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A cautionary note on altered pace of aging in the COVID-19 era

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is highly age-dependent due to hi-jacking the molecular control of the immune cells by the severe acute respiratory syndrome-corona virus 2 (SARS-CoV-2) leading to aberrant DNA methylation (DNAm) pattern of blood in comparison to normal individuals. These epigenetic modifications have been linked to perturbations to the epigenetic clock, development of long COVID-19 syndrome, and all-cause mortality risk. I reviewed the effects of COVID-19 on different molecular age markers such as the DNAm, telomere length (TL), and signal joint T-cell receptor excision circle (sjTREC). Integrating the accumulated clinical research data, COVID-19 and novel medical management may alter the pace of aging in adult individuals (<60 years). As such, COVID-19 might be a confounder in epigenetic age estimation similar to life style diversities, pathogens and pathologies which may influence the interpretation of DNAm data. Similarly, the SARS-CoV-2 affects T-lymphocyte function with possible influence on sjTREC levels. In contrast, TL measurements performed years before the SARS-CoV-2 pandemic proved that short TL predisposes to severe COVID-19 independently from chronological age. However, the persistence of COVID-19 epigenetic scars and the durability of the immune response after vaccination and their effect on the ongoing pace of aging are still unknown. In the light of these data, the heterogeneous nature of the samples in these studies mandates a systematic evaluation of the current methods. SARS-CoV-2 may modify the reliability of the age estimation models in real casework because blood is the most common biological sample encountered in forensic contexts.

Dear editor,

In late 2019, the pandemic of coronavirus disease (COVID-19) started to hit the world's countries after its discovery in Wuhan, China [1]. COVID-19 is a global emergency that claimed over 500 million confirmed cases worldwide as of April 17th, 2022 [2]. As a pandemic occurring in the fourth industrial revolution, highly advanced techniques empowered the scrutinized analysis of epigenetic, genetic, and proteomic markers of biological age to classify COVID-19 patients who are at maximal risk of developing complications or even death [3].

Over the past two decades, a plethora of forensic genetic and epigenetic studies suggested that DNA methylation (DNAm) and telomere length (TL) have a remarkable ability to predict the chronological age of the individual using various biological substrates [4–16]. The majority of DNAm models exhibit the lowest standard error of estimates (SEE) that is kept below 5 years [5–13] with the exception of one validation study [4] due to inter-laboratories variations. The TL-based models have higher SEE above 7 years [14,16]. Other methods of age estimation have been elucidated using the thymic function as measure of immunological maturation with age. It is quantified by a non-replicating circle of DNA called signal joint T-cell receptor excision circle (sjTREC) in naïve T cells using real-time polymerase chain reactions (q-PCR). The sjTREC was found to be negatively correlated to the chronological age albeit the models computed for sjTREC produced SEE of age estimation ranging between 7 years up to ~12 years [14,15].

Because the systematic evaluation of DNA methylation signature of COVID-19 in relation to the biological age is currently a new scientific inquiry [17–36], this correspondence is organized around three overarching medico-legal concerns that need to be addressed for

understanding the influence of COVID-19 on forensic age estimation in survivors. These concerns include: (1) the possibilities of biological and/or epigenetic age acceleration [17–24], TL attrition [17,24,35,36], and altered thymic function with its closely related marker (sjTREC) that may be involved in the pathogenesis of COVID-19 [30–34]; (2) the presence of population differences in both vulnerability and the outcome of the infection such as mortality and long COVID syndrome which has ramifications on the precision of age estimates by the forensic models [37–41]; (3) the protective effect exerted by mRNA vaccines and drugs like metformin, rapamycin, and anti-androgens as potential lifespan-extending against COVID-19 and subsequently the deceleration of epigenetic age [21,42,43].

The chronological age and/or co-morbid conditions like obesity are independent significant risk factors for COVID-19 severity and mortality rates [44]. The inappropriate immune responses induced by COVID-19 in the elderly and obese individuals are related to the immune-senescence and inflammaging in their baseline health status whereas the increased body weight regardless of the age patients results in early initiation of thymic senescence [44–47]. There is an abundance of clinical and epigenetic studies that highlighted the association between the viral life cycle of HIV [48] as well as different types of coronavirus, including SARS-CoV-2 and the molecular mechanisms linked to the host immune response to viral infections [48–53]. The findings of several studies showed significant differential DNA methylation pattern in brain [29] and blood samples associated in COVID-19 patients [22–24,27–29]. The comparative DNA methylation profiling of severe COVID-19 revealed an altered genome-wide methylome signature at 44 CpG sites denoted as the epigenetic susceptibility loci for respiratory

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failure cases [28]. Balnis et al. [23] found that the global mean methylation values were not significantly different between the COVID-19 patients and control group individuals from the pre-pandemic period. Nevertheless, there is a differential DNAm pattern between COVID and non-COVID-19 individuals where the hypomethylated regions found in proximity to gene promoter regions are associated with the acute illness in comparison with the pre-pandemic controls. Patients with more severe disease course, however, exhibited a predominantly hypermethylation profile of the immunosuppressive genes [28,54]. This finding suggests an aberrant immune cell-based epigenetic signature of COVID-19 have been linked to immune dysfunction [23,27], perturbations to the epigenetic clock, development of long COVID-19 syndrome, and all-cause mortality risk in several studies [17–20]. As such, COVID-19 might be a confounder in epigenetic age estimation similar to life style diversities, pathogens and pathologies that may influence the forensic interpretation of DNAm data in a previous report [53].

