined using a threshold of $p < 0.05$.

Shareable abstract (@ERSpublications)

Epigenetic blood biomarkers of cellular and replicative senescence may improve the clinical assessment of COPD patients, particularly for those at a higher risk of death <https://bit.ly/3nEM7tp>

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We calculated age acceleration and grim age acceleration by regressing the DNAmSkinBlood or DNAmGrimAge on chronological age, respectively. Higher residual values indicated faster age acceleration for both clocks. Then for each clock, we ranked our cohort based on the residuals. We selected participants within the top and bottom 25%, and characterised them as acceleration and de-acceleration, respectively. The subsets were used for additional Cox analyses ($p < 0.05$). Furthermore, the regression of DNAmTL on chronological age was used as a measurement of DNAmTL shortening; negative residual values indicated shorter DNAmTL relative to that expected based on the chronological age. We performed a Cox analysis by ranking and selecting participants within the top and bottom 25% for DNAmTL shortening, and characterised them as short and long DNAmTL, respectively.

To determine the relationship between methylation age and mortality (non-survivors *versus* survivors), we used linear models. We conducted these analyses in two ways: first, by regressing DNAmSkinBlood, DNAmGrimAge and DNAmTL only on chronological age; and second, by adjusting our models for age, sex, smoking status, hospitalisation due to acute exacerbation of COPD (yes/no), corticosteroid use, time of blood collection (pre/during/post exacerbation) and cell proportions.

