Biological Age Predictors

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Age is a major risk factor for chronic noncommunicable diseases. It is a recognized contributor to severe COVID-19 and associated complications. However, many studies have suggested that it is biological rather than chronological age that underlies the development of numerous diseases. People age at a different pace, which is determined not only by genetic predisposition but also by external factors, such as socioeconomic factors and lifestyle. The likelihood of aging-associated diseases and mortality varies even among people of the same age. Certain combinations of biomarkers are more reliable predictors of biological age or mortality

Keywords: biological age ; molecular clock ; age-related diseases

1. Introduction

Age is a major risk factor for chronic noncommunicable diseases, such as heart disease [1], cancer [2], chronic obstructive pulmonary disease [3], Alzheimer's disease [4], etc. It is a recognized contributor to severe COVID-19 and associated complications [5]. However, many studies have suggested that it is biological rather than chronological age that underlies the development of numerous diseases. People age at a different pace, which is determined not only by genetic predisposition but also by external factors, such as socioeconomic factors and lifestyle. The likelihood of aging-associated diseases and mortality varies even among people of the same age; hence, it could be reflective of their biological age.

The last 15 years saw the emergence of various biological age markers. Ideally, they should correlate with chronological age and be predictive of age-related diseases and mortality. Clinicians use several tests as markers of biological age: maximal oxygen consumption, forced expiratory volume in 1 s, vertical jump, grip strength, whole-body reaction time, unilateral distance, sit-and-reach test, systolic blood pressure, waist circumference, and soft lean mass [6]. Certain inflammatory markers have also been associated with age: IL-6, IL-8, IL-15, IL-1β, TNFα [7][8][9], lipid profile (HDL cholesterol, LDL cholesterol, triglycerides [7][8][10][11]), glucose metabolism profile (glycohemoglobin (Hba1c) and glucose (fasted or oral glucose tolerance test (OGTT) [12]), insulin and C-peptide [13]. Kidney function indicators, such as creatinine, cystatin C, urea, and albumin, have also been associated with age [14]. Microbiome analysis is another way of assessing biological age, since the microbiome has been significantly associated with age [15].

Aging leads to increased genome instability, which can be evaluated using micronucleus assay [16]. Age has also been associated with telomere length [17] and an increase in reactive oxygen species [18]. However, the most common marker of biological age is DNA methylation. It is widely used in forensic medicine as the most reliable age estimator. Other age-associated epigenetic markers could be changes in miRNA concentrations [19], histone modifications [20], and chromatin remodeling [18].

Individually, these markers are not informative due to their non-specificity. Moreover, changes in their levels can be a manifestation of age-associated conditions, rather than an indication of age. These markers are effective estimators in large study cohorts; however, they may vary significantly at the individual level in clinical practice [21]. To overcome these limitations, artificial intelligence has been used to create models that consider a variety of factors. These models are widely used in clinical practice. They can predict mortality from all causes and the incidence of major aging-associated diseases, including hypertension, diabetes, cardio-vascular diseases, stroke, cancer, and dementia [22][23].

2. Biological Age Predictors

2.1. Clinical Parameters and Blood Biochemistry as Markers of Aging

Blood Biochemistry-Based Calculators

Individually, most clinical biomarkers are insufficiently sensitive to measure the pace of aging and biological age. Studies, however, have shown that certain combinations of biomarkers are more reliable predictors of biological age or mortality.

Putin E. et al. developed the first blood marker-based model of aging using a group of 21 deep neural networks (DNNs) that were trained on more than 60,000 samples from common blood biochemistry and cell count tests [10]. For each patient, they used only 41 biomarkers; nonetheless, the DNN group achieved a rather small interval of mean absolute error (MAE) = 5.55 years (r = 0.91, R^2 = 0.82). The top 10 biomarkers included albumin, erythrocytes, glucose, alkaline phosphatase, hematocrit, urea, RDW, cholesterol, alpha-2-globulin, and lymphocytes. Mamoshina P. et al. [24] presented a new aging clock trained on the data from several populations. The most effective predictor achieved an MAE of 5.94 years despite being trained on fewer features (21 vs. 41). It is likely that ethnically diverse aging clocks are more accurate than conventional ones in predicting chronological age and measuring biological age. The most important blood biochemistry parameters for all three populations were albumin, glucose, urea, and hemoglobin.

