

**FRAILTY PHENOTYPE: A CLINICAL MARKER OF AGE ACCELERATION IN THE
OLDER HIV-INFECTED POPULATION**

Matilde Sánchez-Conde^{*,1,2}, Javier Rodríguez-Centeno³, Fernando Dronda¹, Juan C López⁴,
Zaida Jiménez⁵, Juan Berenguer⁴, María J Pérez-Elías¹, Santiago Moreno^{1,2}, Berta Rodés³ &
Fátima Brañas⁵

¹Infectious Diseases & HIV Department, University Hospital Ramo' n y Cajal, Madrid, Spain

²Research Institute, University Hospital Ramo' n y Cajal (IRYCIS), Madrid, Spain

³Research Institute, University Hospital La Paz (IdiPAZ), Madrid, Spain

⁴HIV Unit, University Hospital Gregorio Marañón, Madrid, Spain

⁵Geriatrics Department, University Hospital Infanta Leonor, Madrid, Spain

*Author for correspondence: msconde@gmail.com

14 **Abstract**

15 **Aim:** To evaluate the association between DNA methylation and frailty in the HIV-infected
16 population and to investigate the usefulness of assessing frailty as a clinical marker to identify age
17 acceleration.

18 **Methods:** Frailty was assessed according to Fried's frailty phenotype. DNA methylation was
19 analyzed in 10 frail patients, and compared with 10 robust control patients, all with HIV. Predicted
20 age was inferred using the Weidner's formula. Age acceleration was assessed using the difference
21 between predicted and chronological age.

22 **Results:** HIV-infected frail patients had significantly higher biological predicted ages than
23 chronological ages (mean acceleration: 10.3 years; $p = 0.012$).

24 **Conclusions:** We link age Acceleration and frailty in an older HIV population. Frailty could be
25 used in this population for implementing specific clinical approaches.

Introduction

The HIV-infected population is aging with an increased prominence of age-related comorbid illnesses relative to the non-HIV general population. Characteristics of aging are not homogeneous among HIV-infected patients; there is heterogeneity in the decline in health and functional ability among individuals during aging so, the identification of patients who are at risk of unhealthy aging has become relevant for the care of the elderly HIV-infected population [1,2].

Frailty is a geriatric syndrome that results from an age-associated decline and increased vulnerability. The detection of frailty is of paramount importance because it allows us to identify persons at risk of adverse health events [3-6]. Frailty has been proven to be related to worse clinical prognosis (morbidity, falls, and death) but with a chance of successful outcome if detected early [7,8]. The prevalence of frailty in community-dwelling adults older than 65 years is close to 7% [5,6,9,10]; the prevalence of frailty in the HIV-infected population could be up to twice that of the noninfected who are 10 years older [11-13]. An association between HIV infection and higher functional impairment among older HIV-infected patients has also been previously suggested [14-17].

Changes in the DNA methylation levels at specific sites in the genome is a known epigenetic hallmark of aging [18,19]. There is vast and extensive variation regarding the DNA methylation profile during aging from embryonic to adult cells. These epigenetic modifications can make important contributions to the development of age-related diseases [20]. Different epigenetic clocks have been developed to accurately measure biological age through DNA methylation [21-23], thereby allowing us to calculate age acceleration by assessing the difference between age

predicted by DNA methylation or epigenetic age used as a surrogate of biological age and chronological age. Age acceleration is a predictor of mortality, independently of health status, lifestyle, and known genetic factors [19,24-26].

In addition, there are few previously published data from general population-based cohorts that show an association between age acceleration measured by DNA methylation with frailty and impaired physical function [27-29]. In the HIV-infected population, the measure of DNA methylation has been shown to increase biological age even if viral replication is suppressed [30-32]. However, the association between DNA methylation and frailty has not yet been assessed in this population.

In this study, we assess the relationship between epigenetic age acceleration and frailty status in HIV-infected older individuals with the hypothesis that frail older HIV-infected patients should be biologically older.

Methods

Study design and patient population:

From June 2014 to June 2015, we performed a cross-sectional study in which we included 117 consecutive patients attending the clinics in two university hospitals. This population was screened for the prevalence of frailty, tested for factors associated with frailty, and evaluated for physical function [14]. The inclusion criteria were confirmed HIV infection, age ≥ 55 years at the time of recruitment, and regular follow-up at the HIV clinics.

