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Investigating the association of measures of epigenetic age with COVID-19 severity: evidence from secondary analyses of open access data

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Summary

BACKGROUND: Epigenetic modifications may contribute to inter-individual variation that is unexplainable by presently known risk factors for COVID-19 severity (e.g., age, excess weight, or other health conditions). Estimates of youth capital (YC) reflect the difference between an individual's epigenetic – or biological – age and chronological age, and may quantify abnormal aging due to lifestyle or other environmental exposures, providing insights that could inform risk-stratification for severe COVID-19 outcomes. This study aims to thereby a) assess the association between YC and epigenetic signatures of lifestyle exposures with COVID-19 severity, and b) to assess whether the inclusion of these signatures in addition to a signature of COVID-19 severity (EPICOVID) improved the prediction of COVID-19 severity.

METHODS: This study uses data from two publicly-available studies accessed via the Gene Expression Omnibus (GEO) platform (accession references: GSE168739 and GSE174818). The GSE168739 is a retrospective, cross-sectional study of 407 individuals with confirmed COVID-19 across 14 hospitals in Spain, while the GSE174818 sample is a single-center observational study of individuals admitted to the hospital for COVID-19 symptoms (n = 102). YC was estimated using the (a) Gonseth-Nusslé, (b) Horvath, (c) Hannum, and (d) PhenoAge estimates of epigenetic age. Study-specific definitions of COVID-19 severity were used, including hospitalization status (yes/no) (GSE168739) or vital status at the end of follow-up (alive/dead) (GSE174818). Logistic regression models were used to assess the association between YC, lifestyle exposures, and COVID-19 severity.

RESULTS: Higher YC as estimated using the Gonseth-Nusslé, Hannum and PhenoAge measures was associated with reduced odds of severe symptoms (OR = 0.95, 95% CI = 0.91–1.00; OR = 0.81, 95% CI = 0.75 - 0.86; and OR = 0.85, 95% CI = 0.81–0.88, respectively) (adjusting for chronological age and sex). In contrast, a one-unit increase in the epigenetic signature for alcohol consumption was associated with 13% increased odds of severe symptoms (OR = 1.13, 95% CI = 1.05–1.23). Compared to the model including only age, sex and the EPICOVID signature, the additional inclusion of PhenoAge and the epigenetic signature for alcohol consumption improved the prediction of COVID-19 severity (AUC = 0.94, 95% CI = 0.91–0.96 versus AUC = 0.95, 95% CI = 0.93–0.97; p = 0.01). In the GSE174818 sample, only PhenoAge was associated with COVID-related mortality (OR = 0.93, 95% CI = 0.87–1.00) (adjusting for age, sex, BMI and Charlson comorbidity index).

CONCLUSIONS: Epigenetic age is a potentially useful tool in primary prevention, particularly as an incentive towards lifestyle changes that target reducing the risk of severe COVID-19 symptoms. However, additional research is needed to establish potential causal pathways and the directionality of this effect.

Introduction

Among the many identified risk factors for Coronavirus disease (COVID-19) severity, such as sex, obesity, diabetes or hypertension, COVID-19 severity is strongly associated with age, with older individuals having a notably higher risk of mortality [1]. However, much inter-individual variation exists, even within age groups, that is not explainable by presently known risk factors [2, 3]. One potential explanation for the inter-individual variation could be differences in individuals' epigenetic profiles.

Epigenetic modifications – which can be induced by environmental and lifestyle behaviors and subsequently alter gene expression – have been implicated in the pathophysiology of COVID-19 severity [4], with epigenetic factors potentially contributing to COVID-19 susceptibility by interfering with viral replication and infection [5]. For example, recent evidence suggests that epigenetic regulation of interferons and inflammatory signaling modulates expression of the ACE2 gene (a gene responsible for the production of the angiotensin converting enzyme 2); it is via ACE2 enzyme receptors that SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2, the virus responsible for COVID-19) enters the human body [4, 6]. Likewise, epigenetic modifications have also been linked with the severity of response to infection, particularly in relation to inflammation and the so-called "cytokine storm" [7]. Epigenetic modifications, in particular estimates of epigenet-

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ic age, could thereby help explain the observed variation in COVID-19 symptom severity. To this effect, estimates of epigenetic age have been linked to adverse health outcomes such as cardiovascular disease, dementia and mortality [8]. Moreover, the difference between epigenetic and chronological age has been shown to be a consequence of lifestyle and environmental exposures [9, 10].

