BRIEF REPORT

OPEN

Association of Epigenetic Age and Outcome in Critically Ill Patients

Archana Sharma-Oates, PhD[1]
Jack Sullivan, PhD[2,3]
Daniel Pestana, MSc[4]
Claudia C. dos Santos, MD[5]
Alexandra Binnie, MD[6]
Janet M. Lord, PhD[2,3]

OBJECTIVES: DNA methylation can be used to determine an individual's biological age, as opposed to chronological age, an indicator of underlying health status. This study aimed to assess epigenetic age in critically ill patients with and without sepsis to determine if higher epigenetic age is associated with admission diagnosis or mortality.

DESIGN: Secondary analysis of whole blood DNA methylation data generated from a nested case–control study of critically ill septic and nonseptic patients.

SETTING: Four tertiary care hospitals in Canada.

INTERVENTIONS: None.

PATIENTS: Critically ill patients with and without sepsis.

MEASUREMENTS AND MAIN RESULTS: Epigenetic age was derived from DNA methylation data using the Hannum and PhenoAge algorithms and deviation from the patient's chronological age in years was determined. Of the 66 patients with sepsis, 34 were male (51.5%), the mean age was 65.03 years and 25 patients (37.8%) died before discharge. Of the 68 nonseptic patients, 47 were male (69.1%), the mean age was 64.92 years and 25 (36.7%) died before discharge. Epigenetic age calculated using the PhenoAge algorithm showed a significant age acceleration of 4.97 years in septic patients ($p = 0.045$), but no significant acceleration in nonseptic patients. Epigenetic age calculated using the Hannum algorithm showed no significant acceleration in the septic or nonseptic patients. Similarly, in the combined septic and nonseptic cohorts, nonsurvivors showed an epigenetic age acceleration of 7.62 years ($p = 0.004$) using the PhenoAge algorithm while survivors showed no significant age acceleration. Survivor status was not associated with age acceleration using the Hannum algorithm.

CONCLUSIONS: In critically ill patients, epigenetic age acceleration, as calculated by the PhenoAge algorithm, was associated with sepsis diagnosis and mortality.

KEYWORDS: biological age; critical illness; DNA methylation; epigenetic clocks; sepsis

Sepsis is a complex, multisystem disease resulting from an exuberant and prolonged inflammatory response to infection or tissue injury that leads to widespread inflammation-mediated organ damage and immune paresis (1). The pathophysiology of sepsis includes a rapid and large-scale transcriptomic activation of genes involved in inflammation, the so-called "genomic storm" (2). Differentiating the physiologic responses that are common to critical illness versus those that are specific to sepsis remains a key challenge in critical care medicine. In addressing this question, we have previously characterized epigenetic marks, specifically DNA methylation, as potential differential regulators of the transcriptomic response in critical illness and sepsis (3). In that study, we compared DNA methylation profiles in whole blood genomic DNA between septic and nonseptic critically ill patients with similar severity

DOI: 10.1097/CCE.0000000000001044

KEY POINTS

Question: Is accelerated biological aging associated with critical illness subtype (i.e., septic vs. nonseptic critical illness) and mortality?

Findings: Secondary analysis of DNA methylation data, from a study of septic and nonseptic critically ill patients admitted to the ICU, revealed that patients with sepsis and nonsurvivors were significantly biologically older than their chronological ages, by 4.97 and 7.63 years, respectively. Nonseptic patients and those who survived their critical illness episodes showed no increase in biological age.

Meaning: Increased biological age, an indicator of health status, is associated with poor outcomes in critical illness.

of illness and mortality. We found 668 differentially methylated regions corresponding to 443 genes including known sepsis-associated genes (3).

DNA methylation at specific sites has been shown to correlate with chronological age and specific subsets of methylation sites, termed epigenetic clocks, can predict chronological age (4). The degree of deviation between epigenetic age and chronological age has been linked to a range of chronic diseases and mortality (4, 5), allowing the clocks to be used as indicators of biological age. Chronological age is known to influence outcomes in critical illness, including the development of sepsis (6), but the degree to which biological age influences critical illness outcomes has not been considered and could conceivably have a greater impact than chronological age.

