Epigenetic ageing accelerates before antiretroviral therapy and decelerates after viral suppression in people with HIV in Switzerland: a longitudinal study over 17 years

Isabella C Schoepf, Andrés Esteban-Cantos*, Christian W Thorball*, Berta Rodés, Peter Reiss, Javier Rodríguez-Centeno, Carlotta Riebensahm, Dominique L Braun, Catia Marzolini, Marco Seneghini, Enos Bernasconi, Matthias Cavassini, Hélène Buvelot, Maria Christine Thurnheer, Roger D Kouyos, Jacques Fellay, Huldrych F Günthard, José R Arribas*, Bruno Ledergerber*, Philip E Tarr*, and the Swiss HIV Cohort Study*

Summary

Background Accelerated epigenetic ageing can occur in untreated HIV infection and is partially reversible with effective antiretroviral therapy (ART). We aimed to make a long-term comparison of epigenetic ageing dynamics in people with HIV during untreated HIV infection and during suppressive ART.

Methods In this longitudinal study, conducted over 17 years in HIV outpatient clinics in Switzerland, we applied 5 established epigenetic age estimators (epigenetic clocks) in peripheral blood mononuclear cells (PBMCs) in Swiss HIV Cohort Study participants before or during suppressive ART. All participants had a longitudinal set of PBMC samples available at four timepoints (T1–T4). T1 and T2 had to be 3 years or longer apart, as did T3 and T4. We assessed epigenetic age acceleration (EAA) and a novel rate of epigenetic ageing.

Findings Between March 13, 1990, and Jan 18, 2018, we recruited 81 people with HIV from the Swiss HIV Cohort Study. We excluded one participant because a sample did not meet quality checks (transmission error). 52 (65%) of 80 patients were men, 76 (95%) were white, and the median patient age was 43 (IQR 37·5–47) years. Per year of untreated HIV infection (median observation 8·08 years, IQR 4·83–11·09), mean EAA was 0·47 years (95% CI 0·37 to 0·57) for Horvath's clock, 0·43 years (0·3 to 0·57) for Hannum's clock, 0·36 years (0·27 to 0·44) for SkinBlood clock, and 0·69 years (0·51 to 0·86) for PhenoAge. Per year of suppressive ART (median observation 9·8 years, IQR 7·2–11), mean EAA was –0·35 years (95% CI –0·44 to –0·27) for Horvath's clock, –0·39 years (–0·50 to –0·27) for Hannum's clock, –0·26 years (–0·33 to –0·18) for SkinBlood clock, and –0·49 years (–0·64 to –0·35) for PhenoAge. Our findings indicate that people with HIV epigenetically aged by a mean of 1·47 years for Horvath's clock, 1·43 years for Hannum's clock, 1·36 years for SkinBlood clock, and 1·69 years for PhenoAge per year of untreated HIV infection; and 0·65 years for Horvath's clock, 0·61 years for Hannum's clock, 0·74 years for SkinBlood clock, and 0·51 years for PhenoAge, per year of suppressive ART. GrimAge showed some change in the mean EAA during untreated HIV infection (0·10 years, 0·02 to 0·19) and suppressive ART (–0·05 years, –0·12 to 0·02). We obtained very similar results using the rate of epigenetic ageing. Contribution of multiple HIV-related, antiretroviral, and immunological variables, and of a DNA methylation-associated polygenic risk score to EAA was small.

Interpretation In a longitudinal study over more than 17 years, epigenetic ageing accelerated during untreated HIV infection and decelerated during suppressive ART, highlighting the importance of limiting the duration of untreated HIV infection.

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*Contributed equally

Department of Infectious Diseases, Bern University Hospital (I C Schoepf MD, C Riebensahm MD, M C Thurnheer MD) **and Graduate School of Health Sciences** (C Riebensahm), **University of Bern, Bern, Switzerland; University Department of Medicine and Infectious Diseases Service, Kantonsspital Baselland, University of Basel, Bruderholz, Switzerland** (I C Schoepf, Prof P E Tarr MD)**; HIV/AIDS and Infectious Diseases Research Group, Hospital La Paz Institute for Health Research, Madrid, Spain** (A Esteban-Cantos PhD, B Rodés PhD, J Rodríguez-Centeno PhD, Prof J R Arribas MD)**; CIBER of Infectious Diseases, Madrid, Spain** (A Esteban-Cantos, B Rodés, J Rodríguez-Centeno, Prof J R Arribas)**; Precision Medicine Unit, Centre hospitalier universitaire vaudois** (C W Thorball PhD, Prof J Fellay MD)**; Infectious Diseases Service, Lausanne University Hospital University of Lausanne, Switzerland** (Prof M Cavassini MD)**; School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Switzerland** (C W Thorball, Prof J Fellay)**; Amsterdam UMC, location University of Amsterdam, Global Health, Amsterdam, Netherlands** (Prof P Reiss PhD)**; Amsterdam Institute for Global Health and Development, Amsterdam, Netherlands** (Prof P Reiss)**; Department of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland** (D L Braun MD,

Introduction

A major concern in people with HIV is an increased rate of ageing-associated comorbidities compared with HIV-negative people.[1] Because people age at different rates, and because ageing can be accelerated or accentuated in people with HIV, there is considerable interest in distinguishing between chronological age, calendar years elapsed since birth, and biological age, which reflects a person's physiological functions and overall state of health.[2] Research in the general population suggests that ageing-associated biomarkers in blood leucocyte samples (telomere length and epigenetic changes, ie, DNA methylation) are powerful tools for prediction of ageing-associated diseases and of lifespan and DNA methylation seems to be the most precise readout of biologic age currently available.[3]