To assess the biological age of older HIV patients and compare this measure between frail and robust groups, we analyzed the peripheral blood mononuclear cells (PBMCs) from 10 frail HIV

patients and compared them with those of 10 robust control HIV patients matched for age and nadir CD4 (**Figure 1**).

The Ethics Committee of each hospital approved the study. All patients signed informed consent forms.

Data collection

We recorded sociodemographic data, variables related to HIV infection (risk practice for HIV infection, baseline and current immunovirological status, and stage of HIV infection at diagnosis), and the antiretroviral treatment received. We separately recorded the time under tenofovir disoproxil fumarate (TDF) because of the suggested impact of TDF on telomere length, thus producing reduced telomerase activity and potentially accelerated aging.

The recorded laboratory data included HIV-related data (HIV-RNA, nadir and current CD4 count, and CD4/CD8 rate), HCV coinfection (detection of antibodies against HCV) and/or HBV coinfection (detection of active hepatitis B surface antigen), body mass index (BMI), nutritional profile (proteins, albumin), and vitamin D levels.

Frailty

Frailty was assessed according to Fried's frailty phenotype, defined by five functional criteria [3], namely: shrinking (unintentional weight loss of ≥ 4.5 kg or $\geq 5\%$ of body weight during the previous year), weakness (grip strength adjusted for gender and BMI), poor endurance and energy (self-reported exhaustion identified by two questions from the Center for Epidemiologic Studies Depression scale), slowness (based on time to walk 4 meters, adjusting for gender and standing height), and low physical activity level (< 383 kcal/week in men and < 270 kcal/week in women).

using the Minnesota Leisure Time Activity Questionnaire). Patients were considered frail when they met at least three of the five criteria, pre-frail when they met one or two criteria, and robust when they met no criteria.

Collection of PBMCs

Blood samples were processed to obtain the PBMCs by a Ficoll gradient using a partitioned solution of lymphocytes. The cell numbers were counted and divided into aliquots of 5 and 10 million PBMCs per milliliter and were cryopreserved in liquid nitrogen.

DNA methylation analysis, biological age prediction, and age acceleration

Genomic DNA was isolated from PBMC by QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany; GYN) according to the manufacturer's protocol. Subsequently, 1.5 µg of DNA per sample were bisulfite converted using the EpiTect Bisulfite kit (QIAGEN). 10 ng of converted DNA were amplified by PCR in three genomic regions spanning the CpG of interest (cg0228185: ASPA, cg25809905: ITGA2B and cg17861230: PDE4C) using the previously described primers [33]. PCR products were then analyzed by next-generation sequencing (NGS). After the first PCR, a second PCR of 13 cycles was performed using the Access Array Barcode Library for Illumina Sequencers (Fluidigm). Equimolar pools of the final amplicons were sequenced on a MiSeq sequencer (Illumina).

Based on NGS results, we obtained the level of methylation for each CpG of interest [cg0228185 (α); cg25809905 (β) and a CpG site upstream of cg17861230 (γ)] [18]. We analyzed the DNA methylation in these three CpG sites previously described by Carola Weidner, who analyzed the methylation profiles of 102 age-related CpG sites in 151 blood samples. They assessed the high

precision of methylation levels at these three aged-related CpGs in order to calculate the biological prediction age with a higher accuracy than any telomere length methods. The mean absolute deviation from chronological age was less than 5 years being the variations more frequent correlated with clinical and lifestyle parameters which we adjusted in our study. Therefore, Weidner's epigenetic tool provides a biomarker to estimate the state of aging from the blood [18]. We considered the use of Weidner's model instead of other more popular epigenetic estimates such as Horvath's and Hannum's models, because it is less expensive and requires less complex bioinformatic analyses which make it more affordable in the majority of clinical settings attending HIV patients. Besides it shows a good correlation with age in blood samples [34].

Predicted age was inferred using the following formula: Predicted age (in years) = $38.0 - 26.4 \alpha - 23.7 \beta + 164.7 \gamma$. Age acceleration was assessed using the difference between predicted epigenetic and chronological age.

Statistical analysis

We used descriptive statistics to examine participant characteristics, which were expressed as frequency (percent) for categorical variables, mean ($\pm$ standard deviation [SD]) for normally distributed continuous variables, and median (interquartile range) for continuous variables with a skewed distribution.

Continuous variables were compared using the t-test for independent variables. The Mann–Whitney test was used for variables with a non-normal distribution or when the group size was small. The association between qualitative variables was assessed using the chi-square test or the Fisher exact test when the groups were very small. We used linear regression to assess the differences in biological age between frail and robust patients. Differences were considered to be

significant when $p \leq .05$.