According to a recent survey in *Nature*, like other coronavirus variants, 60% of scientists believe that the SARS-CoV-2 virus will very likely become endemic [11]. Identifying long-term targeted therapies are therefore imperative for secondary prevention to reduce COVID-19 severity among individuals infected with the SARS-CoV-2 virus. Of note is that most epigenetic modifications are reversible. Identification of differential patterns of methylation associated with COVID severity could aid in identifying secondary, epigenetic targets of intervention. Moreover, estimates of youth capital (YC), which reflect the difference between an individual's epigenetic and chronological age, may quantify abnormal aging due to lifestyle or other environmental exposures and provide insights that could inform risk-stratification for severe COVID-19 outcomes. The specific aims of the present study are to assess the association between different measures of epigenetic age and COVID-19 severity, as well as the added predictive value when including estimates of youth capital alongside the EPICOVID signature. We hypothesize that older epigenetic age and lifestyle exposures, including tobacco exposure and alcohol consumption, are associated with more severe COVID-19 outcomes.

Methods and materials

Data sources

This study is based on two study samples, referred to here by their Gene Expression Omnibus (GEO) accession references: GSE168739 [12] and GSE174818 [13]. Described in detail by Castro de Moura et al., briefly the GSE168739 is a retrospective, cross-sectional study that included 407 individuals with confirmed COVID-19 from 14 hospitals across Spain, had a BMI <30, not presenting with risk factors for comorbidities (diabetes, hypertension, auto-immune disorders, and chronic cardiovascular or lung diseases), non-smokers (including previous smokers) and less than 61 years of age [14]. COVID-19 severity was categorized into asymptomatic or paucisymptomatic (not hospitalized) and severe (requiring hospitalization including oxygen therapy or mechanical ventilation). The original study further stratified between those requiring oxygen therapies and those requiring mechanical ventilation. Whole blood samples – from which peripheral blood-derived DNA methylation was obtained – were retrospectively collected between March 7th 2020 and September 14th 2020.

In contrast, the second sample (GSE174818) is a single-center observational study of individuals admitted to the hospital for COVID-19 symptoms (n = 102), 18 years or older, who provided consent and were not at risk of imminent death. In this sample, Balnis et al. collected information on COVID-19 severity (intensive care unit [ICU] admittance or non-ICU), mortality status, as well as sociodemographic characteristics, severity indexes (e.g., Charlson comorbidity index), and other biomarkers of interest (e.g., C-Reactive protein [CRP]) [13]. Whole blood samples were collected at the time of study enrollment, succeeding admittance to Albany Medical Center, from April 6th 2020 through to May 1st 2020. Information on non-COVID patients (n = 26) admitted for unrelated respiratory health concerns, as well as healthy control patients (n = 39) identified prior to the COVID-19 pandemic, was also collected in the original study. However, these patients are excluded from the present manuscript. Given that this study is a secondary analysis of publicly available data, the sample size was pre-determined.

Data management and normalization

For both the GSE168739 and GSE174818 samples, DNA methylation was obtained using the Illumina Infinium MethylationEPIC Beadchip array (850K). DNA methylation is an epigenetic modification incorporated via the covalent attachment of a methyl group to the 5' position of the cytosine ring; the location of this chemical modification is termed a 'CpG site'. Beta values quantify the level of methylation at each individual CpG site, with zero representing no methylation and one representing full methylation. Files containing raw data were downloaded from GEO accession and subsequently normalized using an internally adapted version of the quantile normalization that included an eight-sample reference to ensure comparability [15,16]. Beta values were calculated using the reference sample-normalized data. Additional variables on patient-specific outcomes were obtained from the original study authors directly via e-mail (addresses obtained from the description page for each study on the GEO website) (e.g., Charlson comorbidity index, BMI, Fibrinogin, Albumin); details on variable collection are provided in the original manuscript [13,14]. An open science protocol was not prepared, nor registered for the present study.