Different so-called epigenetic clocks have been developed, based on algorithms that take into account changes in DNA methylation at various CpG positions to predict epigenetic age as a surrogate measure of biological age.[3] The first-generation epigenetic clocks, of which the most extensively investigated is Horvath's clock,[4] were originally trained to determine chronological age. Second-generation clocks were developed integrating both DNA

Prof R D Kouyos PhD, Prof H F Günthard MD, Prof B Ledergerber PhD); **Institute of Medical Virology** (Prof R D Kouyos, Prof H F Günthard), **University of Zurich, Zurich, Switzerland; Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland** (Prof C Marzolini PhD); **Division of Infectious Diseases, Kantonsspital St Gallen, St Gallen, Switzerland** (M Seneghini MD); **Division of Infectious Diseases, Ente Ospedaliero Cantonale, University of Geneva and University of Southern Switzerland, Lugano, Switzerland** (Prof E Bernasconi MD); **Division of Infectious Disease, Geneva University Hospital, Geneva, Switzerland** (H Buvelot MD)

Correspondence to: Prof Philip E Tarr, University Department of Medicine and Infectious Diseases Service, Kantonsspital Baselland, University of Basel, 4101 Bruderholz, Switzerland
philip.tarr@unibas.ch

See Online for appendix

Research in context

Evidence before this study

Epigenetic clocks are powerful tools for prediction of ageing-associated diseases and of lifespan in the general population; however, there are little data on the effects of untreated HIV infection and antiretroviral therapy (ART) on epigenetic ageing. We searched PubMed for reports published in English from database inception to June 28, 2021, using combinations of the following keywords: "HIV infection", "antiretroviral therapy", "epigenetic ageing", "epigenetic clocks", "epigenetic age acceleration" and "rates of ageing". We identified three studies showing untreated HIV infection is associated with a significant increase in epigenetic ageing. In addition, we authored two studies that described early positive effects of ART on immune cell reconstitution and epigenetic ageing with observation periods of up to 4 years of viral suppression.

Added value of this study

To our knowledge, our study is the first to investigate epigenetic ageing dynamics in the same people with HIV with longitudinal samples available during more than 8 years of untreated HIV infection and during almost 10 years of suppressive ART. Our results support evidence that untreated HIV infection is accompanied by a large increase in epigenetic ageing. In addition, we documented a significant decrease in epigenetic ageing during suppressive ART, which is sustained over a long period of almost 10 years. We identified a modest contribution of multiple HIV-related, immunological variables, and host genetics to the substantial increase and decrease in epigenetic ageing, which suggests that uncontrolled HIV infection is a major driver of accelerated ageing in people with HIV, and that suppressive ART is the key intervention that can powerfully influence the biological ageing process.

Implications of all the available evidence

By exploring epigenetic ageing in people with HIV and an effective pharmacological intervention, ie, suppressive ART, a better biological understanding of the ageing process can be obtained. More evidence is needed to stratify people with HIV according to their risk for major age-related outcomes, and future research should focus on studying a potential association of epigenetic age acceleration with hard clinical endpoints in the unique setting of HIV.

methylation data and clinical information to predict disease endpoints and mortality more accurately.[5,6] Epigenetic clocks have become attractive biomarkers because the difference between measures of epigenetic age and chronological age, also known as epigenetic age acceleration (EAA), can predict ageing-related comorbidities and mortality in the general population.[7]

Previous cross-sectional studies have reported that people with HIV have more EAA than do individuals without HIV.[2,8,9] We previously documented a reduction in EAA during the first 96 weeks of antiretroviral therapy (ART)[10] and no EAA increase during suppressive ART over a 4-year follow-up period,[11] suggesting that ART has a positive effect on EAA. Similarly, in a longitudinal study over 17 years in 107 people with HIV serving as their own controls, we recorded significant telomere length shortening during untreated HIV infection and no evidence of any telomere length change during suppressive ART.[12] The aim of the current study was to investigate epigenetic ageing dynamics in the same people with HIV with longitudinal samples available during more than 8 years of untreated HIV infection and during almost 10 years of suppressive ART.

Methods

Study design and participants

We used the same participants, samples, and timepoints as in our previous longitudinal telomere length study, for whom detailed inclusion and exclusion criteria were previously reported.[12] Eligible participants had a longitudinal set of peripheral blood mononuclear cells (PBMCs) samples available at four timepoints (T1–T4, figure 1; table 1). T1 and T2 had to be 3 years or longer apart, as did T3 and T4.[12] All HIV RNA values measured between T3 and T4 had to be less than 100 copies per mL. We excluded elite controllers (defined as participants with all HIV RNA values <100 copies per mL).

This study was approved by the local ethics committees of the participating centres (appendix p 1). Participants provided written informed consent including for genetic testing.