Results

We evaluated 117 HIV-infected patients ≥ 55 years old. According to Fried's criteria, the prevalence of frailty was 15.4% (18 patients) in our population. Clinical and functional data were previously published [14].

In this study, we performed DNA methylation analysis and biological age prediction in 10 older frail HIV patients and compared the data with those of 10 HIV older robust patients from our cohort; all included patients were on antiretroviral therapy and were immunovirologically well controlled.

We did not detect any differences at baseline between frail and robust patients; both groups had similar chronological age, nadir CD4 counts, time of HIV infection, and time under antiretroviral treatment. None of the patients were coinfecting with hepatitis B virus (HBV). The nutritional profile was similar except in albumin rates, which were higher in robust patients than in frail patients, although these were within the normal range in both groups. All patients in both groups had been educated to at least primary school level. Moreover, all patients had maximum punctuation in Barthel for activities of the daily living index, which means total functional independence. The main HIV-related and non-HIV related characteristics of both groups are shown in **Tables 1 and 2**, respectively.

When we compared both groups, we observed that frail patients according frailty phenotype had significantly higher biological predicted age than chronological age (mean acceleration age: 10.3 years; $p = 0.012$), whereas robust patients (none frailty criteria according frailty phenotype) did not show acceleration and had similar biological predicted age and chronological age (mean

acceleration age: -0.67 years; $p = 0.63$). Graphic representations of the biological predicted and chronological ages and age acceleration in both groups are shown in **Figure 2** and **Figure 3**.

Discussion

The principal finding of our study is the demonstration of aging accelerated up to 10.3 years in the older HIV-frailty population as measured by DNA methylation. The relationships among DNA methylation and HIV-1 infection have been previously established in some studies [35]. Gross et al. [32] measured the impact of chronic HIV infection on aging by performing a global analysis of the whole-blood DNA methylomes of 137 HIV+ individuals under stable antiretroviral treatment compared with those of 44 matched HIV-negative individuals. These authors found that both chronic and recent HIV infection leads to an average aging advancement of 4.9 years, with expected mortality risk increased by 19%. In another study with participants from the Veterans Aging Cohort Study, Nelson et al. [36] assessed the difference in DNA methylation among antiretroviral treatment (ART)-naïve HIV-positive and HIV-negative individuals and found that DNA methylation age was 11.2 years higher in HIV-positive individuals than in HIV-negative ones. Moreover, Horvath and colleagues have shown that HIV infection leads to an increase in epigenetic age in both brain tissue (7.4 years) and blood (5.2 years) [30], and an association between HIV-infected with neurocognitive disorders (HAND) and higher biological age determined via the epigenetic clock has already been reported in a recent study published by Levine et al. [37].

One critical point that is unique to our study is that we have taken into account the fact that HIV patients are aging heterogeneously [1,38]. In all the studies previously mentioned, the older HIV population has been analyzed as a single group, and the findings from these patient groups have

187 then been extrapolated to the whole population of older HIV-infected patients [30,32,36,37].
188 However, as far as we know, HIV-infected people with the same comorbid conditions can have
189 very different functional aging trajectories, and some of them are more vulnerable to age-related
190 comorbid illnesses than others. Patients show worse clinical prognosis (morbidity, falls, and death)
191 and higher markers of immunosenescence when frailty is clinically detected [10,12,39].
192 Ours is the first study in which the relationship among frailty and epigenetic age acceleration in
193 older HIV adults is showed. We did not detect accelerated aging in the older HIV population who
194 were robust, even though there were no other differences in basal characteristics, duration of HIV
195 infection, time under ART, previous or current tenofovir exposure, or education level among the
196 two groups of patients (frail and robust).
197 The HIV population is getting older but based on our data there is more to understanding in the
198 aging process than simple comorbidities or morbidity. Therefore, the Frailty Phenotype could be
199 a possible clinical marker of biological aging according to DNA methylation and would allow us
200 to identify in the daily clinic those older patients who, beyond their chronological age are more
201 vulnerable to unhealthy aging. Given that frailty can be reversed through clinical interventions
202 (mainly based on physical exercise and adequate nutrition), the approach described in this
203 paragraph could help to screen and design interventions for the older HIV population.
204 Finally, regarding the possibility that nutritional intervention could support an adequate approach
205 to reverse frailty status, we also want to highlight that we found significantly higher albumin rates
206 in robust patients than in the frail, although these values were within the normal range in both
207 groups. We cannot evaluate the real importance of this finding, which should be evaluated
208 carefully in future trials with a greater number of patients, thereby confirming whether dietary
209 changes could help to reverse unhealthy aging [40].