Corley et al. [22] demonstrated that patients with severe COVID-19 had a significantly accelerated epigenetic age in comparison with the control healthy sample using Hovarth multi-tissue clock [5] and GrimAge clock [55]. Another study [20] employed a valid forensic model to estimate the biological age of the study groups namely “Bekaert’s algorithm” [8] which encompassed a set of 4 CpG DNAm markers in the ASPA, PDE4C, ELOVL2, and EDARADD genes analyzed by *Pyrosequencing* the gold standard in forensic analysis. A statistically significant increase in the epigenetic age of individuals suffering from long COVID-19 syndrome ($n = 117$; 10.45 ± 7.29 years) was found in comparison to COVID-19 free individuals ($n = 144$; 3.68 ± 8.17 years) which represents an addition of 5.25 years above the reported SEE of the Bekaert’s algorithm. The acceleration of epigenetic age was deemed 76.6% in the COVID-19 group vs 48.2% in the COVID-19 free sample [20].

Recently, a Spanish study by Cao et al., 2022 [24] reported significant acceleration of epigenetic aging using Hannum [7], PhenoAge [56], skin Horvath and GrimAge [55] clocks and DNAm TL [16] attrition acceleration in young (<50 y) and old (>50 y) COVID-19 patients compared with healthy individuals. Using the Bernardes et al.’s [57] longitudinal cohort, they found that DNAm ages calculated from five clocks and TLs of the samples collected sequentially during the disease process of six patients showed an acceleration of epigenetic aging in the Hovarth age, PhenoAge and GrimAge at the initial phases of COVID-19, and the accumulation of age acceleration was incompletely reversed at later phases of convalescence in certain individuals. Another longitudinal US study by Pang et al. [21] showed an epigenetic age acceleration of individuals above 50 years by 2.1 and 0.84 years using the principal component (PC) of epigenetic age estimates using PhenoAge clock and GrimAge clock as well as deceleration of age after vaccination by Moderna mRNA vaccine by 3.9 years in average in the same age group presenting with mild and moderate severity of the disease [21]. The chronological age clocks did not show significant epigenetic age acceleration after COVID-19. The biological age predictors (PhenoAge and GrimAge clocks) are among the strongest epigenetic age predictors of mortality risk and able to capture the age-dependent perturbation to epigenetic clocks after COVID-19 infection and signals of both immunosenescence and inflammaging [55,56]. In young individuals (age less than 50 years), deceleration of the epigenetic clock can occur due to a robust activation of the immune system [21].

One study argued against the presence of such “biological age acceleration” [25]. Nevertheless, Franzen et al. acknowledge the small sample size and the early collection of blood sample in the disease process from currently infected severe acute respiratory syndrome-corona virus 2 (SARS-CoV-2) patients without reporting their treatment protocol, and the pre-pandemic control samples were not available to the same studied patients group [25].

These conflicting findings may be attributed to the different array platforms used and CpGs sites interrogated as well as their designated capabilities to predict particular outcomes [13,53,58,59]. Testing of

data based on HM450 is not completely overlapping with the markers interrogated with the EPIC BeadChip (only ca. 90% of the sites on the HM450 are covered). The biological age predictions using Hovarth 2013 and Hannum clocks while employing the EPIC BeadChip dropped certain CpGs (19 and 6, respectively), and this might result in a moderate offset of age-predictions [25,58].

Moreover, little overlap in CpGs between the different DNAm clocks because the cell-/tissue-specific differences employed in the training of the models [59]. For example, there are only five CpGs sites that are commonly found in the three epigenetic aging measures: Hannum, PhenoAge, and Horvath DNAm Age measure including: cg05442902 (P2RXL1), cg06493994 (SCGN), cg09809672 (EDARADD), cg19722847 (IPO8), and cg22736354 (NHLRC1) [Levine]. Only EDARADD and NHLRC1 were included in the previously published mimimized CpG forensic models [58]. The forensic Bekaert’s model has two common CpG DNA methylation sites with Hannum DNAm epigenetic clock namely CpG6 ELOVL2 and CpG1 EDARADD genes. FHL2 is a gene highly correlated with chronological age and affected by COVID-19 disease [60]. It is included in Hannum [7] and Han [11] models.

It is important to mention some remarks on the results displayed by Franzen et al. [25] study:

- The dataset used for comparing the performance of different clocks were based on the studies by Blanis et al. [23] and Castro de Moura et al. [28]. Both studies showed clearly an epigenetic age acceleration by the Hovarth et al. [5] and Han et al. [11] models.
- In the original Blanis et al. [23] study, they stated non significant differences in the delta age between control and COVID-19 group without mentioning the model employed. The prepandemic control with unknown health status. They were also significantly older than the pandemic COVID-19 cases. In the original Blanis et al. [23] study, they stated non significant differences between the COVID-19 patients and pre-pandemic control albeit they did not declare the DNAm clock used. Additionally, the prepandemic control group was unknown health status and they were significantly older than the pandemic COVID-19.