Procedures

We measured DNA methylation and calculated EAA at T1 (first available sample before ART start), T2 (last available sample before ART start), T3 (first sample at which HIV RNA was <20 copies per mL) and T4 (most recent available sample during suppressive ART [defined as <100 copies per mL). We explored epigenetic ageing dynamics during the untreated HIV infection phase (T1–T2), the suppressive ART phase (T3–T4), and the transition phase (T2–T3; roughly the time from ART start until viral suppression was attained). We obtained clinical, immunological, and HIV-related variables to evaluate their association with the rate of epigenetic ageing in each study phase including chronological age (in tertiles); sex; smoking status (current, past, or never); HIV viraemia (maximum $\log_{10}$ HIV RNA); CD4 cell count; CD8 cell count; CD4:CD8 ratio at the beginning of each phase (in tertiles); annualised change in CD4 cell count, CD8 cell count, and CD4:CD8 ratio during each phase; cytomegalovirus seropositivity; and hepatitis C seropositivity.

Genomic DNA from cryopreserved PBMCs was purified by use of the NucleoSpin Blood kit (Macherey-Nagel, Dueren, Germany) according to the manufacturer's instructions. Genome-wide DNA methylation patterns

were assessed by use of the Infinium MethylationEPIC BeadChips (Illumina; San Diego, CA, USA) following the manufacturer's instructions. All experiments were done by the Genomics Service of Life&Brain (Bonn, Germany). Raw methylation data were processed and background corrected by use of the noob normalisation method implemented in the minfi R package.[13] All samples were required to satisfy the quality control criteria established in the minfi package. We analysed five different epigenetic clocks: three were first-generation clocks (Horvath's Pan-tissue clock,[4] Hannum's clock,[14] and SkinBlood clock,[15] which predict chronological age based on the DNA methylation levels of 353, 71, and 391 CpG positions, respectively) and two second-generation clocks (PhenoAge and GrimAge). PhenoAge estimates a measure of phenotypic age based on DNA methylation levels at 513 CpG sites;[5] GrimAge is a composite biomarker derived from DNA methylation-based surrogates of seven plasma proteins and smoking pack-years that predicts a time-to-death variable (due to all-cause mortality).[6] Measures of epigenetic age for each estimator were generated after uploading processed DNA methylation data to the Horvath's New Online Methylation Age Calculator software.[4]

Genotyping was done by use of the Global Screening Array v2.0+MD (Illumina, San Diego, CA) or, in the setting of previous Swiss HIV Cohort Study (SHCS) genetic studies using DNA samples obtained from stored PBMCs. All quality control, imputation, and filtering steps were done separately for each batch of samples before merging as described (appendix p 1). Only single nucleotide polymorphisms with a minor allele frequency of more than 1% and missingness of less than 10% in the final merged dataset were retained for the polygenic risk score (PRS) calculations.

Both the European ancestry-specific (EUR) and the trans-ethnic (MRMEGA) PRS for Hannum's clock, PhenoAge, and GrimAge were calculated by use of the summary statistics from the genome-wide association studies by McCartney and colleagues.[16] Summary statistics for Horvarth's clock were not available, only the derivate clock intrinsic EAA. The PRSs were calculated by use of PRSice (version 2.3.5) with all available variants and their respective weights (p=1) with default clumping parameters (r^2=0·1). Because of the low number of samples included in this study, a linkage disequilibrium reference panel consisting of 1675 other SHCS samples were used during clumping. The DNA methylation-PRS included 135.700 (EUR) and 109.946 (MRMEGA) single nucleotide polymorphisms after clumping.

Statistical analysis

We implemented two different measures to explore the effect of HIV on epigenetic ageing. To assess the individual trajectories of epigenetic ageing over time (during untreated HIV infection and during suppressive ART) we calculated EAA, for every sample and epigenetic clock, as the residual result from regressing epigenetic age on chronological age data. Because of the longitudinal nature of data, EAA measures were obtained by use of mixed-effect linear regression models with random intercepts at the individual level.[12,17] Therefore, a positive value of an EAA measure means that epigenetic age is higher than predicted based on chronological age. To assess the longitudinal dynamics of epigenetic ageing in each study phase, we calculated a rate of epigenetic ageing (R), defined as the ratio of the difference between epigenetic age at T(n) and at T(n–1) to the difference between chronological age for the same visits, as reported by Sehl and colleagues.[18]

$$\textit{Rate of epigenetic ageing (R)} = \frac{\textit{epigenetic age (follow up time point n)} - \textit{epigenetic age (baseline time point n-1)}}{\textit{chronological age (follow up time point n)} - \textit{chronological age (baseline time point n-1)}}$$

By this definition, R=1 means that epigenetic age increases 1 year per chronological year. R greater than 1