Our study has some limitations. Similar to most of the studies cited in our discussion, it had a small sample size. Despite this, we detected a relationship between frailty and age acceleration in older HIV-infected patients. We believe that this finding was possible because we performed an exhaustive clinical, functional, and geriatric evaluation, which allowed us the possibility to properly select the controls. However, future trials with larger sample sizes are needed to confirm our findings.

Conclusions

In conclusion, ours is the first study in which accelerated aging and frailty have been linked in an older HIV population. This finding is important in clinical practice because we have demonstrated that it is possible to easily evaluate frailty as a clinical marker of accelerated aging and, consequently, implement specific approaches to address frailty in the HIV-infected population, thereby avoiding future adverse events and disability.

Future Perspective

The current HIV care model is centered on HIV-related clinical and analytical measures, on ART, and on comorbidities. In this model, the older HIV population is considered as a homogeneous group as if because of their chronological age they should have the same health characteristics and problems. However, HIV patients with the same chronological age and comorbid conditions can have very different functional aging trajectories. In our study, we were the first to find that age acceleration measured by DNA methylation in older HIV adults is linked with frailty, which is intrinsically defined by physical function. Nonfrail, older HIV adults did not suffer from age

acceleration so the effect of frailty on the biological aging among older HIV adults was stronger than the effect of the HIV itself.

Our work is the first step toward the change the HIV care model needs. The new HIV care model has to take into account frailty and physical function in older HIV adults as clinical markers of biological ageing. This should allow us to detect those older HIV adults who are at risk of unhealthy ageing regardless of their chronological age.

Summary points

- Age acceleration is a predictor of mortality, independently of health status, lifestyle, and genetic factors. DNA methylation at specific genomic sites is a known epigenetic hallmark of accelerated aging.
- Frailty has been proven to be related to worse clinical prognosis.
- We demonstrated 10.3 years accelerated aging in the older HIV-infected frail population (measured through DNA methylation)
- Nonfrail older HIV adults did not suffer from age acceleration so the effect of frailty on the biological aging among older HIV adults was stronger than the effect of the HIV itself.
- We propose frailty as a clinical marker of biological aging which allows us to identify in the daily clinic those older patients who, beyond their chronological age are more vulnerable to unhealthy aging.
- This is the first study that explores and demonstrates its association with frailty in older HIV adults.

References

1. Bunting E, Rajkumar C, Fisher M. The human immunodeficiency virus and ageing. *Age Ageing*. 2014;43:308–310.
2. Guaraldi G, Orlando G, Zona S, Menozzi M, Carli F, Garlassi E. Premature Age-Related Comorbidities Among HIV-infected Persons Compared With the General Population. *Clin Infect Dis*. 2011 Dec; 53(11):1120–6.
3. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: Evidence for a phenotype. *J Gerontol A Biol Sci Med Sci*. 2001 Mar;56(3):146-156.
4. Bandeen-Roche K, Ferrucci L, et al. Phenotype of frailty: characterization in the women's health and aging studies. *J Gerontol A Biol Sci Med Sci*. 2006;61(3):262–6.
5. Santos-Eggimann B, Cuenoud P, Spagnoli J, Junod J. Prevalence of frailty in middle-aged and older community-dwelling Europeans living in 10 countries. *J Gerontol A Biol Sci Med Sci*. 2009;64(6):675–81.
6. Abizanda P, Sánchez-Jurado PM, Romero L, Paterna G, Martínez-Sánchez E, Atienzar-Núñez P. Prevalence of frailty in a Spanish elderly population: the Frailty and Dependence in Albacete Study. *J Am Geriatr Soc*. 2011;59(7):1356–9.
7. Liu CK, Fielding RA. Exercise as an Intervention for Frailty. *Clin Geriatr Med*. 2011 Feb;27(1):101–10.
8. Bray NW, Smart RR, Jakobi JM, Jones GR. Exercise prescription to reverse frailty. *Appl Physiol Nutr Metab*. 2016 Oct;41(10):1112–6.
9. Danon-Hersch N, Rodondi N, Spagnoli J, Santos-Eggimann B. Prefrailty and chronic morbidity in the youngest old: an insight from the Lausanne cohort Lc65+. *J Am Geriatr Soc*. 2012;60(9):1687–94.
10. Macklai NS, Spagnoli J, Junod J, Santos-Eggimann B. Prospective association of the SHARE-operationalized frailty phenotype with adverse health outcomes: evidence from 60+ community-dwelling Europeans living in 11 countries. *BMC Geriatr*. 2013;3(13):3.
11. Brothers TD, Kirkland S, Guaraldi G, Falutz J, Theou O, Johnston BL, et al. Frailty in people aging with human immunodeficiency virus (HIV) infection. *J Infect Dis*. 2014 Oct 15;210(8):1170-9.
12. Desquilbet L, Jacobson LP, Fried LP, et al. A frailty-related phenotype before HAART initiation as an independent risk factor for AIDS or death after HAART among HIV-infected men. *J Gerontol A Biol Sci Med Sci*. 2011;66(9):1030–8.