Epigenetic signatures

Epigenetic age was assessed using three individual measures; epigenetic age as proposed by Hannum et al. [17] (Hannum), Horvath et al.[18] (Horvath), Levine et al.[19] (DNAm PhenoAge; referred to as PhenoAge in the present study) and Gonseth-Nusslé et al.(Gonseth-Nusslé). These individual epigenetic signatures represent first- (Horvath and HannumHa) and second-generation (Gonseth-Nusslé, and PhenoAge) estimates of epigenetic age. Whereas first-generation signatures were maximized to predict chronological age, second-generation signatures maximize "biological age" and subsequent disease prediction. To this effect, the Gonseth-Nusslé epigenetic signature accounts more for lifestyle effects, while the PhenoAge signature maximizes disease prediction. Lifestyle exposures, and tobacco and alcohol consumption were also assessed using epigenetic signatures.

Briefly, the lifestyle signatures for tobacco and alcohol consumption were determined by generating hundreds of thousands of models using data from a general population-based cohort (n = 694) [20], which included random combinations of CpGs identified in the literature as explanatory variables and respectively the number of cigarettes smoked per day or the number of standard glasses drunk per week as dependent variables. To minimize the risk of false find-

ings, each random model went through a stepwise procedure based on Bayesian Information Criterion (BIC) statistics, and the CpG combination that maximized the goodness-of-fit (r-squared) was finally selected as the epigenetic signature. In a second step, an epigenetic age formula was estimated using a conditional regression framework to account for the contribution of lifestyle exposures on epigenetic age (Patent reference: EP 22 162 216.0), thereby identifying CpG sites associated with chronological age without controlling for lifestyle exposures. Signatures were calibrated using a subset of the original population (n = 442), and then validated using data from the remaining subset (n = 248). The epigenetic signature for alcohol consumption was then transformed to correspond to units of alcohol consumed, whereby a score of 12 equates to a consumption of 12 standard units of alcohol per week. CpGs identified for inclusion in the Gonseth-Nusslé epigenetic age signature were conditional on maximizing the association between the residual of the age-CpG model with lifestyle exposures. The epigenetic signatures Horvath, Hannum, and PhenoAge have been described previously [17–19]. Youth capital was calculated as the difference between epigenetic age and chronological age, such that a higher youth capital denotes a lower epigenetic age compared to the chronological age. The EPICOVID signature, described previously by Castro de Moura et al., is an epigenetic signature composed of 44 CpGs identified as being associated with the clinical severity of COVID-19 [14].

Ethics approval and consent to participate

Ethics approvals from the institutional ethics review boards, and written informed consent from all participants were obtained in the previous studies included in this work [13, 14].

Statistical analysis

Age, BMI, the Charlson comorbidity index (an ordered variable ranging from 0 to 11) and all epigenetic signatures, including youth capital, were considered as continuous variables. Only sex was considered as a dichotomous (male/female) variable. In case of missing data, cases were planned to be excluded from statistical analyses, although data were non-missing for variables of interest (age, sex, and epigenetic signatures). The association between youth capital, as the independent variable of interest, and COVID-19 severity, as the primary dependent variable of interest, was assessed using logistic regression models; model assumptions were tested [21]. Separate models were used for each measure of youth capital (e.g., Hannum, Horvath, PhenoAge, and Gonseth-Nusslé), as well as for epigenetic signatures of lifestyle exposures, including alcohol consumption (GSE168739 and GSE174818 samples), and tobacco consumption (only the GSE174818 sample). To assess the predictive capacity of models to predict severity status when including youth capital and other epigenetic signatures, confidence intervals and point estimates for the receiving operator curve (ROC) summary area under the curve (AUC) measure were estimated using 10-fold cross-validation with the *cvAUC* package. Sensitivity – i.e., the ability of the model to correctly identify true positives – and specificity – the ability of the model to correctly identify true negatives – values (according to a threshold of 0.5) are also reported. Due to the likely overestimation of youth capital with increasing age, and the known influence of age on COVID-19 severity, all models were adjusted for chronological age and sex [22].

All analyses were carried out using R Studio (R version 4.0.2) [23].