Population affinity has been also implicated in the DNA methylation differences among individuals [41,61]. A study by Ahmad [40] showed a clear distinction of the overall distribution of patients from the different geographic regions in which the Middle Eastern and Northern American patients exhibited a younger average age values than individuals inhabited the EU and Asian countries [40]. The age estimation models based on different populations showed different age related CpG markers [10,56,61].

SARS-CoV-2, as an RNA virus, is capable of hijacking the epigenetic landscape of host immune cells to suppress the host antiviral response [22,30,49–51]. These epigenetic mechanisms are closely linked to the control of lymphopoiesis and the immune response depending on the disease severity [22–24,27,28,50,57]. Therefore, obvious cell-type shifts in the composition of blood cell have been correlated with the severity of COVID-19 disease due to the effect of COVID-19 DNAm signature. For example, a low count of lymphocytes (particularly, the CD4+ and CD8+) and decreased production of naïve T-cells are implicated in the prediction and prognosis of severe COVID-19 [31–33]. Pang et al. [21] observed increasing CD4+ Naïve T cells in COVID-19 patients under 50 years of age. Furthermore, sjTREC levels were slightly higher in young individuals suffering from severe COVID-19 with extensive lung affection than control sample due to thymus hyperplasia ($p = 0.02$, $n = 24$) [34]. These evidences are suggesting that sjTREC values may be also affected by the SARS-CoV-2 infection and may play a role in influencing biological age differences in survivors relative to their chronological age. While it has not been conclusively established yet that sjTREC have a different quantity due to acute infection, I have presented some evidences from the literature that such possibility may exist.

The aforementioned studies [17–24] may refer to a sudden alteration

of biological age in a large sectors of COVID-19 survivors of forensically relevant age (younger than 60 years) after severe COVID-19 disease as captured by certain epigenetic clocks [20–24] which reflects the efficiency of the immunological response in combating the infection. Moreover, severe COVID-19 might be associated with durable scars to the epigenome and alterations to epigenetic clocks in blood [24].

As regards the TL, it was significantly shortened in the cases of COVID-19 in comparison to the control in three studies [24,35,36] and the long COVID compared with the COVID-free groups in a fourth study [20]. The DNAm and TL age markers were thought to be closely related as the telomere attrition leads to differentiation instability via DNA methylation [27,62,63]. However, Mongelli et al. [20] and Marioni et al. [63] studies suggested that both markers are regulated and work independently. Corley et al. [22] reported non significant decrease in DNAm-based telomere length in severe COVID-19 (n = 9) compared with the negative control sample. The TL measurements performed years before the SARS-CoV-2 pandemic proved that short TL predisposes to severe COVID-19 independently from age [35,36]. Moreover, Mongelli [20] showed that altered biological age computed from the DNAmAge as well as short telomere length coexist in the post-COVID-19 group. Nevertheless, the population differences in TL such as known populations with shorter TL [14] are still a possible factor responsible for interpopulation variability in response to the COVID-19 disease in different age groups.

This cautionary note would require a well-concerted collaboration among worldwide forensic researchers for rigorous validation of the previously published population data, age estimation standards, and revising the most implicated biological age markers in this process. Moreover, evaluation of a large set of CpGs is preferable to detect the effect of COVID-19 on the epigenome landscape [20]. The search for markers quantifying the chronological age independently from the biological age which is affected by the disease processes and genetic variants, could have a great influence on the accuracy of forensic age estimation [64]. I also infer that more caution should be exerted with blood samples as source of DNAm values since most systems/ methods are based on peripheral whole blood samples or blood stains as important specimens within the forensic contexts [20,25]. Severe COVID-19 in patients younger than 65 years induced molecular signatures of aging in human tissues other than blood [29]. More importantly, the analysis of whole blood samples do not capture the changes existing in other tissue compartments relevant to the pathogenesis of the COVID-19 [23]. Therefore, the effect on epigenetic age estimates from forensic biological materials other than blood is still unknown.

In future studies, it is important to mention the date of data collection or sampling for timely correlation with the global pandemic situation, the past history of confirmed SARS-CoV-2 infection, its severity and the outcome, the intake of certain drugs that may reverse the aging process [25], and finally the vaccination status as well as its type [21]. The persistence of COVID-19 epigenetic scars and the durability of the immune response after vaccination are still unknown information [20, 21,26,52]. Altogether, these confounding factors may influence the reliability of the age estimation models and correct interpretation of results in the diverse forensic scenarios. The inter-population differences and inter laboratories variations may also affect the technique selection and interpretation of results [4,65–67]. We, researchers in the forensic disciplines, are active in reporting the best practices in forensic case-work and announcements of potential sources of error to anticipate any problems affecting the accuracy and reliability of forensic investigations.

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