13. Leng SX, Margolick JB. Understanding frailty, aging, and inflammation in HIV infection. *Curr HIV/AIDS Rep*. 2015 Mar;12(1):25-32.
14. Brañas F, Jiménez Z, Sánchez-Conde M, et al. Frailty and physical function in older HIV-infected adults. *Age Ageing*. 2017;46(3):522-526.
15. Greene M, Justice AC, Covinsky KE. Assessment of geriatric syndromes and physical function in people living with HIV. *Virulence*. 2016 Jul 4;8(5):1–13.
16. Greene M, Covinsky K, Astemborski J, et al. The relationship of physical performance with HIV disease and mortality. *AIDS*. 2014;28(18):2711–9.
17. Erlandson KM, Allshouse AA, Jankowski CM, et al. Comparison of functional status instruments in HIV-infected adults on effective antiretroviral therapy. *HIV Clin Trials*. 2012;13(6):324–34.
18. Weidner C, Lin Q, Koch C, et al. Aging of blood can be tracked by DNA methylation changes at just three CpG sites. *Genome Biol*. 2014 Feb 3;15(2):R24.
19. Christiansen L, Lenart A, Tan Q, et al. DNA methylation age is associated with mortality in a longitudinal Danish twin study. *Aging Cell*. 2016 Feb;15(1):149–54.
20. Gensous N, Bacalini MG, Pirazzini C, et al. The epigenetic landscape of age-related diseases: the geroscience perspective. *Biogerontology*. 2017;18(4):549–59.
21. Hannum G, Guinney J, Zhao L, et al. Genome-wide Methylation Profiles Reveal Quantitative Views of Human Aging Rates. *Mol Cell*. 2013 Jan 24;49(2):359–67.
22. Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol*. 2013;14(10):R115.
23. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. 2018 Jun;19(6):371-384.
24. Marioni RE, Shah S, McRae AF, et al. DNA methylation age of blood predicts all-cause mortality in later life. *Genome Biol*. 2015 Jan 30;16:25.
25. Perna L, Zhang Y, Mons U, Holleczer B, Saum K-U, Brenner H. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. *Clin Epigenetics*. 2016 Jun 3;8:64.
26. Horvath S, Pirazzini C, Bacalini MG, Gentilini D, Di Blasio AM, Delledonne M, et al. Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. *Aging (Albany NY)*. 2015 Dec 15;7(12):1159–70.