Results

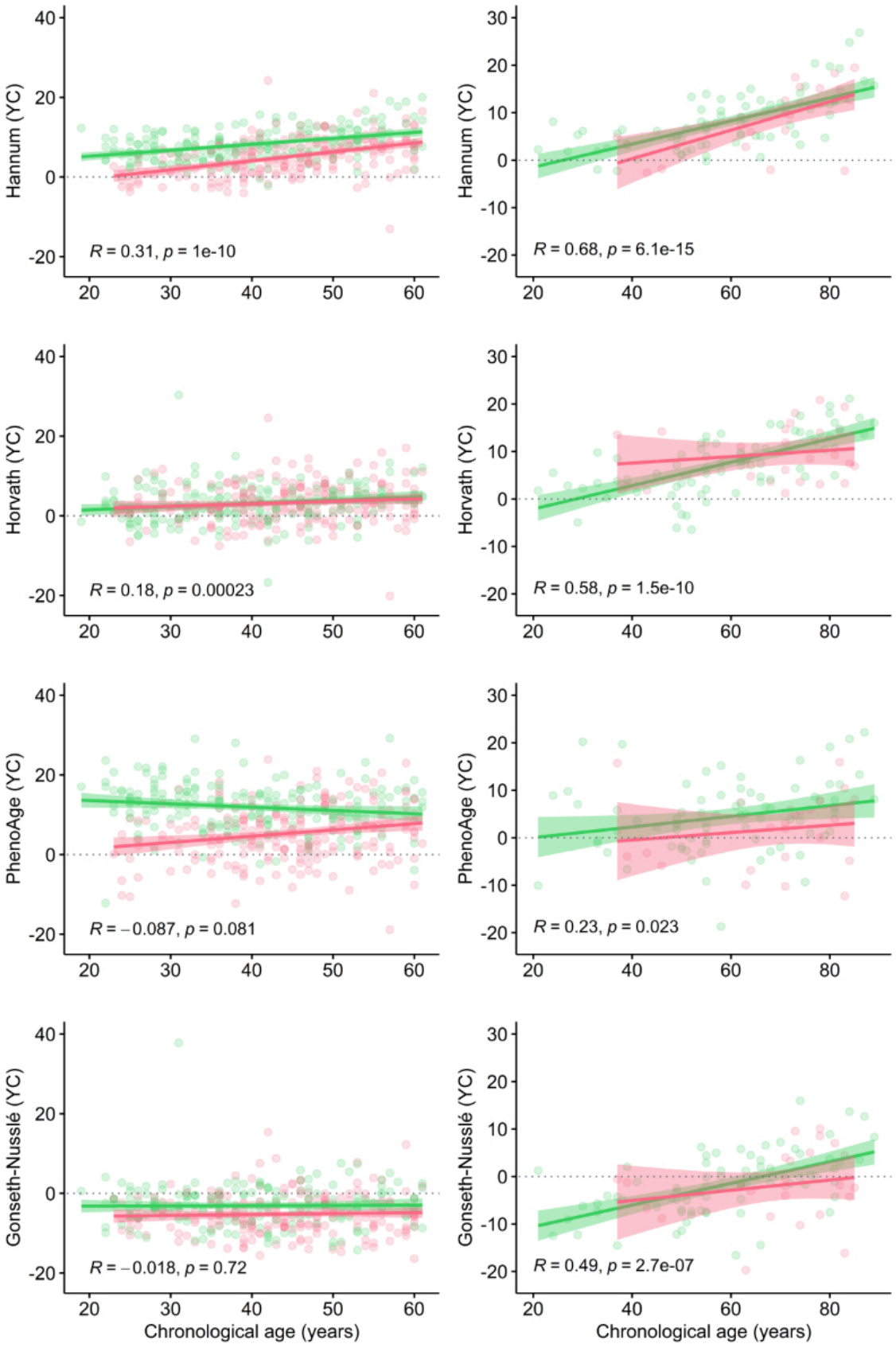
Descriptive statistics for the GSE168739 and GSE174818 studies are provided in table 1. Participants included in the GSE168739 study and who had severe clinical symptoms were on average older than those without symptoms or paucisymptomatic, had a higher average epigenetic age, and a greater proportion were male (67.6%) (table 1).

Similar sample distributions were observed for the GSE174818 study. A higher correlation between youth capital and chronological age was observed for the GSE174818 sample compared to the GSE168739 sample (figure 1). In comparison to individuals with less severe symptoms, youth capital was lower for those with severe symptoms; similarly, individuals who died had lower youth capital in comparison to those who survived (table 1 and figure 1). The epigenetic age estimated by Gonseth-Nusslé et al. was the only measure that reported an average epigenetic age that was older than the reported chronological age (table 1). Across all measures of epigenetic age, youth capital improved with chronological age (figure 1).

Table 1:
Descriptive table of study characteristics, stratified by severity.

