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Concept Paper

Biological Age versus Chronological Age in the Prevention of Age Associated Diseases

Gian Andrea Rollandi [1,*], Aldo Chiesa [1], Nicoletta Sacchi [1], Mauro Castagnetta [1], Matteo Puntoni [1], Adriana Amaro [2], Barbara Banelli [2], Ulrich Pfeffer [2,*]

1. Ente Ospedaliero Ospedali Galliera, Genova, Italy; E-Mails: gianandrea.rollandi@galliera.it; aldo.chiesa@galliera.it; nicoletta.sacchi@galliera.it; mauro.castagnetta@galliera.it; matteo.puntoni@galliera.it
2. IRCCS Ospedale Policlinico San Martino, Genova, Italy; E-Mails: adriana.amaro82@gmail.com; banellib.epigenetics@gmail.com; patologia.molecolare.integrata@gmail.com

* **Correspondences:** Gian Andrea Rollandi, Ulrich Pfeffer; E-Mails: gianandrea.rollandi@galliera.it; patologia.molecolare.integrata@gmail.com

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Abstract

Aging is associated with an increased incidence of major diseases, including cancer, cardiovascular, neurodegenerative, metabolic and autoimmune diseases. Primary prevention and early diagnosis of these diseases have a dramatic impact on incidence, outcome, quality of life and are commonly applied as age-dependent indications based on evidence of efficacy for specific groups of the aging population. They likely contribute to the observed increase in life expectancy through the reduction of incidence and the retardation of the onset of age-associated diseases. In the present article, we develop the hypothesis that age-dependent preventative measures and diagnostic screenings might perform better if biological age were used instead of chronological age. This is based on the observation that there are individual differences in age-associated decline in performance that are reflected by measurable biological indicators, such as telomere length, signal joint T-cell

receptor rearrangement excision circles, and specific DNA-methylation and gene expression events.

Keywords
Signal joint T-cell receptor rearrangement excision circle; telomeres; DNA-methylation; gene expression; cellular senescence; aging

1. Introduction

Age is a major risk factor for many diseases such as cancer [1, 2], cardiovascular diseases [3, 4], diabetes [5], and neurodegenerative syndromes [6], as these diseases show clear age-related incidence rates. The age-related risk can be explained by: i) the age-dependent number of stem cell divisions for specific tissues [7], likely not only for cancer; ii) life-long exposure to environmental insults, including diet [8, 9], radiation [10] and chemical factors [11, 12], and lifestyle [13, 14]; and iii) the, at least in part, genetically determined [15, 16] efficacy of endogenous repair systems such as the DNA-repair machinery [17, 18]. Chronic inflammation increases with age and is likely both a cause and a consequence of age-related (patho) physiological decline [19, 20]. The psychological stress that accumulates over a lifetime and the related resilience also contribute to aging [21]. A genetic predisposition for age-related diseases strongly affects longevity [22], an effect that is difficult to discern from true genetically determined longevity that is not mediated by disease susceptibility. Nonetheless, genome-wide association studies have delivered several genetic variants that independently affect longevity [23-26]. Differences in longevity, whether determined by endogenous genetic factors or environmental exposure, likely correlate with the physical performance state at any given age. Some people may “look” (perform) younger or older than the mean population of the same chronological age. Genetic variability and history of exposure to environmental stresses probably determine these differences, but this has not yet been studied in a systematic way. In this hypothesis article, we argue that a systematic analysis of differences in individual performance status can and should affect medical decision making. Diagnostic and therapeutic interventions, especially in the preventative setting, are often prescribed by applying age-thresholds. To mention a few, cardiovascular preventative drugs are described for the population over 50 years of age [27], mammographic breast cancer screening is recommended for women over 50 while screening of the younger population is highly debated [28, 29], and colonoscopy for the prevention of colon cancer is reserved for the elderly [30]. Age thresholds are implemented on a rationale considering objective health effects, associated costs, [31] and the risk of overdiagnosis [32]. Personalization of preventative screening invariably includes consideration of the person’s age [31]. Here we postulate that personalized medicine should consider “biological age” instead of “chronological age,” especially for the personalization of preventative measures in healthy subjects who do not present disease specific biomarkers.