The models laid the foundation for the following calculators: Aging.AI 1.0 (r = 0.91, Rsq = 0.82, MAE = 5.5 years), Aging.AI 2.0 (r = 0.79, Rsq = 0.63, MAE = 6.2 years), and Aging.AI 3.0 (r = 0.8, Rsq = 0.65, MAE = 5.9 years) [25]. The predictors use various combinations of input parameters: albumin, glucose, alkaline phosphatase, urea, erythrocytes, cholesterol, RDW, alpha-2-globulins, hematocrit, alpha-amylase, lymphocytes, ESR, total and direct bilirubin, gamma GT, creatinine, LDH, total protein, alpha-1 globulins, beta globulins, gamma globulins, triglycerides, chlorides, HDL-C, LDL-C, calcium, potassium, sodium, iron, hemoglobin, MCH, MCHC, MCV, platelets, leukocytes, ALT, AST, basophils, eosinophils, monocytes, and neutrophils. The parameters are measured in whole blood, plasma or blood serum.

Several authors have used the above predictors in their studies. Cohen [26] used 10 biomarkers from Aging.AI (albumin, glucose, alkaline phosphatase, urea, erythrocytes, cholesterol, RDW, alpha-2 globulins, hematocrit, and lymphocytes) to predict chronological age in cohorts from the Women's Health and Aging Study I &II (WHAS), the Baltimore Longitudinal Study on Aging (BLSA), Invecchiare in Chianti (InCHIANTI) and publicly available cross-sectional data from a representative sample of the American population from the National Health and Nutrition Examination Survey (NHANES). The performance in all four data sets was not as robust, with MAE ranging from 12.7 (NHANES) to 17.4 (BLSA). The authors excluded the possibility that the results were due to the use of 10 biomarkers rather than 41 and suggested that it could be caused by the absence of children in the cohorts and the differences in ethnic, socioeconomic, and environmental backgrounds. Overall, the results were consistent with those reported by Putin E. et al. [10] and showed the model's tendency to underestimate the age of individuals over 70 years of age, i.e., it lacked discriminatory power in older age ranges.

Psychological status-based calculation of biological age using medical history and self-estimation of physiological and emotional states.

Currently, there are extremely few papers on psychological markers of aging. However, they deserve further investigation, particularly due to the non-invasive nature of the associated procedures. Repeatedly, biological aging has been shown to lead to cognitive decline. Diagnosed cognitive dysfunction is a predictor of unsuccessful aging and mortality; however, it has a low predictive power in younger people. Zhavoronkov et al. used deep neural networks (DNNs) to classify human behavior for biological age prediction [27]. They presented two new models, PsychoAge and SubjAge, which were similar to the aging clock. To predict chronological and subjective age, they trained the DNNs on a set of 50 modifiable behavioral features based on anonymous surveys of U.S. residents from the Midlife in the United States (MIDUS). After filtering and exclusion, the final dataset comprised 6071 samples. DNNs were able to accurately predict age, with MAE = 6.7 years for chronological age and MAE = 7.3 years for subjective age. Both PsychoAge and SubjAge have also been shown to be predictive of the risk of all-cause mortality. For both models, the top five important variables were related to sex life in the past 10 years, marital status, health limitations on vigorous activity, and intake of prescription blood pressure drugs. Headache frequency in the past 30 days ranked 5th in PsychoAge and 9th in SubjAge. Neuroticism, one of the five most commonly used personality traits, was the only one present among the top 25 features in PsychoAge. Openness and extraversion, another "big fiver", were the only personality traits in SubjAge.

2.2. Age Predictors Based on Molecular and Genetic Markers

2.2.1. Transcriptome-Based Age Predictors

Peters M. et al. were among the first to successfully use transcriptome analysis to predict age [28]. They investigated 14,983 whole-blood samples from people of European ancestry. To calculate the "transcriptomic" age based on age-related differential gene expression, the authors used Illumina HumanHT-12 (v3/v4) and identified 1497 genes that produced highly correlated results in the discovery and replication stages. The R^2-values for chronological age and

predicted transcriptomic age were below 0.6; however, the average absolute difference between the predicted and chronological age was 7.8 years.

Ren X. et al. developed RNAAgeCalc, an age calculator based on transcriptional activity across 30 different tissues [29]. They used genome-wide and transcript-level gene expression data from 9662 samples available from the Genotype-Tissue Expression (GTEx) Program (V6 release). Tumor samples (n = 102) were omitted. Across all tissues, 1616 genes were identified as age-related. Transcriptional age acceleration was significantly correlated with mutation burden, mortality risk and cancer stage in several types of cancer from the TCGA database. Despite the above advantages, RNAAgeCalc produced rather high median errors for the predicted transcriptional age and chronological age (7–10 years, for most tissues).