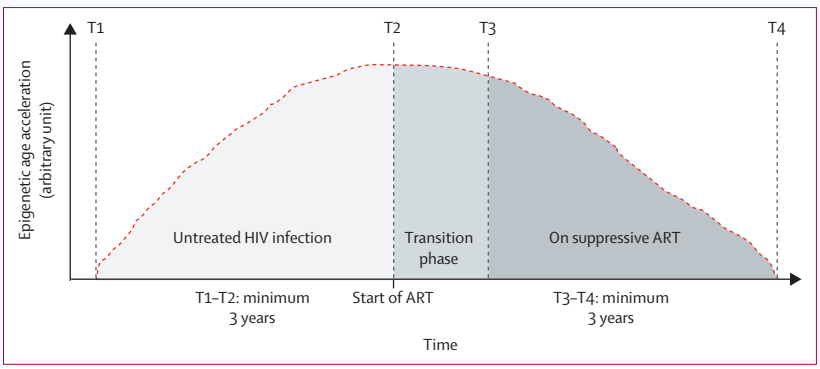

Figure 1: **Study hypothesis and timepoints T1–T4 for measurement of DNA methylation**
Median date was Aug 19, 1997, at T1 (IQR Dec 6, 1994, to May 1, 1999), March 21, 2006, at T2 (March 16, 2004, to Feb 12, 2008), June 1, 2007, at T3 (March 17, 2006, to Jan 26, 2010), and April 11, 2017, at T4 (Feb 14, 2017, to June 27, 2017). In each participant, we measured epigenetic age acceleration in the first available sample before ART start (T1) and in the last available sample before ART start (T2), in the first available sample after attaining viral suppression (T3), and in the last available sample during suppressive ART (T4). The light grey shaded area indicates the transition phase from ART start until viral suppression was attained (T2 to T3). The dark grey area indicates the suppressive ART phase (T3 to T4). ART=antiretroviral therapy.

	T1–T2	T2–T3	T3–T4
CD4 cell count, cells per µL	517·5 (367·0 to 678·5)	294·0 (224·0 to 362·5)	428·5 (320·5 to 554·0)
CD4 annual change, cells per µL	–27·0 (–51·6 to –13·1)	76·6 (26·7 to 145·7)	26·6 (9·8 to 38·0)
CD8 cell count, cells per µL	762·0 (590·5 to 951·0)	941·5 (675·0 to 1232·5)	775·5 (597·0 to 1008·5)
CD8 annual change, cells per µL	21·5 (–14·6 to 53·1)	–73 (–244·4 to 25·2)	–7·4 (–29·8 to 10·1)
CD4:CD8 ratio	0·7 (0·4 to 1·0)	0·3 (0·2 to 0·4)	0·5 (0·4 to 0·8)
CD4:CD8 ratio annual change, cells per µL	–0·04 (–0·09 to –0·03)	0·15 (0·06 to 0·23)	0·05 (0·02 to 0·06)
HIV RNA, log copies per mL	4·92 (4·57 to 5·31)	4·86 (4·34 to 5·24)	1·65 (1·53 to 1·78)

Data are median (IQR).

Table 1: **Immunovirological and HIV-related status in each phase**

indicates that epigenetic ageing is advancing faster than chronological ageing, and R less than 1 indicates that epigenetic ageing is advancing more slowly than chronological ageing.

We visualised EAA trajectories with individual spaghetti plots and linear regressions for each of the three phases. Mixed-effects multilevel regression with random intercept and slope of EAA were applied for the two phases before and during suppressive ART. Results were visualised as linear predictions from average marginal effects. We also present linear predictions from average marginal effects for receiving ART by sex and from models including three phases. For the three-phase predictions, we had to choose average timings of T1–T4 which resulted in discontinuities of lines at T2 and T3. Rates of epigenetic ageing for each clock were averaged for each phase and presented with 95% CIs. We then assessed effect modifications by including the different levels of the 12 clinical variables in univariable linear regression for each phase and clock. Results are presented graphically with significant variables highlighted before and after Bonferroni correction for multiple testing (appendix pp 6–9). Normal distributions were checked with graphical methods and Shapiro-Wilk W tests. Data management and analyses were done with Stata/SE 17.0 (StataCorp, College Station, Texas, USA).

We calculated slopes of clocks (EAA per year) separately between T1 and T2 and between T3 and T4, and assessed associations of EAA slope with HIV-related events (captured in SHCS since 1988), comorbidities (captured in SHCS since 2000), and with cumulative exposure to the major three drug classes (boosted protease inhibitors, non-nucleoside reverse transcriptase inhibitors, and integrase inhibitors) until T4.

	Study population (n=80)
Sex	
Male	52 (65%)
Female	28 (35%)
Age, years	43 (37·5–47)
Ethnicity	
White	76 (95%)
Black	1 (1·3%)
Hispanic	1 (1·3%)
Asian	2 (2·5%)
Mode of HIV transmission	
Heterosexual	34 (42·5%)
Men who have sex with men	27 (33·8%)
Injection drug use or other	19 (23·8%)
BMI, kg/m^2	22·9 (20·9–25·9)
Diabetes	2 (2·5%)
Hypertension	16 (20%)
Dyslipidaemia	42 (52·5%)
Alcohol†	
None or light	60 (75%)
Moderate or severe	20 (25%)
Cocaine use (any in previous 6 months)	2 (2·5%)
Estimated duration of HIV infection at ART start, years	15·4 (11·4–20·1)
Smoking	
Never	21 (26·3%)
Current	45 (56·3%)
Past	14 (17·5%)
Cytomegalovirus seropositivity	59 (73·8%)
Hepatitis C virus seropositivity	20 (25%)
Cumulative exposure to boosted protease inhibitors during phases 2 and 3, years	0·87 (0–7·22)
Cumulative exposure to non-nucleoside reverse transcriptase inhibitors during transition phase and on suppressive ART phase, years	3·58 (0–8·69)
Cumulative exposure to integrase inhibitors during transition phase and on suppressive ART phase, years	0·23 (0–7·22)

Data are n (%) or median (IQR). SHCS=Swiss HIV Cohort Study. *Defined as the last SHCS routine visit before or at ART start. †Defined as none or mild versus moderate or heavy as less or more than 40 g (men) or 20 g (women) until 2012 and using the AUDIT-C questionnaire from 2013. ART=antiretroviral therapy.

Table 2: **Characteristics of study participants at baseline***

Role of the funding source

The funders had no role in study design, data collection, analysis, interpretation, or writing of the report.