2. The Concept of Biological Age

We define “biological age” as the individual physiological performance status that imperfectly correlates with “chronological age.” Simply, chronological age equals the years a person has lived and biological age is how old he or she seems, performs and/or feels. Alex Comfort was the first to address aging differences in the “aging rate” in men in 1969 [33]. The following search for biomarkers of aging has, however, met with limited success [34, 35], although it was clear from the beginning that biological age is associated with disease risk [36]. The complexity of aging, considering endogenous and environmental factors, has stimulated the development of multifactorial models [37-43]. The aim of these attempts was the prediction of mortality with limited applicability to prevention medicine (for a recent review see [44]).

Many factors show some association with aging and might serve for constructing models for the prediction of biological age. As discussed above, these factors can be intrinsic or environmental. Intrinsic, genetic factors have been identified but are not strong. Many of them are correlated with the risk for specific diseases that might limit one's lifespan, whereas very few show any disease independent effects on longevity. The latter are expected to correlate with biological age. Markers of exposure can be useful but their effect is limited by the fact that a double correlation is considered; the exposure correlates with the marker and with lifespan and/or performance limitations but the marker might not necessarily correlate in a significant manner with lifespan or performance due to the lack of transitivity of correlation [45] (Figure 1).

3. Which Markers?

Research on biological age markers has proposed many different markers and marker combinations (for a recent review see [46]). Usually, the markers are better if they are causally associated with the event for which they are a marker. If a specific type of cancer depends on the presence of a specific mutation, then the mutation is the best marker. Yet causal relations are rarely so straightforward. The complex nature of biological systems and their potential perturbations limit the value of markers that measure just one perturbation that might well be compensated by other features of the complex system. We, therefore, propose to abandon markers that are associated with lifespan and performance limiting environmental factors and to use instead of a set of markers that are directly associated with chronological age. Markers of chronological age, widely used in forensic science to estimate the age of biological specimens [47], try to closely match chronological age but fail to do this perfectly. We hypothesize that the discrepancy between age and marker estimated age (1- correlation efficient) is due to noise in the measure and to differences in biological age.