Mayer D. et al. developed a binarized transcriptomic aging (BiT age) clock, which is currently among the most accurate transcriptome-based age predictors [30]. They processed 1020 publicly available RNA-seq samples for adult C. elegans, 900 of which were used to train and test the model. The transcriptome data were binarized to reduce noise: with a count per million above the median of the corresponding sample, the value of each gene was set to 1; otherwise, it was set to 0. BiT age does not require age discretization and allows assessment of the effect of single gene expression changes on the predicted age. To demonstrate the applicability of the novel method, the authors used the same human datasets as Fleischer et al.; however, binarization before calculating the elastic net regression significantly improved the results: R^2 = 0.92; the Pearson correlation = 0.96 (p = 7.87e-73), the Spearman correlation = 0.96 (p = 9.31e-73); MAE = 6.63 years; MAD = 5.24 years; and RMSE = 8.41 years. The model also predicted that the patients with Hutchinson-Gilford progeria syndrome (HGPS) were significantly older. BiT age comprises 141 predictor genes, among which the forkhead transcription factor FOXO1—a regulator of the aging process in C. elegans and mammals—is positively correlated with age, which serves as further evidence of the evolutionary conservation of transcriptional mechanisms that regulate longevity [31].

2.2.2. Predictors Based on the Peripheral Blood Proteome

Lehallier B. et al. developed a bioinformatics approach by analyzing venous plasma from 4263 healthy people aged 18–95 years [32]. Before processing, plasma was treated with ethylenediaminetetraacetic acid (EDTA). The authors used the SomaScan aptamer technology for high-precision proteomic analysis. They found a significant sex-related difference in 895 out of 1379 proteins that changed with age ($q < 0.05$). Lehallier et al. concluded that aging is a series of biologically motivated surges in plasma protein levels. The test in 1446 individuals provided a 0.97 Pearson correlation coefficient between the chronological age and predicted age. The authors also demonstrated that deviations from the plasma proteomic clock were correlated with clinical and functional changes.

2.2.3. Metabolome-Based Age Predictors

Van den Akker E. et al. used 56 serum biomarkers and proton nuclear magnetic resonance (1H-NMR) to build metaboAge, a metabolomics-based age predictor of an individual's biological age. The predictor achieved a high correlation coefficient between the predicted and chronological age, with an average mean absolute error of 7.3 years and R^2 = 0.654. MetaboAge also proved effective in predicting current and future cardiovascular and metabolic health and functionality in older individuals [33].

2.2.4. Age Predictors Based on T-Cell DNA Rearrangements

With age, the number of episomal DNA molecules, or signal joint T-cell receptor (TCR) excision circles (sjTREC), declines in a log-linear fashion. This is a manifestation of a persistent thymus involution that starts soon after birth: the thymus transforms into adipose tissue and loses its function. Zubakov et al. used the sjTREC number as the only predictor in a linear regression model, which explained a large share of highly statistically significant total age variance (R^2 = 0.835, $p = 8.16 \times 10^{-215}$, standard error of the estimation ± 8.9 years) [34].

2.2.5. Microbiome-Based Age Predictors

Galkin F, et al. developed an aging clock by analyzing more than 4000 metagenomic profiles of people aged 18–90 years. Floro'clock (R^2 = 0.5, Rsq = 0.3, MAE = 5.9 years) uses whole-genome sequences of the intestinal lumen microbiota [35].

Huang S. et al. assessed the accuracy of several age prediction models based on oral, gut, and skin microbiome samples. The prediction ability differed in three models (mean ± standard deviation): the skin microbiome, 3.8 ± 0.45 years; the oral microbiome, 4.5 ± 0.14 years; the gut microbiome, 11.5 ± 0.12 [36].

Several promising age prediction strategies, such as those based on the assessment of DNA damage [37], have not been tested in large cohorts and cannot be considered reliable methods of evaluating the pace of aging. Certain age prediction methods are no longer in use. For instance, telomere length is currently not viewed as a biomarker of human aging due to its hypervariability across human tissues [38].

Currently, the predictors based on molecular and genetic markers present a promising approach to biological age prediction. However, further research is needed for their clinical application.