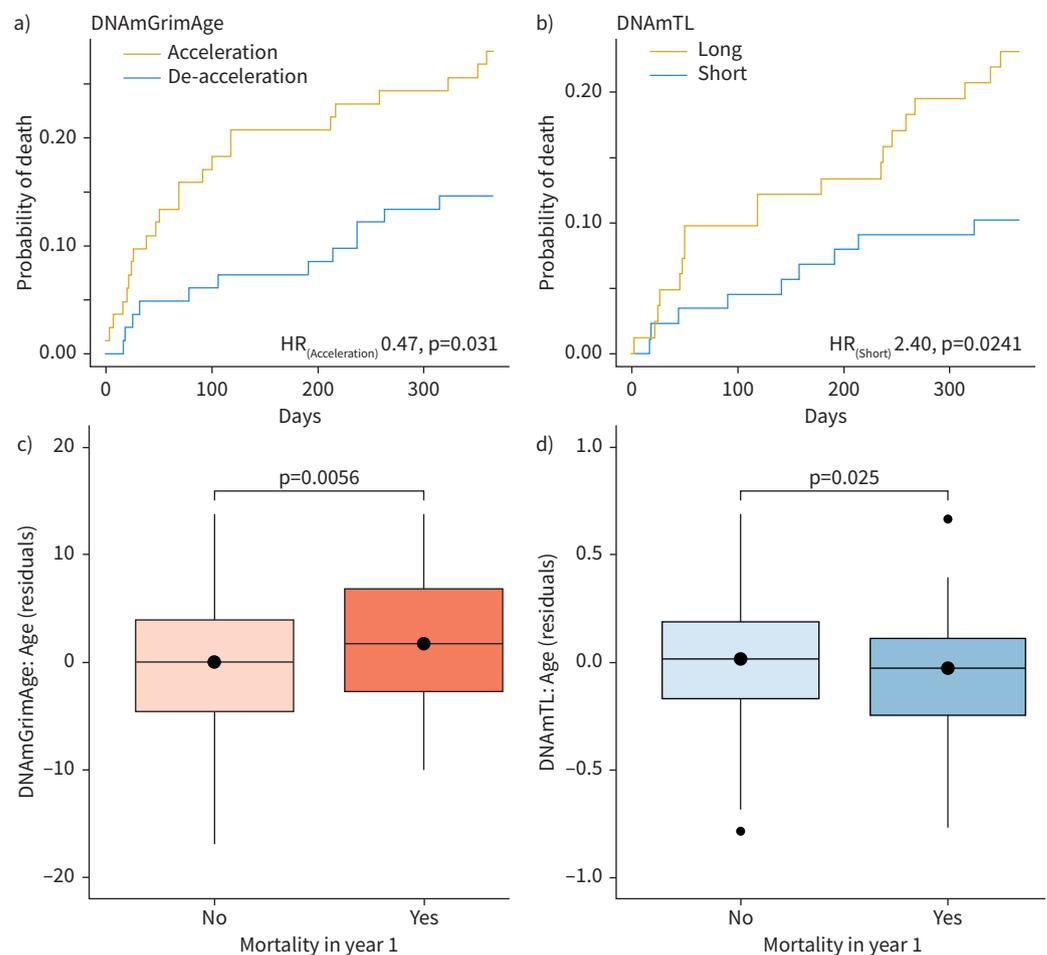


FIGURE 1 Epigenetic measurements of mortality and telomere length in COPD. The association between the epigenetic measurements and mortality is shown for **a)** DNAmGrimAge grim age acceleration groups (acceleration and de-acceleration groups) and **b)** DNAmTL telomere shortening groups (short and long groups) over 1 year. Hazard ratio (HR) and p-values in (a) and (b) correspond to the likelihood ratio test (Cox analysis). Reference groups for the hazard ratio in (a) and (b) correspond to the de-acceleration, and long telomere groups, respectively. Boxplots show **c)** grim age acceleration (residuals from the regression of DNAmGrimAge on chronological age) and **d)** telomere shortening estimate (residuals from the regression of DNAmTL on chronological age) plotted against mortality in year 1 (no refers to survivors, $n=264$; yes to deceased, $n=63$). p-values in (c) and (d) correspond to the linear model.

Non-survivors were chronologically older ($p=0.017$) and more likely to have experienced a severe COPD exacerbation ($p=0.004$) compared to survivors. Cigarette smoking, blood cell proportions, sex and use of corticosteroids were similar between the two groups ($p>0.05$). Adjustments for these factors in a multivariable Cox model showed that DNAmGrimAge ($p=3.93\times 10^{-03}$) and DNAmTL ($p=0.034$) were significantly related to mortality. Univariate Cox analyses showed that DNAmGrimAge acceleration ($p=0.031$) and the short DNAmTL group ($p=0.024$) were associated with a higher probability of mortality (figure 1a and b). DNAmSkinBlood was strongly correlated with chronological age ($R=0.787$, $p=3.34\times 10^{-79}$) and after adjustments for chronological age, this variable was no longer significantly associated with mortality ($p=0.380$). In contrast, DNAmGrimAge, which also was strongly related to chronological age ($R=0.789$, $p=9.34\times 10^{-71}$), was independently associated with 1-year mortality ($p=5.60\times 10^{-03}$) (figure 1c). DNAmTL demonstrated a significant correlation with chronological age ($R=-0.480$, $p=3.20\times 10^{-20}$); non-survivors had shorter DNAmTL compared to survivors ($p=0.025$) (figure 1d). After adjusting for potential confounders, the non-survivor group still showed significant DNAmGrimAge acceleration ($p=0.020$), while the effect of DNAmTL shortening weakened slightly ($p=0.061$). Interestingly, none of the epigenetic clocks were associated with hospitalisation for acute COPD exacerbation ($p>0.05$).

We showed that two blood epigenetic biomarkers, DNAmGrimAge and DNAmTL, were associated with 1-year mortality in patients with COPD, while other epigenetic clocks [13], such as DNAmSkinBlood, failed to predict mortality after adjustments for chronological age. One potential explanation for this observation is that DNAmGrimAge, dissimilar to other epigenetic clocks [5, 9], captures information on epigenetic regulation of important mortality- and age-related features, such as smoking pack-years and inflammatory plasma proteins (C-reactive protein, adrenomedullin, plasminogen activation inhibitor 1 and growth differentiation factor) in its calculator [10]. These features may have enhanced its performance in a population of patients with COPD, which is an inflammatory disorder. To our knowledge this is the first report of these clocks as a potential biomarker for COPD mortality. Our findings also highlight the importance of replicative senescence, which was estimated by DNAmTL, in overall COPD mortality. A previous study from our group demonstrated short peripheral blood telomere length as a risk factor for mortality in COPD [14]. We extend those findings by showing that epigenetic regulation of telomere length also contributes to poor outcomes in COPD. Telomere length has also been associated with lung function [15] and risk of exacerbation [14].

Our study was limited by several factors. First, these findings remain exploratory until further validation in additional cohorts is performed. Second, we were unable to assess the relationship between epigenetic ageing clocks and mortality beyond 1 year. Third, our cohort was enriched for patients hospitalised with acute exacerbation of COPD and, therefore, these results may not be generalisable for patients with stable or mild COPD.

In conclusion, our findings support the idea that COPD is an age-accelerated condition and that epigenetic blood biomarkers of cellular and replicative senescence may improve the clinical assessment of COPD patients, particularly for those at a higher risk of death.

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