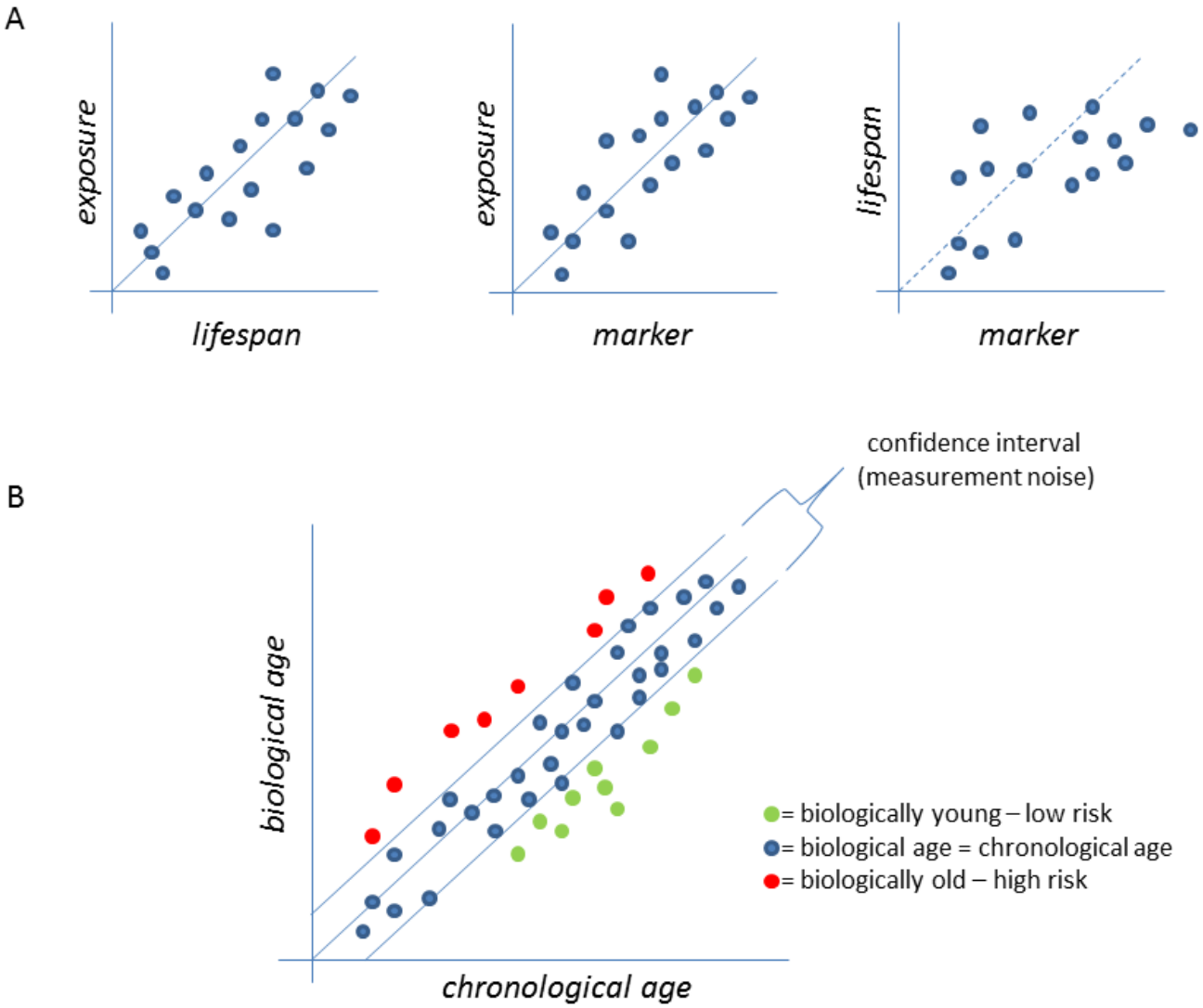

Figure 1 Markers for biological age determination. (A) Exposure to environmental risk factors correlates with lifespan and with exposure markers. Since these correlations are not perfect and since correlations are not transitive, exposure markers do not necessarily correlate with lifespan. It is therefore necessary to develop markers that directly correlate with life expectancy. (B) The concept of biological versus chronological age. Markers of biological age correlate with chronological age, yet single subjects show some variation. Within a confidence interval, variation must be considered "noise,"; above and beneath this confidence interval we find subjects who show discordance between biological and chronological age. This discordance is expected to be associated with risk for age-related diseases and longevity.

4. Forensic Markers of Chronological Age

We propose here the introduction of chronological age forensic markers for the determination of biological age in a manner that is as much as possible independent of the processes that limit lifespan by increasing the risk for specific diseases. Recently, forensic science has introduced new markers that can be analyzed by minimally invasive methods in a cost-effective manner.

4.1 Telomere Length

Telomere length has been associated with the replicative history of human cells [48] as well as with aging and longevity [49, 50], but this association is still not clear from the epidemiological point of view [51]. The relationship between cell division and net alteration in telomere length is not a constant one and distinct patterns of telomere length change can predominate [52]. Telomere length depends on the activity of the enzyme that replicates telomeres, the telomerase, which is present with variable activities in the population [53]. Telomere length can be determined through a luminescence-based hybridization method after DNA restriction [54]. More recently, polymerase chain reaction (PCR)-based assays have been introduced [55, 56].