Here we carried out a secondary analysis of our original data (3) using two epigenetic clocks: one developed by Hannum that is specific for DNA derived from blood cells and was designed to predict chronological age (4) and a second termed the PhenoAge clock which was developed to relate DNA methylation profiles to risk of chronic disease and mortality (5).

MATERIALS AND METHODS

This study is a secondary analysis of existing DNA methylation data from a nested case–control study, the Epigenetic Profiling in Severe Sepsis study (3), which analyzed samples from patients in the multicenter prospective observational trial DNA as a Prognostic Marker in ICU patients (DYNAMICS; NCT01355042) (7). Adult septic and nonseptic ICU patients were recruited from four Canadian ICUs between November 2010 and May 2014. Briefly, written informed consent was obtained from the patient, or their substitute decision-maker if the patient was unable to provide consent. Patients who were younger than 18 years, pregnant or breastfeeding, or receiving palliative care were excluded. Septic ICU patients were those admitted to ICU with confirmed or suspected infection along with greater than or equal to one organ dysfunction and greater than or equal to three signs of systemic inflammatory response syndrome. Nonseptic ICU patients were those admitted to ICU with: 1) multiple traumas, 2) acute neurocritical illness (i.e., intracranial, subarachnoid, or subdural hemorrhage), or 3) nonseptic shock (i.e., cardiogenic shock, heat shock, pulmonary embolism, burns, hypovolemia). All patients were expected to remain in the ICU for at least 72 hours at the time of admission. DNA samples were collected on day 1 of ICU admission and DNA methylation analysis was conducted as previously described (3).

The DNA methylation analysis was approved by the McMaster University Research Ethics Board in November 2010 (number:12-216). All participants gave informed consent and procedures were followed in accordance with the ethical standards of the research ethics board and with the Helsinki Declaration of 1975.

DNA Methylation Data Analysis

The preprocessing steps were as described in our original publication (3). The ENMix R Bioconductor package was used to estimate epigenetic age using the Hannum (4) and PhenoAge (5) epigenetic clocks. Epigenetic age was regressed against chronological age and epigenetic age acceleration was determined as the residual of epigenetic age minus chronological age.

Statistical Analysis

All analyses were performed in the R statistical programming environment, R, version 4.1.0. A multiple regression model was initially generated, using all

cases, to assess the impact of epigenetic age in patients admitted to ICU. Multiple regression models were then generated to explore the relationship between epigenetic age and independent variables including phenotype (sepsis or nonsepsis), age, and outcome (survivor vs. nonsurvivor). A p value of less than or equal to 0.05 was considered significant.

RESULTS

Patient Demographics

DNA methylation data were analyzed for 66 patients with critical illness secondary to sepsis and 68 with nonseptic critical illness, who had previously been matched for age, severity of illness, and 28-day mortality. In our primary analysis, we confirmed that there were no significant differences in clinical characteristics between the two groups, including the number of baseline comorbidities (see supplementary Table 1 in reference [3]). Of the septic patients, 34 were male (51.5%), the mean age was 65.03 (SD = 15.95) years and 25 patients (37.8%) died before discharge. Of the 68 nonseptic patients, 47 were male (69.1%), the mean age was 64.92 (SD = 13.64) years and 25 (36.7%) died before discharge (**Table 1**).

DNA Methylation Age Analysis

Using the Hannum clock, no significant age acceleration was identified in septic patients ($p = 0.07$) or nonseptic patients (Table 1). Using the PhenoAge clock, however, septic patients showed an age acceleration of 4.97 years ($p = 0.045$) whereas nonseptic patients showed no age acceleration.

We then assessed the association between epigenetic age and mortality (Table 1). Using the Hannum clock there was no significant age acceleration amongst survivors or nonsurvivors ($p = 0.102$). Using the PhenoAge clock, nonsurvivors showed an average age acceleration of 7.63 years ($p = 0.004$) while survivors showed no age acceleration. These findings were corroborated by independent multiple regression analyses of the septic and nonseptic subgroups, which confirmed a significant age acceleration amongst both septic ($p < 0.017$) and nonseptic ($p < 0.027$) nonsurvivors using the PhenoAge but not the Hannum clock (Table 1).