		GSE168739 (n = 407)		GSE 157103 (n = 102)	
		Asymptomatic / paucisymptomatic (n = 194)	Severe (n = 213)	Alive (n = 77)	Dead (n = 25)
		Mean (SD); Q1,Q3	Mean (SD); Q1,Q3	Mean (SD); Q1,Q3	Mean (SD); Q1,Q3
Chronological age (years)		39.4 (10.7); 31.0, 47.0	44.6 (9.4); 39.0, 51.0	58.8 (16.3); 50.0, 72.0	69.3 (14.1); 64.0, 78.0
Epigenetic age	Gonseth-Nusslé	42.5 (11.8); 33.5, 51.8	50.0 (10.7); 42.3,57.1	61.0 (14.7); 52.2, 69.7	71.3 (14.7); 67.0,82.7
	Horvath	36.3 (10.9); 28.3, 44.6	41.3 (10.2); 34.0, 48.5	51.6 (13.2); 43.5, 60.4	60.1 (14.5); 55.0, 69.8
	Hannum	31.1 (10.2); 23.7, 38.7	39.5 (8.4); 34.0, 44.5	50.6 (13.1); 44.3, 59.8	60.0 (11.2); 58.1, 65.8
	PhenoAge	27.1 (14.3); 16.5, 37.2	39.3 (10.4); 32.9, 46.2	54.4 (16.2); 46.0, 65.3	67.5 (15.1); 66.1, 75.1
Youth capital	Gonseth-Nusslé	–3.1 (5.3); –5.9, –0.3	–5.42 (5.5); –8.5, –2.3	–2.1 (7.3); –7.0, 2.1	–1.9 (7. 6); –6.6, 4.0
	Horvath	3.0 (4.9); 0.2, 5.9	3.3 (5.1); 0.3, 6.6	7.4 (6.1); 3.3, 11.8	9.5 (5.9); 3.6, 14.2
	Hannum	8.3 (4.7); 5.6, 10.8	5.1 (4.8); 1.9, 8.0	8.0 (6.0); 3.3, 12.0	9.1 (6.7); 4.8, 13.0
	PhenoAge	12.2 (8.0); 8.5, 15.6	5.2 (7.0); 0.7,9.7	4.4 (7.5); –0.5, 8.6	1.8 (7.7); –4.1, 7.5
Females; n (%)		153 (78.9)	69 (32.4)	29 (37.7)	10 (40.0)

Figure 1: Correlation between youth capital and chronological age. Plots depict the correlation between measures of youth capital (y-axis) and chronological age (x-axis) (assessed using Spearman's rank correlation coefficient). Plots on the left-hand side correspond to COVID-19 severity in the GSE168739 sample; plots on the right-hand side correspond to COVID-19 severity in the GSE174818 sample. For all plots, the green line represents less severe symptoms, and the red line represents severe symptoms/death.



Epigenetic age and symptom severity

Improved youth capital was associated with reduced odds of severe symptoms for the Gonseth-Nusslé, Hannum and PhenoAge measures (OR = 0.95, 95% CI = 0.91–1.00; OR = 0.81, 95% CI = 0.75–0.86; and OR = 0.85, 95% CI = 0.81–0.88, respectively), albeit not Horvath (table 2). Moreover, when adjusting for age and sex, the PhenoAge youth capital was associated with reduced odds of COVID-associated mortality (OR = 0.93, 95% CI = 0.86–0.99; unadjusted OR = 0.96, 95% CI = 0.90–1.02). This association persisted when additionally adjusting for BMI, the Charlson comorbidity index. A weak association was also observed for Hannum's youth capital, although not significant (p = 0.09). The epigenetic signature (ES) for alcohol consumption, was associated with COVID-19 severity in the GSE168739 study. For example, a one-unit increase in the ES of alcohol consumption was associated with a 13% increased odds of severe symptoms (OR = 1.13, 95% CI = 1.05–1.23). This association remained even when adjusting for youth capital. No lifestyle exposures were associated with COVID-associated mortality in the GSE174818 sample.

In comparison with the base model (adjusting only for age and sex), the AUC value was significantly improved when also including the ES of alcohol consumption and the PhenoAge youth capital estimate (AUC = 0.79, 95% CI = 0.74–0.83 versus 0.85, 95% CI = 0.81–0.88, respectively: p <0.001) (figure 2). At a threshold of 0.5, the base model had a sensitivity of 75.8% and specificity of 70.4%, while the model, including the PhenoAge youth capital and the ES for alcohol consumption had a sensitivity of 80.4% and specificity of 80.8%. Finally, the inclusion of the epigenetic signature for alcohol consumption and the PhenoAge youth capital with the EPICOVID signature improved the model performance (AUC = 0.94, 95% CI = 0.91–0.96 versus AUC = 0.95, 95% CI = 0.93–0.97; p = 0.01).