4.2 Signal Joint T-Cell Receptor Excision Circles

Human individual age can accurately and reliably be estimated through the analysis of T-cell DNA rearrangements in blood cells [57, 58]. T-lymphocytes express T-cell receptors (TCR) that are adapted for the specific recognition of antigens through DNA-rearrangements. In this process, intervening DNA sequences in the TCR loci are deleted and circularized into episomal DNA molecules, also called signal joint TCR excision circles (sjTRECs) [59]. The δRec-ψJα sjTREC arises through an intermediate rearrangement in the TCRD/TCRA locus in developing TCRαβ+ T-lymphocytes. The number of sjTRECs declines in a log-linear fashion with increasing human age, as a consequence of thymus involution that starts shortly after birth [60]. sjTRECs thus correlate with age although there is some influence of diseases and their therapies that alter thymic function and eventually lead to thymic regeneration [61, 62]. SjTREC levels can be monitored on archival DNA by a simple Taqman qPCR protocol in which the sjTREC DNA is amplified in comparison to the single copy albumin (ALB) gene following the protocol described by Zubakov et al. [57]. Figure 2 shows the analysis of sjTRECs for 82 healthy bone marrow donors collected at the Galliera Hospital in Genova, Italy. The inverse correlation of the relative amount of sjTREC DNA molecules with chronological age is evident (r^2= 0.63); however, it is also clear that the values for single individuals greatly scatter around the central diagonal of perfect correlation.