TABLE 1.
Epigenetic Age Acceleration Differences by Mortality in ICU Patients

Subset		PhenoAge				Hannum			
All Cases	Number of Cases	Coefficient Estimate	SE	t	Pr (>|t|)	Coefficient Estimate	SE	t	Pr (>|t|)
Chronological age (all cases)	134	0.962	0.083	11.548	$< 2 \times 10^{-16}$	0.795	0.051	15.726	$< 2 \times 10^{-16}$
Phenotype (septic)	66	4.970	2.452	2.027	0.045	2.724	1.488	1.830	0.070 NS
Outcome (expired)	50	7.628	2.587	2.948	0.004	2.585	1.571	1.646	0.102 NS
Septic cases only									
Chronological age (all septic cases)	66	0.99	0.11	8.90	1.1×10^{-12}	0.745	0.084	8.867	$< 2 \times 10^{-16}$
Outcome (expired)	25	8.88	3.61	2.46	0.017	4.672	2.743	1.703	0.094 NS
Nonseptic cases only									
Chronological age (all nonseptic cases)	68	0.87	0.13	6.71	6.08×10^{-9}	0.850	0.048	17.567	$< 2 \times 10^{-16}$
Outcome (expired)	25	8.26	3.64	2.27	0.027	0.401	1.359	0.295	0.769 NS

NS = not significant.

The table displays the results of multiple regression analysis investigating the association between epigenetic age and independent variables, including phenotype (sepsis) and survival. The initial model includes all cases, while separate models are presented for septic and nonseptic patients showing the influence of survival status on epigenetic age. Coefficient estimate indicates age acceleration in the number of years.

DISCUSSION

Using the PhenoAge epigenetic clock, which was designed to measure biological aging, accelerated epigenetic age was observed in septic patients admitted to ICU, but not in nonseptic critically ill patients with matching illness severity. Similarly, accelerated epigenetic age was observed using the PhenoAge epigenetic clock in nonsurvivors of both septic and nonseptic critical illnesses. No significant age acceleration was seen with the Hannum clock, though the data did approach significance in the comparison of septic versus nonseptic patients ($p < 0.07$). These results suggest that increased biological age is associated with poorer outcomes amongst critically ill patients, including sepsis and death. Epigenetic age acceleration in patients with COVID-19, measured using various epigenetic clocks, indicates higher acceleration in severe cases than in moderate ones (8), reinforcing the influence of biological age on critical illness outcomes.

In this study, the effect of epigenetic age was significant only for the PhenoAge clock and not the Hannum clock. The Hannum clock was built using DNA methylation data with the goal of predicting chronological age (4). Conversely, the PhenoAge clock was built using a combination of clinical biomarkers and DNA methylation data and has been shown to outperform first-generation clocks, including Hannum, for the prediction of chronic disease as well as all-cause mortality (5). Age acceleration detected by the PhenoAge clock is, therefore, more likely to be driven by underlying clinical conditions that subsequently influence outcomes in critical illness. Alternatively, since we do not know how quickly de novo DNA methylation occurs it is possible that sepsis may trigger de novo methylation in genes associated with chronic disease that are overrepresented in the PhenoAge clock.

Biological aging is accompanied by changes that could underlie the increased risk of infection and sepsis. Reduced immunity as a result of immunosenescence will increase susceptibility to infection and reduce the ability to regulate the inflammatory response (9). The PhenoAge clock was shown to detect immunosenescence parameters such as the frequency of naive and exhausted CD8 T cells (5). Our previous analysis of differentially methylated regions in septic versus nonseptic critically ill patients revealed several differentially methylated genes in sepsis that are involved in inflammatory responses, including complement component 3, myeloperoxidase, and major histocompatibility complex isotypes (3). Another study of 24 patients with sepsis reported 161 sites that were differentially methylated in association with age, many of which were located in genes associated with immune responses (10).

A limitation of our study is that lifestyle characteristics such as smoking status were not captured in the original study data and could have influenced both epigenetic age and the likelihood of sepsis and poor outcomes. Furthermore, while our two cohorts did not differ in the number of underlying comorbidities (3), they may have differed qualitatively.