Discussion

All measures of epigenetic age, except for the Horvath signature, were associated with COVID-19 symptom severity. However, the association across individual epigenetic signatures was not homogenous, as a stronger association with COVID-19 severity was observed for both the Hannum and PhenoAge signatures. The inclusion of PhenoAge youth capital and the EpiAlc signature alongside the age, sex, and the EPICOVID signature modestly improved the predictive capacity for COVID-19 severity. Finally, only the PhenoAge-based measure of youth capital was associated with an elevated COVID-related mortality.

The results from the present study support evidence for an association between epigenetic age and COVID-19-related outcomes. The observed variation in the strength of association across epigenetic signatures likely reflects differences in how the individual signatures were developed, but may also capture different aging processes [24]. For instance, the epigenetic age signature by Horvath et al. was developed using 51 different tissue and cell types and a 27k DNA methylation array, uses 353 age-associated CpG sites, and was optimized to measure chronological age [25]. In contrast, Hannum et al. used blood-derived DNA, an Illumina 450k micro-array platform, and subsequently identified 71 CpG sites associated with chronological age [17]. Gonseth-Nusslé et al. similarly used blood-derived DNA, but used an EPIC 850k micro-array platform, and identified chronological age-associated CpGs (n = 11) that were conditional on maximizing the contribution by epigenetic signatures of lifestyle exposures (patent reference: EP 22 162 216.0). Of the epigenetic signatures included in the present study, the DNAm PhenoAge signature is the only one that specifically incorporated measures of inflammation and immune system reactivity in its initial creation [19]. Recent evidence has pointed towards a so-called cytokine storm to be at the root of severe COVID-19 outcomes [26]. To this effect, age-associated changes to the immune system modify the immune response, particularly contributing to over activity, which may help to explain the worse COVID-19 symptomology observed among the elderly [26]. This could, in turn, explain why the DNAm PhenoAge signature – but not the other signatures – was associated with COVID-related mortality. Importantly, recent evidence based on Mendelian Randomization tech-

Figure 2: ROC curves for COVID-19 severity discrimination (GSE168739). The orange, solid line corresponds to the base model (age and sex); the green, short-dashed line corresponds to the model adjusting for age, sex, the epigenetic signature for alcohol and PhenoAge youth capital; the blue, dashed line corresponds to the model adjusting for age, sex, the epigenetic signature for alcohol consumption, PhenoAge youth capital, and the EPICOVID signature.

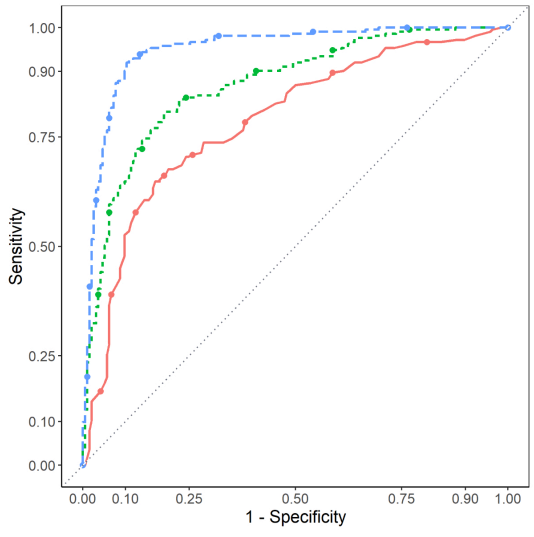


Table 2:
Logistic regression results for the association between youth capital and COVID-19 severity (GSE168739). Presented coefficients represent odds ratios and 95% confidence intervals. COVID-19 severity is defined according to hospitalization status (yes/no).

		Gonseth-Nusslé	Horvath	Hannum	PhenoAge
Unadjusted model		0.92 (0.8–0.95)	1.01 (0.97–1.05)	0.85 (0.80–0.89)	0.85 (0.82–0.88)
Adjusted model	Youth capital	0.95 (0.91–1.00)	1.00 (0.96–1.05)	0.81 (0.75–0.86)	0.85 (0.81–0.88)
	Chronological age	1.05 (1.03–1.07)	1.05 (1.02–1.07)	1.09 (1.06–1.13)	1.06 (1.03–1.08)
	Sex	6.62 (4.17–10.71)	7.49 (4.77–11.99)	4.64 (2.83–7.70)	8.07 (4.77–13.64)

niques suggests that epigenetic age does not lead to increased COVID-19 severity [27].