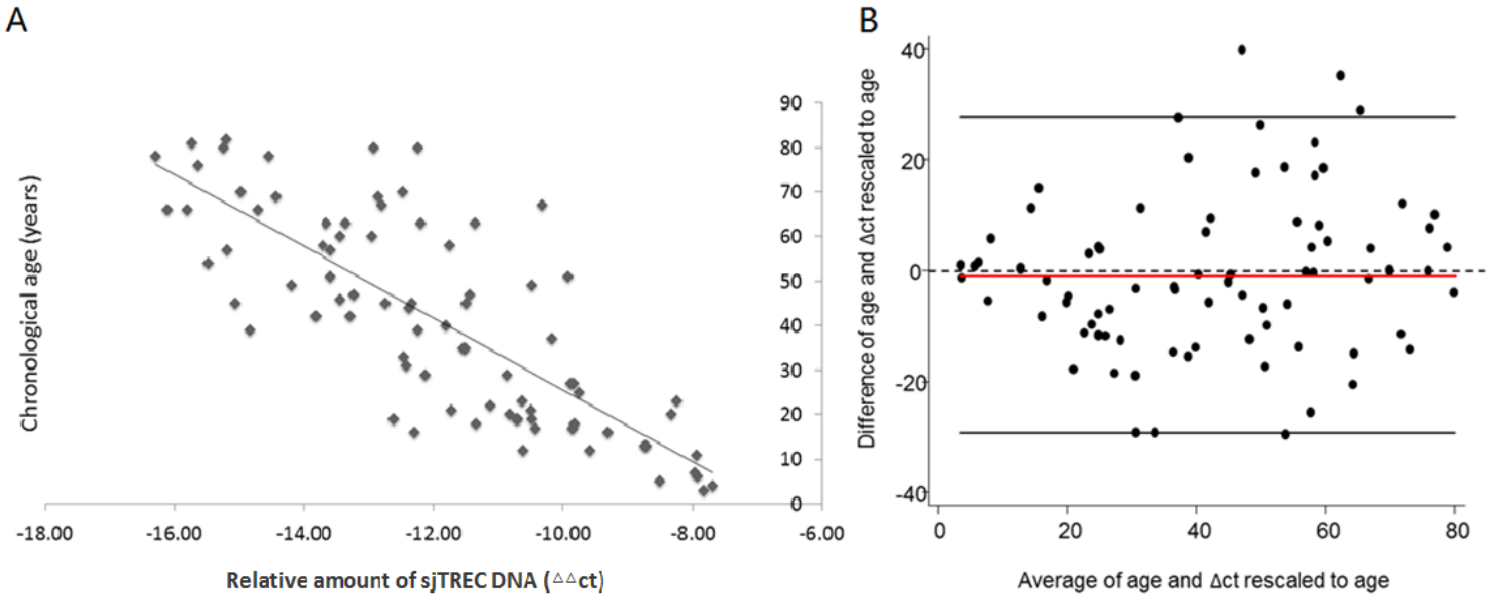

Figure 2 sjTREC as a marker of biological age. (A) Linear regression analysis shows an inverse correlation between sjTREC molecules, as determined by semi-quantitative polymerase chain reaction performed as described by Zubakov et al. [57], and chronological age (linear regression: coeff. = -8.04, p <0.001, r^2 = 0.63). Data were obtained from DNA samples of 82 healthy bone marrow donors, ranging from 18 to 55 years of age. For data points related to those under 18 and over 55 years of age, samples are obtained from donors' relatives or from patients tested for HLA-related pathology susceptibility upon informed consent. Considerable deviation from a perfect correlation is observed for many subjects likely indicating discordance between biological and chronological age. Similar correlations are expected for the other markers discussed that should be combined to determine biological age. (B) Exploratory analysis of agreement, by means of Bland-Altman plot, between chronological age and ΔCT (relative amount measured by qPCR) of sjTREC, rescaled to age. The mean difference (red line) is -0.86 (95%CI: -3.99, 2.27), the correlation between difference and mean values is not significant (Pitman's Test of difference in variance: r=.16, p=.2). A larger difference is strongly noticeable at the center of the distribution, while at the borders (youngest and oldest subjects), the difference between chronological age and ΔCT seems to be lower. Limits of agreement (reference range for difference): -29.330 to 27.613; mean difference: -0.86 (CI -3.99 to 2.27); range: 3.5 to 80.

4.3 DNA Methylation and Transcription

Biological aging is a consequence of developmental programs and organ maintenance that imply, or may even rely on, DNA methylation events. Hence, DNA methylation can be used as an age estimator. Although the precise mechanisms that link aging and DNA methylation are not entirely clear, intracellular alterations leading to a loss of cellular identity and alterations affecting the number and viability of stem cells and DNA methylation are thought to be interrelated. DNA

methylation therefore is an independent marker of all types of aging [63, 64]. Several sets of age-associated DNA methylation markers have been developed [65-71] and the association of DNA methylation determined biological age with human diseases has been shown [71-77]. DNA methylation appears to outperform other markers of biological age [46]. The biological "clocks" developed by Levine [71], Horvath [68], Hannum [63], and their co-workers are based on 513, 353 and 71 methylation sites (CpGs), respectively.

Less numerous panels that have been developed for forensic science appear more practical for routine use. Zubakov's team has recently described the human age estimation using DNA methylation in cells from peripheral blood [58]. They identified 75 significantly differentially methylated CpG sites [58] in addition to the already well validated markers in the ELOVL2 and FHL2 genes [78, 79]. Yi and colleagues identified several age-associated DNA-methylation markers and developed a multigene score that strongly correlated with chronological age in the cohort analyzed (r=0.96) [80]. DNA-methylation levels can be assessed by differential digestion by methylation sensitive restriction enzymes or, more accurately, by bisulfite (pyro-) sequencing [81]. A high throughput sequencing approach combined with machine learning for age prediction has allowed for the identification of 16 age-associated methylation markers with a mean error of 3.8 years. Marioni and co-workers established a multigene DNA-methylation signature that is strongly associated with aging [82] and could be validated in independent cohorts [74].

Methylation can directly affect gene expression and it is possible to exploit their inverse correlation to develop a transcriptomic signature that also contemplates the expression of genes with differential, age-associated methylation [83]. The age-associated gene expression signature was shown to be associated with biological features linked to aging, such as blood pressure, cholesterol levels, fasting glucose, and body mass index [83]. Yet in contrast to the aforementioned measures, transcriptomics requires isolation of RNA that is much less stable than DNA and therefore presents a logistic challenge in everyday practice (RNase free environment and procedures).