In conclusion, epigenetic age, as measured by the PhenoAge algorithm at ICU admission, may identify patients at higher risk of poor outcomes in critical illness. It will be important to ascertain if these data outperform current clinical scores, persist over time, and are associated with long-term outcomes. Although DNA methylation analysis is time-consuming and expensive, there are other biological aging clocks developed on routine clinical biochemistry data that could be applied in the clinical setting at no extra cost (5).

ACKNOWLEDGMENTS

We thank Dr Peter Keane for his independent review of the statistical aspects of our study.

1 *School of Biosciences, University of Birmingham, Birmingham, United Kingdom.*

2 *Institute of Inflammation and Ageing, University of Birmingham, Birmingham, United Kingdom.*

3 *NIHR Surgical Reconstruction and Microbiology Research Centre, University Hospital Birmingham and University of Birmingham, Birmingham, United Kingdom.*

4 *Algarve Biomedical Center, Research Institute (ABC-RI), University of Algarve Campus Gambelas, Faro, Portugal.*

5 *Critical Illness and Injury Research Centre, Unity Health, Toronto, Canada.*

6 *William Osler Health Centre, Toronto, Canada.*

Dr. Sharma-Oates, Dr. Sullivan, and Mr. Pestana analyzed the data, Dr. Lord conceptualized the study, Drs. Sharma-Oates and Lord wrote the initial draft of the article, Drs. Binnie and dos Santos generated DNA methylation data, with Mr. Pestana edited and reviewed the article and all authors approved the final version.

Dr. Sullivan is supported by the National Institute for Health and Care Research Surgical Reconstruction and Microbiology Research

Centre. The views expressed here are those of the authors and not necessarily those of the National Institute for Health and Care Research, National Health System, or the Department for Health and Social Care. Dr. dos Santos is funded by the Canadian Institutes of Health Research (EpiGen Marks for Sepsis, MOP106545).

For information regarding this article, E-mail: j.m.lord@bham.ac.uk

REFERENCES

1. Lord JM, Midwinter MJ, Belli A, et al: The systemic immune response to trauma: An overview of pathophysiology and treatment. *Lancet* 2014; 384:1455–1465
2. Xiao WZ, Mindrinos MN, Seok JH, et al; the Inflammation and Host Response to Injury Large-Scale Collaborative Research Program: A genomic storm in critically injured humans. *J Exp Med* 2011; 208:2581–Y2590
3. Binnie A, Walsh CJ, Hu P, et al; Epigenetic Profiling in Severe Sepsis (EPSIS) Study of the Canadian Critical Care Translational Biology Group (CCCTBG): Epigenetic profiling in severe sepsis: A pilot study of DNA methylation profiles in critical illness. *Crit Care Med* 2020; 48:142–150
4. Hannum G, Guinney J, Zhao L, et al: Genome-wide methylation profiles reveal quantitative views of human aging rates. *Mol Cell* 2013; 49:359–367
5. Levine ME, Lu AT, Quach A, et al: An epigenetic biomarker of aging for lifespan and healthspan. *Aging (Albany NY)* 2018; 10:573–591
6. Martin GS, Mannino DM, Moss M: The effect of age on the development and outcome of adult sepsis. *Crit Care Med* 2006; 34:15–21
7. Jackson Chornenki NL, Coke R, Kwong AC, et al: Comparison of the source and prognostic utility of cfDNA in trauma and sepsis. *Intensive Care Med Exp* 2019; 7:29
8. Cao X, Li WJ, Wang T, et al: Accelerated biological aging in COVID-19 patients. *Nat Commun* 2022; 13:2135
9. Aiello A, Farzaneh F, Candore G, et al: Immunosenescence and its hallmarks: How to oppose aging strategically? A review of potential options for therapeutic intervention. *Front Immunol* 2019; 10:2247
10. Lang X, Shen L, Zhu T, et al: Role of age-related changes in DNA methylation in the disproportionate susceptibility and worse outcomes of sepsis in older adults. *Front Med* 2022; 9:822847