Epigenetic age is not sufficient to triage patients in the hospital as it is not as effective as the EPICOVID signature or interferon-based detection tests [28]. However, these signatures could serve to inform prevention efforts aimed at lifestyle management to prevent severe COVID-19 symptomology [29]. To this effect, the Gonseth-Nusslé signature also represents a convenient way of capturing the impact of exposure to lifestyle even when these exposures have not been adequately assessed in a given study. Recently, promising results have demonstrated the reversibility of epigenetic age. For example, a randomized clinical trial that targeted sleep, diet, physical activity, stress, and the gut microbiome found that at the end of the 8-week treatment program, participants in the intervention arm reduced their epigenetic age (measured using the Horvath signature) by an average of nearly two years [30]. Another clinical study targeted epigenetic age by repurposing pharmacological therapies, and administering individual-based doses of rhGH, DHEA, and metformin [31]. At the end of a 1-year study protocol, epigenetic age was reduced by an average of 2.5 years; an effect that persisted for up to six months post-study [31]. Unfortunately, considering the very small sample size (n = 10), further validation is needed. Targeting epigenetic modifications associated with COVID-19 severity could also inform the development of medications (e.g., "epidrugs") to limit the severity of symptoms or in the identification of currently existing drug therapies that could be repurposed for tertiary prevention (i.e., the reduction of symptom severity) [32]. Finally, while DNA methylation techniques based on microarrays remain expensive and slow, emerging techniques – such as nanopore sequencing – could accelerate DNA methylation sequencing for use in a triage setting [33].

Strengths and limitations

The present study uses two separate, publicly available datasets to investigate the association between epigenetic age and COVID-19 severity. Both included studies were restricted to individuals identified in a hospital setting with COVID-19 diagnosis. The GSE168739 sample excluded individuals who smoked tobacco, presented with comorbidities (e.g., BMI ³30), and were 61 years of age or older at time of inclusion. Results are thus not generalizable to the broader general population, particularly those known to be at higher risk of experiencing more severe COVID-19 symptomology. Furthermore, given the restriction to non-smokers it was not possible to investigate the contribution of tobacco consumption to COVID-19 severity. However, although there was no evidence for an association between the epigenetic signature of tobacco consumption and symptom severity in the GSE174818 sample, this could be due to the limited power to detect small effect sizes. Finally, the restriction of the GSE168739 sample to a population without known risk factors for COVID-19 severity, but also disease in the general population, may have contributed to the weaker association between the Gonseth-Nusslé epigenetic age and COVID-19 symptom severity.

In comparison to other epigenetic ages, the Gonseth-Nusslé estimated overall poorer youth capital, while the epigenetic age estimated using Hannum's signature was overall younger. Such global differences could be influenced by platform effects, insomuch that the Hannum score was built based on a 450K assay, while the Gonseth-Nusslé score was based on the 850K EPIC assay. Moreover, while epigenetic age measures may capture different underlying aging processes, estimates of epigenetic age may be subject to measurement error or survivor bias, particularly among the elderly. El Khoury et al. recently demonstrated the systematic underestimation of epigenetic age among the elderly [22]. In theory, if such an effect is not present, youth capital should remain uncorrelated with chronological age; in the present study, although the Hannum signature was most strongly associated with COVID-19 severity in the GSE168739 sample, youth capital was also correlated with chronological age; demonstrating a dependence on chronological age ($R^2 = 0.3$). In contrast, youth capital remained weakly associated with chronological age when measured using the Gonseth-Nusslé or PhenoAge signatures ($R^2 = 0.02$ and 0.07, respectively). Another limitation of the present study is the lack of longitudinal measures of epigenetic age. It is thus unclear whether factors external to epigenetic age influence COVID-19 severity, which subsequently increases epigenetic age, or whether higher epigenetic age prior to infection plays a role in disease severity. Contributing to this incertitude, the stability of epigenetic mechanisms and signatures of epigenetic age is not well understood. Finally, it is necessary to note that the analyses included within this study are secondary analyses based on datasets that have additionally been used in two prior publications[34,35]. Complementing the results of these previous publications, this study investigates novel signatures of epigenetic age, tobacco, and alcohol consumption in association with COVID-19 outcomes.