4.4 SNVs

Many studies have addressed genetic determinants of longevity and genome-wide association studies have identified many single nucleotide variants (SNV) that are associated with long life expectancy. Several of these SNVs have been confirmed in thorough validation studies [23-25]. A variant of the telomerase RNA component (TERC, rs3772190) has been found to be associated with leukocyte telomere length and longevity [26]. We hypothesize that the association with longevity might be determined by slower aging for the carriers of the longevity alleles. Hence, these alleles would also be associated with a lower biological age, yet this has not been shown so far. This hypothesis is sustained by the finding that the difference between biological and chronological age appears to develop gradually. Its accumulation determines longevity and reflects lifetime extending interventions such as dietary restriction [84].

4.5 Immunosenescence and Inflamm-Aging

Interleukin-6 (IL-6), a pro-inflammatory and pro-angiogenic cytokine, is present in the plasma at higher levels in elder as compared to younger subjects [85]. Similarly, other pro-inflammatory cytokines, coagulation factors, homocysteine, acute phase proteins, stress hormones, reactive

oxygen species, and lipoprotein-A are present at elevated levels in the elderly [20]. Inflammation plays a crucial role in almost all age-related diseases, like cancer, atherosclerosis, cardiovascular disease, type II diabetes, and neurodegeneration, yet inflammation markers, in particular, IL-6, are present at elevated levels even in healthy centenarians. IL-6 or other inflammation markers could therefore contribute to the definition of biological age but they are too heavily influenced by the actual inflammation state of the subjects, for example infections, to yield a reliable marker.

4.6 Other Markers of Aging

Immunoglobulins (Ig) are post-translationally modified by glycosylation that regulates their function. After analyzing a cohort of over 5,000 subjects, Kristic and colleagues described an alteration of the complex pattern of immunoglobulin glycosylation associated with age that explained 58% of the variation in chronological age [86]. Ig glycosylation affects Ig function, thus contributing to immunosenescence [87]. The sensitivity of this method allows for age determination using bloodstains [88]. The analysis of the gut microbiome allowed for the identification of bacterial patterns associated with biological age [89] that might reflect age-dependent decline in gastro-intestinal functions [90].

5. Conclusions

The introduction of biological age instead of chronological age could affect medical decisions that rely on age thresholds. Age is associated with risk for disease, and preventative measures like diagnostic procedures or pharmacological therapy are usually applied in consideration of the persons' age. Age as a risk factor is mediated by an age-related performance that varies within a given age group. Biological age is therefore expected to be more closely associated with risk.

Guidelines for mammographic screenings to prevent metastasization of breast cancer generally recommend screening of women over 50 years of age and restrict screening of women between 40 and 49 to those with additional risk factors [91]. Pharoah et al. have proposed the introduction of genetic risk factors to personalize screening programs [92, 93]. Introduction of biological instead of chronological age could further personalize mammographic screenings and it could do so in a cost-neutral manner since the inclusion of women under 50, yet "older" than their chronological age, would likely be compensated by women over 50 that are "younger" than their age. At the same time, the astonishingly limited effect of mammographic screening [94] could be enhanced because of a risk-oriented personalization. The same would apply to other screening programs such as colonoscopy for colorectal or prostate cancer prevention as well as pharmacological prevention such as low-dose NSAIDS for cardiovascular disease prevention. At the same time, biological age could become a powerful tool for the physician to induce changes in the lifestyles and diets of patients who, in the absence of acute disease, present an increased biological age. It is therefore justified to think that in the future, clinical decision making can replace chronological age by the assessment of the biological age of patients.

Author Contributions

All authors contributed to the conceptual development and read, revised an approved the final manuscript. AC, AA, BB, MC performed pilot-experiments to sustain the hypothesis, MP analyzed

data and performed simulations, GAR, NS and UP supervised the discussion, UP wrote a draft manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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