Results

Between March 13, 1990, and Jan 18, 2018, we assessed 81 participants for eligibility. We excluded one participant because a sample did not meet quality checks (transmission error). We recruited and analysed 80 participants with stored PBMC samples available at the four timepoints (figure 1 and table 1), ie, a total of 320 DNA methylation measurements. At baseline (last routine HIV clinic visit before or at ART start) 65% of participants were men, 95% were white, and the median patient age was 43 years (table 2). The median interval was 8·08 years (IQR 4·83–11·09) between timepoints T1 and T2 (untreated HIV infection), 1·35 years (1·01–2·25) between T2 and T3 (transition phase), and 9·81 years (7·16–11·01) between T3 and T4 (suppressive ART).

As shown in figure 2, observed EAA trajectories showed considerable intraindividual and interindividual variability, and EAA variability (EAA amplitude on the y-axis) seems largest during the transition phase. All clocks except for GrimAge show an EAA increase during untreated HIV infection, a steep EAA decline immediately after ART start, and a lesser EAA decline during suppressive ART.

We show predicted EAA results for each epigenetic clock during untreated HIV infection and during suppressive ART (appendix p 4). During untreated HIV infection, the mean annualised EAA was 0·47 years according to Horvath's clock (95% CI 0·37 to 0·57),

0·43 years according to Hannum's clock (0·30 to 0·57), 0·36 years according to SkinBlood clock (0·27 to 0·44), 0·69 years according to PhenoAge (0·51 to 0·86), and 0·10 years according to GrimAge (0·02 to 0·19). During suppressive ART, the mean annualised EAA was –0·35 years according to Horvath's clock (–0·44 to –0·27), –0·39 years according to Hannum's clock (–0·50 to –0·27), –0·26 years according to SkinBlood clock (–0·33 to –0·18), –0·49 years according to PhenoAge (–0·64 to –0·35), and –0·05 years according to GrimAge (–0·12 to 0·02). We found no evidence of any difference between female and male participants in predicted EAA during untreated HIV infection and suppressive ART (appendix pp 2–3). When we added the transition phase to the prediction models, the beneficial effect on EAA appears to start with ART initiation, ie, before viral suppression is attained. Furthermore, EAA benefit appears quantitatively larger during the transition phase than during the suppressive ART phase (appendix pp 10; figure 3). Again, GrimAge showed little EAA change over time, and PhenoAge exhibited the largest changes.

Using the rate of epigenetic ageing (R),[18] results were qualitatively and quantitatively similar to results obtained using EAA measures: mean R was greater than 1 during untreated HIV infection for all epigenetic clocks, and mean R was less than 1 during the transition phase and during suppressive ART, indicating an acceleration of epigenetic ageing during untreated HIV infection and deceleration of epigenetic ageing after ART initiation and viral suppression (figure 4, appendix pp 5, 11). For example, according to PhenoAge, participant annualised mean R was 2·38 (2·09 to 2·67) during untreated HIV infection, –4·23 (–5·47 to –3·0) during the transition phase, and 0·72 (0·55 to 0·89) during suppressive ART.

We found little effect modification by 12 clinical ageing-associated variables, and inconsistent results between individual epigenetic clocks (appendix pp 6–7). After Bonferroni correcting for multiple testing, during untreated HIV infection, there was a trend for an association of Horvath-R greater than 1 with greater annualised increase in CD8 cells. In the transition phase, Horvath-R less than 1 was associated with female sex; and there was a trend for an association with PhenoAge-R less than 1 and greater annualised CD4 increase. During suppressive ART, none of the clinical co-factors remained significantly associated with any rate of epigenetic ageing, after correction for multiple testing (appendix pp 6–7).

During untreated HIV infection, there were 32 HIV-related events, but comorbidities were not yet reliably captured in the SHCS database (appendix p 12). Only PhenoAge EAA acceleration and GrimAge EAA acceleration were associated with HIV-related events (appendix p 14). During suppressive ART, there were four HIV-related events (appendix p 13) and 16 comorbidities (appendix p 15). Only Horvath EAA deceleration was associated with HIV-related events (appendix p 14). We found no consistent evidence for any association of EAA with cumulative exposure to boosted protease inhibitor (297·5 person-years exposure), non-nucleoside reverse transcriptase inhibitors (364 person-years) and integrase inhibitors (100·1 person-years). Only PhenoAge EAA deceleration was significantly associated with cumulative non-nucleoside reverse transcriptase inhibitors exposure (appendix p 16).