2.3. Epigenetic Clocks

The first generation of epigenetic clocks comprised a set of CpG sites and used chronological age as reference. Bocklandt S. et al. were the first to calculate biological age by measuring methylation in CpG loci [39]. They identified 88 loci in or near 80 age-correlated genes. Methylation in three sites had the highest correlation with age and the widest distribution of values. The findings were validated in a different cohort. The authors developed a regression model based only on loci located in EDARADD and NPTX2 (error = 5.2 years). In 2013, Hannum G. et al. developed an epigenetic clock based on 71 methylation markers and clinical parameters (gender and BMI). The Hannum's model produced an error of 3.9 years for the primary cohort and 4.9 years for the validation cohort. Although the authors focused on white blood cells (WBC), the model proved applicable to other human tissue types [40]. Horvath S. et al. carried out a more comprehensive analysis of methylation and developed a multi-tissue predictor measuring methylation levels in various types of tissues, such as whole blood, peripheral blood WBC, umbilical cord blood, brain tissues, neurons and glial cells, buccal epithelium, gastrointestinal tract, heart, lungs, kidneys, saliva, placenta, etc. Using multivariate regression, the model automatically selected 353 methylation sites, which make up the predictor. It showed a fairly high accuracy on both the training set (age correlation = 0.97, error = 2.9 years) and test set (age correlation = 0.96, error = 3.6 years). The pace of aging in different tumor tissues was significantly accelerated (by an approximate average of 36 years), while the pluripotent stem cells had a DNAm age close to zero [41]. In 2014, Weidner et al. [42] developed a model based on 102 CpG methylation sites in blood. To facilitate clinical application, they focused on three methylation sites in the highly age-correlated genes—ITGA2B, ASPA, and PDE4C (training set, MAD = 5.4 years; validation set, MAD = 4.5 years). In 2018, Horvath S. et al. were able to improve their model [43]. They used different tissue; specifically, they increased the sensitivity for fibroblasts, since skin biopsy and isolation of fibroblasts are widely used in progeria research. The new age estimator comprised 391 CpGs. It has been used in several studies to calculate life expectancy or assess all-cause mortality [44][45][46] and analyze the association between biological age and aging-associated diseases [47][48][49][50]. Hun Y. et al. carried out a comparative analysis of biological age models based on various methods of measuring CpGs, such as 450 k Beadchip platform, CpG pyrosequencing, droplet digital PCR (ddPCR), and bisulfite barcoded amplicon sequencing. The ddPCR-based model was the most accurate in predicting epigenetic age in an independent validation sample, which could be due to the fact that the PCR is generally characterized by low error rates [51]. Galkin F. et al. used deep neural networks and data from 17 studies to develop an aging model comprising 1000 sites, with an MAE of 2.77 years [52].

Second-generation epigenetic clocks were the next step in the quest for more accurate and robust biomarkers of aging, including clinical characteristics. Researchers were not satisfied with the existing epigenetic clocks that used chronological age as a surrogate measure of biological age and did not factor in CpG sites, the methylation of which was not strongly associated with age. In 2018, Levine M. et al. developed a new epigenetic biomarker of aging—DNA-m PhenoAge [53]. First, they built a model that calculated phenotypic age based on nine clinical markers most significantly associated with mortality (albumin, creatinine, serum glucose, c-reactive protein, lymphocyte percentage, mean cell volume, red cell distribution width, and alkaline phosphatase) and chronological age. During the second stage, they selected 513 CpG sites that were the most accurate in predicting phenotypic age and that formed DNA-m PhenoAge. The model showed a significant correlation with all-cause mortality, age-related diseases, cardiovascular diseases, coronary artery disease, incidence of and mortality from lung cancer, Alzheimer's disease, etc. Out of 513 CpGs, 41 CpGs were the same as in the Horvath DNAm age measure and six CpGs as in Hannum's clock. In 2019, Lu A. et al. proposed a modified GrimAge model that was developed in two stages [54]. First, they identified DNAm biomarkers of physiological risk and stress factors (adrenomedullin, C-reactive protein, plasminogen activation inhibitor 1 (PAI-1), and growth differentiation factor 15 (GDF15)). They then combined them into one complex biomarker, DNAm GrimAge, and carried out a large-scale meta-analysis. The authors demonstrated that DNAm GrimAge was an accurate predictor of time-to-death, time-to-cancer, time-to-CVD, time-to-fatty liver, and time-to-menopause.

Thus, epigenetic clocks are among the most promising biomarkers of biological age and powerful predictors of lifespan. However, there are approximately 28 million CpGs in the human genome, and the above models only used approximately

20,000 CpGs available in 27 K, 450 K, and EPIC. Publicly available whole-genome bisulfite sequencing databases would greatly facilitate the development of even more accurate epigenetic clocks [55].

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