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Research letter

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Epigenetic Blood Biomarkers of Aging and Mortality in COPD

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To the editor:

Chronic Obstructive Pulmonary Disease (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 characterized 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 aging 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 aging. 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 2 major teaching hospitals in Vancouver, 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 hospitalized for AECOPD were followed for 1-year post-enrollment during which vital status was determined. Blood samples were collected at the time of enrollment. Of these participants, 264 survived (155 current and 89 former smokers, mean exposure 41 ± 13 pack years) and 63 died (31 current and 26 former smokers, mean 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 normalization steps have been previously described [8]. Datasets were combined before the normalization 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, hospitalization due to AECOPD, 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 $P<0.05$.

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 characterized 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 characterized 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, hospitalization due to AECOPD (yes/no), corticosteroid use, time of blood collection (pre/during/post AECOPD), and cell proportions.

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 ($P=3.93x10^{-03}$) 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 1b)**. DNAmSkinBlood was strongly correlated with chronological age (R=0.787, $P=3.34x10^{-79}$) 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, $P=9.34x10^{-71}$), was independently associated with 1-year mortality ($P= 5.60x10^{-03}$) (**Figure 1c**). DNAmTL demonstrated a significant correlation with chronological age (R=-0.480, $P=3.20x10^{-20}$); 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 AECOPD hospitalization (*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 such as 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 (TL) as a risk factor for mortality in COPD [14]. We extend those findings by showing that epigenetic regulation of TL also contributes to poor outcomes in COPD. TL 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 aging clocks and mortality beyond 1 year. Third, our cohort was enriched for patients hospitalized with AECOPD and therefore these results may not be generalizable 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.

References

1. Tsuji T, Aoshiba K, Nagai A. Alveolar Cell Senescence in Patients with Pulmonary Emphysema. *Am J Respir Crit Care Med* American Thoracic Society - AJRCCM; 2006; 174: 886–893.

2. Singh D, Agusti A, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Criner GJ, Frith P, Halpin DMG, Han M, López Varela MV, Martinez F, Montes de Oca M, Papi A, Pavord ID, Roche N, Sin DD, Stockley R, Vestbo J, Wedzicha JA, Vogelmeier C. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease: the GOLD science committee report 2019. *Eur Respir J* 2019; 53: 1900164.

3. Magd G-EM, Entesar AS, Manal HR, Eman SM, Mona AH. Telomere length in chronic obstructive pulmonary disease. *Egypt J Bronchol* 2015; 9: 20–26.

4. Elizabeth C-L, Sara C-R, Adriana E-J, de-Torres JP, María-José P, Armando A-J, Bartolomé C, Ciro C. Telomere shortening and accelerated aging in COPD: findings from the BODE cohort. *Respir Res* [Internet] 2017 [cited 2021 Jun 10]; 18Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5390353/.

5. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S, Klotzle B, Bibikova M, Fan J-B, Gao Y, Deconde R, Chen M, Rajapakse I, Friend S, Ideker T, Zhang K. Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013; 49: 359–367.

6. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013; 14: R115.

7. Leung JM, Chen V, Hollander Z, Dai D, Tebbutt SJ, Aaron SD, Vandemheen KL, Rennard SI, FitzGerald JM, Woodruff PG, Lazarus SC, Connett JE, Coxson HO, Miller B, Borchers C, McManus BM, Ng RT, Sin DD. COPD Exacerbation Biomarkers Validated Using Multiple Reaction Monitoring Mass Spectrometry. *PLoS One* 2016; 11: e0161129.

8. Hernandez Cordero AI, Yang CX, Obeidat M, Yang J, MacIsaac J, McEwen L, Lin D, Kobor M, Novak R, Hudson F, Klinker H, Dharan N, Man SP, Sin DD, Kunisaki K, Leung J, INSIGHT START Pulmonary and Genomic Substudy Groups. DNA methylation is associated with airflow obstruction in patients living with HIV. *Thorax* 2020; .

9. Horvath S, Oshima J, Martin GM, Lu AT, Quach A, Cohen H, Felton S, Matsuyama M, Lowe D, Kabacik S, Wilson JG, Reiner AP, Maierhofer A, Flunkert J, Aviv A, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Ferrucci L, Matsuyama S, Raj K. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. *Aging (Albany NY)* 2018; 10: 1758–1775.

10. Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, Hou L, Baccarelli AA, Li Y, Stewart JD, Whitsel EA, Assimes TL, Ferrucci L, Horvath S. DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging (Albany NY)* 2019; 11: 303–327.

11. Lu AT, Seeboth A, Tsai P-C, Sun D, Quach A, Reiner AP, Kooperberg C, Ferrucci L, Hou L, Baccarelli AA, Li Y, Harris SE, Corley J, Taylor A, Deary IJ, Stewart JD, Whitsel EA, Assimes TL, Chen W, Li S, Mangino M, Bell JT, Wilson JG, Aviv A, Marioni RE, Raj K, Horvath S. DNA methylation-based estimator of telomere length. *Aging (Albany NY)* 2019; 11: 5895–5923.