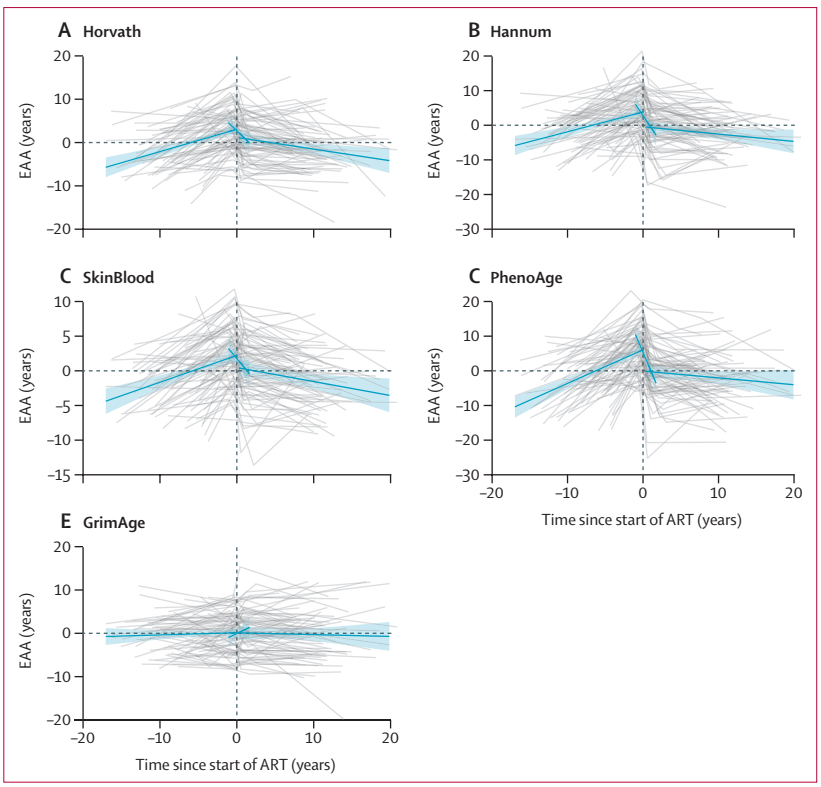

Figure 2: **EAA over time for different epigenetic clocks**
Spaghetti plots combine data for the phase of unsuppressed HIV infection (T1 to T2), transition phase (T2 to T3), and during suppressive ART (T3 to T4). The blue line corresponds to the solid linear regression line across all timepoints and the blue shaded area indicates 95% CI. EAA=epigenetic age acceleration. ART=antiretroviral therapy.

Of the 80 participants, 22 (28%) by Horvath, 28 (35%) by Hannum, 22 (28%) by SkinBlood, 34 (43%) by PhenoAge, and 28 (35%) by GrimAge showed an EAA increase despite suppressive ART, as suggested by the observed EAA trajectories (figure 2). EAA increase was associated with comorbidities for Hannum and PhenoAge, but not the other clocks (appendix p 15).

The variability of EAA in the three phases explained by host genetic background was 0–2·1% for EUR DNA methylation PRS and 0–4·9% for MRMEGA DNA methylation-PRS for the different epigenetic clocks (appendix p 17), with similar results for the rates of ageing (data not shown). EAA variability explained by DNA methylation-PRS in our study was similar to EAA variability explained by the DNA methylation-PRS in the reference paper from the general population (1·07–1·38%).[16] We found a small association between

DNA methylation-PRS (EUR and MRMEGA) and EAA according to the different epigenetic clocks in the three phases, except for a significant association of PhenoAge in the transition phase with DNA methylation-PRS MRMEGA (data not shown). Similarly, we found a small association between DNA methylation-PRS and the rates of epigenetic ageing, except for a trend for PhenoAge in the transition phase and during suppressive ART with DNA methylation-PRS MRMEGA (data not shown).

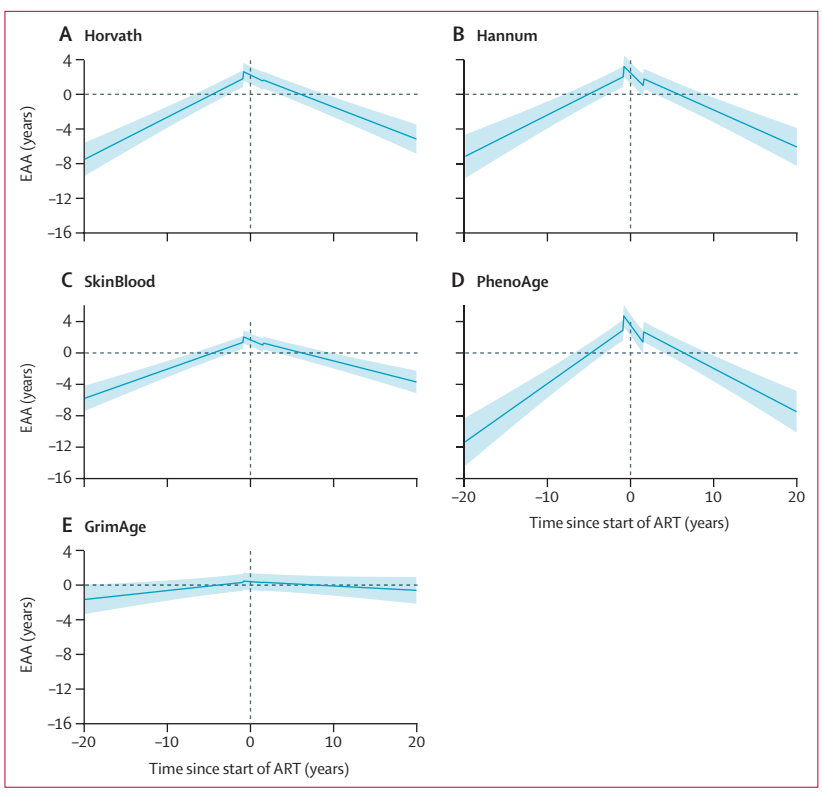

Figure 3: **EAA over time predictions for different epigenetic clocks**
Transition phase (ART start) and predicted EAA added for each of the 3 phases (untreated HIV infection, transition phase, suppressive ART phase) for the different epigenetic clocks. EAA=epigenetic age acceleration. ART=antiretroviral therapy.