Conclusions

Consideration of epigenetic age does not meaningfully improve the prediction of COVID-19 severity, but is a potentially useful tool in primary prevention, particularly as an incentive towards lifestyle changes that target reducing the risk of severe COVID-19 symptoms. Therefore, the development and extension of epigenetic-based tools towards routine clinical care should be encouraged, particularly in the context of chronic disease prevention. Unfortunately, the results from the present study cannot disentangle the relationship between COVID-19 severity and epigenetic age. To address this limitation, longitudinal studies are required to understand the interplay between epigenetic age and COVID-19 severity.

Availability of data and materials

The datasets analyzed during the current study are available in the GEO Accession repository:

GSE174818[13]: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE174818

GSE168739[14]: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE168739

Statistical code and the epigenetic signatures described in this study (that is, those created by genknowme) are available upon request for non-commercial use by contacting genknowme at labo[at]genknowme.com.

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Authors' contributions: JDC: Conceptualization, methodology, formal analysis, writing – Original draft, MB: Investigation, writing – review & editing, SB and SGN: writing – review & editing, supervision, funding acquisition.

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Potential competing interests
The authors SGN and SN declare the following competing interests: A patent filed March 15, 2022 as a European Patent Application, reference number: EP 22 162 216.0. Financial interests in Genknowme. The remaining authors have no competing interests to declare: JDC, MB.

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Appendix

Figure S1: Correlation between chronological age and epigenetic ages (Horvath, Hannum, and Gonseth-Nusslé). Plots A-C correspond to COVID-19 severity in the GSE168739 sample; D-E correspond to COVID-19 severity in the GSE174818 sample. For all plots, the blue line represents less severe symptoms, and the red line represents severe symptoms/death.

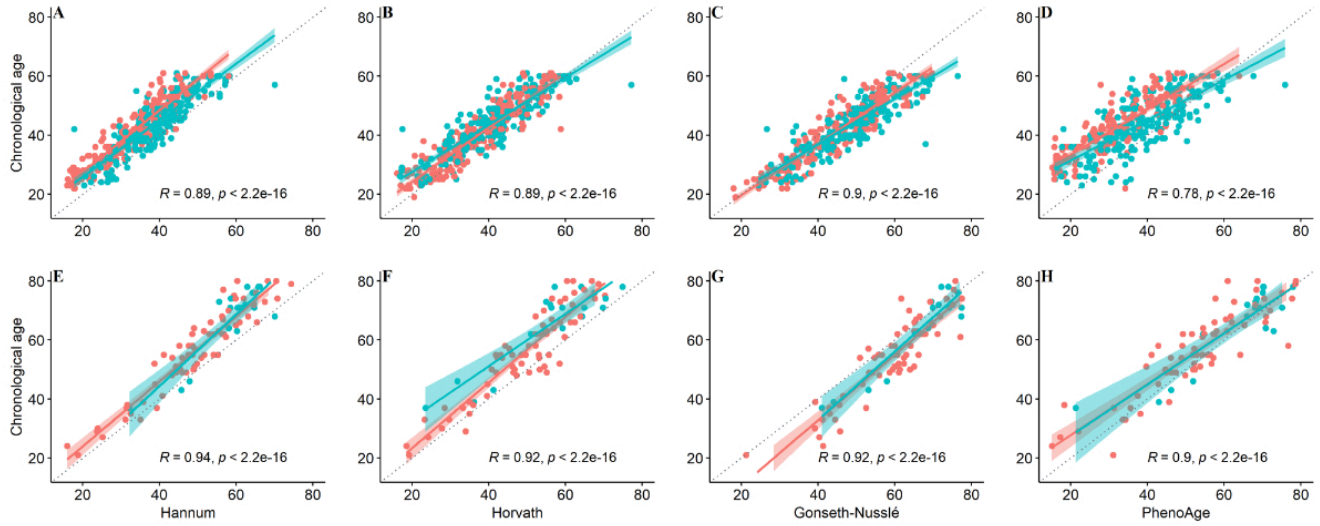

Figure S2: Youth capital stratified by categories of age and severity.
Legend: Red boxplot corresponds to youth capital calculated using the Gonseth-Nusslé signature; green to those calculated with Hannum signature; teal to Horvath signature; and purple to the youth capital when using PhenoAge signature.