12. Therneau TM, Grambsch PM. The Cox Model. In: Therneau TM, Grambsch PM, editors. *Modeling Survival Data: Extending the Cox Model* [Internet] New York, NY: Springer; 2000 [cited 2021 Jul 9]. p. 39–77Available from: https://doi.org/10.1007/978-1-4757-3294-8_3.

13. Hillary RF, Stevenson AJ, McCartney DL, Campbell A, Walker RM, Howard DM, Ritchie CW, Horvath S, Hayward C, McIntosh AM, Porteous DJ, Deary IJ, Evans KL, Marioni RE. Epigenetic measures of ageing predict the prevalence and incidence of leading causes of death and disease burden. *Clinical Epigenetics* 2020; 12: 115.

14. Jin M, Lee EC, Ra SW, Fishbane N, Tam S, Criner GJ, Woodruff PG, Lazarus SC, Albert R, Connett JE, Han MK, Martinez FJ, Aaron SD, Reed RM, Man SFP, Leung JM, Sin DD. Relationship of Absolute Telomere Length With Quality of Life, Exacerbations, and Mortality in COPD. *Chest* 2018; 154: 266–273.

15. Rode L, Bojesen SE, Weischer M, Vestbo J, Nordestgaard BG. Short telomere length, lung function and chronic obstructive pulmonary disease in 46 396 individuals. *Thorax* BMJ Publishing Group Ltd; 2013; 68: 429–435.

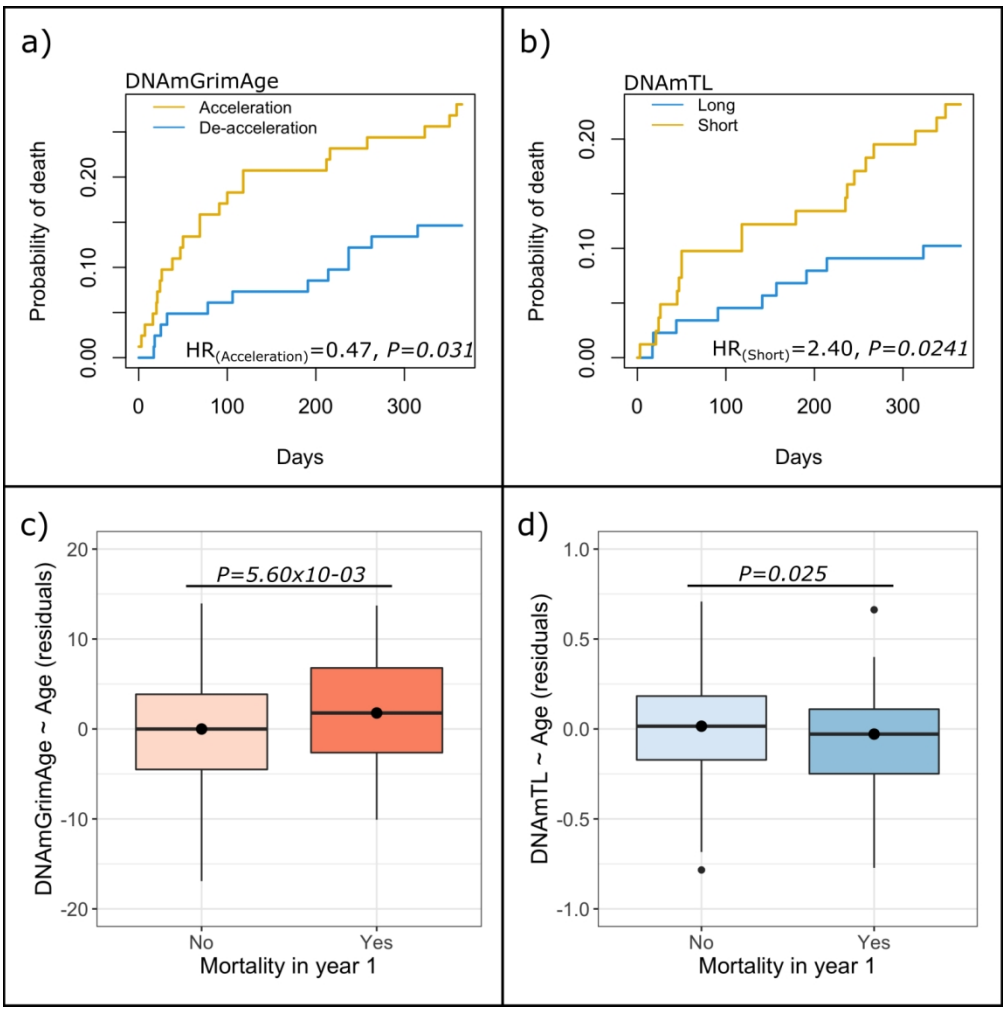

Figure 1. Epigenetic measurements of mortality and telomere length in COPD. The association between the epigenetic measurements and mortality is shown for (a [DNAmGrimAge]) grim age acceleration groups (acceleration [yellow line] and de-acceleration groups [blue line]) and (b [DNAmTL]) telomere shortening groups (short [yellow line] and long [blue line]) over one year. Hazard ratio (HR) and P-values in a and b plots correspond to the Likelihood ratio test (Cox analysis). Reference groups for the HR 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 = survivors [n=264], Yes = deceased [n=63]). P-values at the top of the plots correspond to the linear model.