Discussion

Our longitudinal study investigating epigenetic ageing in people with HIV who served as their own controls during a median duration of more than 8 years of untreated HIV infection and almost 10 years of suppressed HIV infection has three major findings. First, untreated HIV infection was accompanied by a significant increase in epigenetic ageing with each of the two approaches we applied (EAA and the rate of epigenetic ageing). This confirms and extends previous findings from studies that included observation periods of 6, 6·7, and 2·9 years of untreated HIV infection.[18–20] Second, although the early beneficial effects of ART on immune cell reconstitution[21] and EAA and R decrease are well reported in studies with observation periods up to 4 years of viral suppression,[10–11] we extend these findings by reporting a significant EAA and R decrease that is sustained during long-term, suppressive ART. Third, although our sample size was small, we identified a small contribution of multiple HIV-related variables, immunological variables, and host genetics (individual PRS for DNA methylation), to the substantial increase in epigenetic ageing during untreated HIV infection, and decrease in epigenetic ageing during suppressed HIV infection. These data suggest that uncontrolled HIV infection is a major driver of accelerated ageing in people with HIV, and thereby identifies individuals at high risk of accelerated ageing. Conversely, our long-term results confirm how suppressive ART is the key intervention that can powerfully improve the biological ageing process in people with HIV. When HIV infection is controlled by suppressive ART, the measured epigenetic age tends to equate the epigenetic age equivalent to the person's chronological age. Unfortunately, even though HIV treatment guidelines have long recommended early ART start, prolonged periods of uncontrolled HIV infection remain common, even in high-income countries: almost half of participants were late presenters when they enrolled in the SHCS (appendix p 18).

Previous evidence suggested a benefit of ART on ageing biomarkers, including a significant reversal in EAA after starting ART,[10,22,23] a telomere length increase after 96 weeks

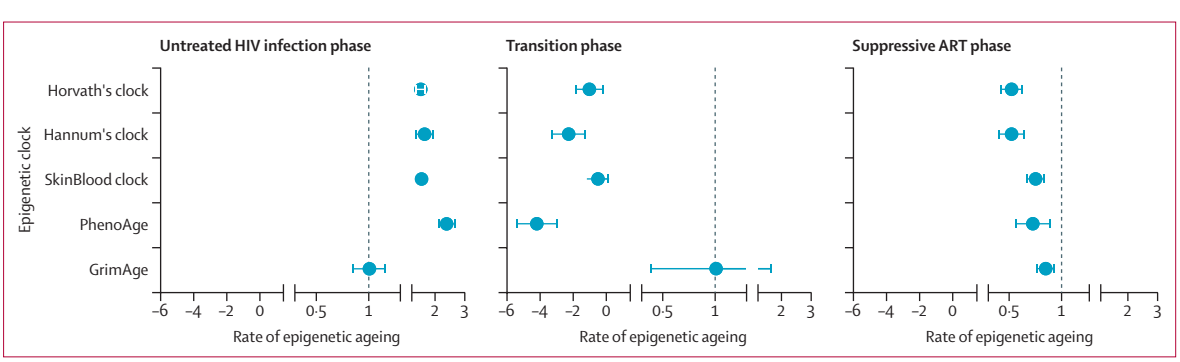

Figure 4: **Rates of epigenetic ageing for different epigenetic clocks**
Data are mean, error bars indicate 95% CI. Rate of epigenetic ageing (R) was calculated as reported by Sehl and colleagues.[18] The vertical dashed line (R=1) indicates that 1 year of epigenetic age increases per 1 year chronologically, with R greater than 1 suggesting faster or accelerated ageing, and R less than 1 suggesting slower or decelerated ageing (see appendix p 5 with x-axis adjusted to the respective scale).

of ART,[24,25] prevention of telomere length shortening by early ART in people with primary HIV Infection,[26] and stability of EAA during 4 years of suppressive ART.[11] Our study confirms and extends these reports by demonstrating, in the same individuals who served as their own controls, significant EAA and R increase during untreated chronic HIV infection and a reduction of EAA and R during almost 10 years of suppressive ART. Similarly, in the same patients and samples as analysed in the present report, we reported a median annual telomere length decrease of 2·12% during untreated HIV and no evidence of any telomere length decrease during suppressive ART.[12]

Our results appear robust, because 2 different epigenetic ageing measures (EAA and R) and 5 different epigenetic clocks gave qualitatively and quantitatively similar results. Additional strengths of our study include the extensive longitudinal study period of more than 17 years, allowing each participant to serve as their own control during phases of untreated and well controlled HIV infection. We selected extended periods between DNA methylation measurements to minimise false positive results due to assay variability or short-term intraindividual variability. Moreover, participants were selected from a well established prospective observational study, the SHCS, which has a rich database that includes extensive phenotypic and genotypic data. Except for an association of sex with Horvath-R (only during the transition phase), we found little evidence for confounding of epigenetic ageing dynamics by multiple HIV-related, immunological factors and host genetic background that can influence epigenetic age. Research into epigenetic ageing in people with HIV and research into a powerful pharmacological intervention (ie, suppressive ART) could contribute to a better biological understanding of the ageing process in people with HIV and serve as an important paradigm for general population ageing research, given the increasing prevalence of ageing-related conditions in the general population as a consequence of increasing inflammatory metabolic disorders such as obesity and diabetes. To our knowledge, ART is the only therapeutic intervention available that influences the epigenetic ageing process to a similar degree.

Our results were similar with each of the epigenetic clocks except for GrimAge. GrimAge showed only minimal changes in EAA or R during untreated HIV infection and suppressive ART. This is in line with previous studies,[11,20] which found that GrimAge performs qualitatively differently from the other epigenetic clocks in people with HIV. There might be several possible explanations for this. First, differences in the design of each epigenetic clock and the paucity of CpG overlap among them suggest that the different clocks could capture distinct features of biological ageing.[27] An investigation documented that mitochondrial activity, stem-cell composition, and nutrient sensing affect epigenetic ageing measured by SkinBlood, but other hallmarks of ageing (such as cellular senescence, telomere attrition, or genomic instability) did not.[28] However, the underlying biological mechanisms that are linked with GrimAge are incompletely defined, with a link to current smoking, BMI, and educational attainment.[17] Second, while a mendelian randomisation analysis has pointed to a causal effect of lymphocyte counts on the Hannum and GrimAge estimates,[16] we and others have previously reported that GrimAge is the epigenetic clock least affected by changes in leukocyte composition,[10,20] which is well known to be markedly altered during HIV infection. Thus, we could speculate that HIV infection might affect GrimAge early after HIV acquisition, and that these changes remain stable over time, even after many years of suppressive ART. Despite the little variation of GrimAge during HIV infection, VACS cohort investigators showed that GrimAge predicted all-cause mortality and physiological frailty better than the other epigenetic clocks, after adjusting for potential confounders.[29]

Our results are limited by sample size due to strict selection criteria[12] (each participant was required to have longitudinal samples available at four defined timepoints, each >3 years apart). Therefore, and because the SHCS only started capturing comorbidities after 2000, the small, inconsistent association of epigenetic changes with HIV-related, immunological, and genetic variables should be interpreted cautiously and validated in larger, more diverse populations. Interindividual variability in EAA dynamics was considerable, but less than our previous telomere length results.[12,26] As we measured DNA methylation only in blood, our results cannot answer the question of whether EAA detected in PBMCs reflects ageing processes of the whole person or reflects predominantly the state of immune activation in peripheral blood. Therefore, it is unknown whether the apparent rejuvenating effect of ART (reflected by negative EAA values and R values <1) can be extrapolated to other tissues. Nonetheless, a study[30] has shown how EAA correlates between 11 different tissues in the same individual; EAA measured in blood cells correlated significantly with EAA measured in the spleen and bone marrow, but less so with EAA measured in the liver.[30]

Another limitation of our results includes the small number of women, people older than 65 years, and ethnicities other than White. Results should only be extrapolated to these populations cautiously.

Finally, an important avenue for future research includes correlating the epigenetic changes observed in this study to hard clinical outcomes, such as cardiovascular events, but also healthy ageing—this requires a much larger study population and will probably be limited by the cost of DNA methylation measurement.

In conclusion, we show that EAA is significantly increased during 8 years of untreated HIV infection. By contrast, ART appears to initiate a reversal of EAA that is sustained for almost 10 years of viral suppression. The effects of untreated HIV infection and of ART on epigenetic ageing appear to be clinically relevant due to their large effect compared with the smaller effects of

selected clinical, immunological, and HIV-related variables and host genetics. Thus, ART appears to have a key beneficial effect on epigenetic ageing, and thereby could powerfully contribute to healthy ageing and mitigate concerns about accelerated ageing in people with HIV.

Contributors

ICS, AEC, BR, JRA, BL, and PET contributed to study design. ICS, CR, DLB, CM, MS, EB, MC, HB, MCT, HFG, and PET contributed to patient recruitment. AEC, BR, JRA, BL, and PET contributed to data acquisition. ICS, AEC, CWT, BR, PR, RDK, JF, JRA, BL, and PET analysed the data. ICS, AEC, CWT, BR, JRA, BL, and PET verified the underlying data and drafted the manuscript. All authors contributed to critical review and revision of the manuscript. ICS, AEC, CWT, JRA, BL, and PET had final responsibility for the decision to submit for publication.

Declaration of interests

ICS received a lecture fee to institution from ViiV, outside the submitted work. BR declares personal fees from Gilead and non-financial support from ViiV Healthcare, outside the submitted work. DLB reports honoraria paid to himself for advisory boards and lectures from Gilead, Merck, and ViiV, outside the submitted work. CM declares personal fees from ViiV, MSD, and Pfizer, outside the submitted work. EB received consultant fees and travel grants to institution from Gilead Sciences, MSD, ViiV, Pfizer, AbbVie, Ely Lilly, and Moderna, outside the submitted work. HFG has been an adviser or member of a drug safety monitoring board (with personal fees), outside of the submitted work for Merck, Gilead Sciences, ViiV, GSK, Janssen, Johnson & Johnson, and Novartis and has received unrestricted research grants and a travel grant from Gilead Sciences. JRA has received personal fees from Gilead, Janssen, ViiV, MSD, Aelix, and Theranos, outside the submitted work. BL received personal fees from Kantonsspital Baselland, Liestal, Switzerland, during the conduct of the study, and reports personal fees from Gilead, outside the submitted work. PET received grants, and educational and advisory fees to institution from Gilead, MSD, and ViiV, outside the submitted work. All other authors declare no competing interests.

Data sharing

Deidentified individual participant data used in the study can be made available for investigators upon request to the corresponding author.

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