27. Breitling LP, Saum K-UU, Perna L, Schöttker B, Holleczeck B, Brenner H. Frailty is associated with the epigenetic clock but not with telomere length in a German cohort. *Clin Epigenetics*. 2016 Feb 26;8:21.
28. Marioni RE, Shah S, McRae AF, et al. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. *Int J Epidemiol*. 2015 Aug;44(4):1388–96.
29. Bellizzi D, D'Aquila P, Montesanto A, et al. Global DNA methylation in old subjects is correlated with frailty. *Age (Omaha)*. 2012;34(1):169–79.
30. Horvath S, Levine AJ. HIV-1 infection accelerates age according to the epigenetic clock. *J Infect Dis*. 2015 Nov 15;212(10):1563-73.
31. Boulias K, Lieberman J, Greer EL. An Epigenetic Clock Measures Accelerated Aging in Treated HIV Infection. *Mol Cell*. 2016 Apr 21;62(2):153-155.
32. Gross AM, Jaeger PA, Kreisberg JF, et al. Methylome-wide Analysis of Chronic HIV Infection Reveals Five-Year Increase in Biological Age and Epigenetic Targeting of HLA. *Mol Cell*. 2016;62(2):157–68.
33. Endo K, Li J, Nakanishi M, et al. Establishment of the MethyLight Assay for Assessing Aging, Cigarette Smoking, and Alcohol Consumption. *Biomed Res Int*. 2015;2015:451981.
34. Freire-Aradas A, Phillips C and Lareu MV. Forensic Individual Age Estimation with DNA: From Initial Approaches to Methylation Tests. *Forensic Sci Rev* 2017;29(2):121-144.
35. Zhang X, Justice AC, Hu Y, et al. Epigenome-wide differential DNA methylation between HIV-infected and uninfected individuals. *Epigenetics*. 2016;11(10):750–60.
36. Nelson KN, Hui Q, Rimland D, et al. Identification of HIV infection-related DNA methylation sites and advanced epigenetic aging in HIV-positive, treatment-naïve U.S. veterans. *AIDS*. 2017;31(4):571–5.
37. Levine AJ, Quach A, Moore DJ, et al. Accelerated epigenetic aging in brain is associated with pre-mortem HIV-associated neurocognitive disorders. *J Neurovirol*. 2016;22(3):366–75.
38. Greene M, Justice AC, Covinsky KE. Assessment of geriatric syndromes and physical function in people living with HIV. *Virulence*. 2016 Jul 4;8(5):1–13.
39. Desquilbet L, Margolick JB, Fried LP, et al. Relationship between a frailty-related

347 phenotype and progressive deterioration of the immune system in HIV-infected men. J
348 Acquir Immune Defic Syndr. 2009 Mar 1;50(3):299–306.

349 40. Szarc vel Szic K, Declerck K, Vidaković M, Vanden Berghe W. From inflammaging to
350 healthy aging by dietary lifestyle choices: Is epigenetics the key to personalized nutrition?.
351 Clin Epigenetics. 2015 Mar 25;7:33.

352

TABLES

Table 1. HIV-related patient characteristics

Characteristics	Frail N = 10	Robust N = 10
Sex: Male (N)	7	8
Chronological Age, y, mean (SD)*	60.6 (3.1)	59.9 (3.8)
HIV transmission category [#] (N)		
• IDU	2	2
• Heterosexual	7	5
• MSM	1	2
• NK	0	1
CDC [#] stage (B or C) (N)	8	7
Nadir CD4+ T-lymphocytes/μL, median (IQR)*	137 (43-218)	109 (15-306)
Actual CD4+ T-lymphocytes/μL, median (IQR)*	584 (413-859)	718 (537-800)
CD4/CD8 rate, median (IQR)*	0.98 (0,1-1)	1 (0,9-1,2)
Duration HIV infection, y, median (IQR)*	17.2 (8.6-22.4)	18,42 (11.6-18.4)
Years on ART [#] , y, median (IQR)*	16.9 (7.1-19.2)	14.9 (10.2-18.06)
Months on tenofovir, median (IQR)*	56.5 (19-88)	70 (1-87)
Current tenofovir (N)	5	5

All patients were on HAART and virologically suppressed.

[#]Abbreviations: IDU, intravenous drug user; MSM, men who have sex with men; NK, not known; CDC, Centers for Disease Control and Prevention; ART, antiretroviral therapy; SD, standard deviation.

*No differences were found among both groups when comparing chronological age; nadir and actual CD4+ T-lymphocytes; CD4/CD8 rate; duration HIV infection; years on ART; months on Tenofovir.

Table 2. Non-HIV-related patient sociodemographic and baseline characteristics

Characteristics	Frail N = 10	Robust N=10
Education, (N)		
• Illiterate	0	0
• Primary education	3	2
• High school	5	6
• University degree	2	2
Smoker (N)	3	3
HCV coinfection* (N)	2	5
BMI [#] index, median (IQR)	24.5 (19,7-29,7)	24.9 (21,4-27,5)
Albumin g/dl, median (IQR)**	4,1 (4-4,2)	4,7 (4,1-5)
Vitamin D IU, median (IQR)	20.8 (16,9-35,5)	24.3 (12,5-45,3)
Barthel index ^{\$} (BI) maximum punctuation	100	100

[#]Abbreviations: BMI index, body mass index. *HCV coinfection, detection of antibodies against HCV.

**p=0.04

^{\$} Barthel index corresponds to the patient's current level of ability for activities of daily living. Maximum punctuation of 100 assesses total functional independence.

FIGURES

Figure 1: Study Design

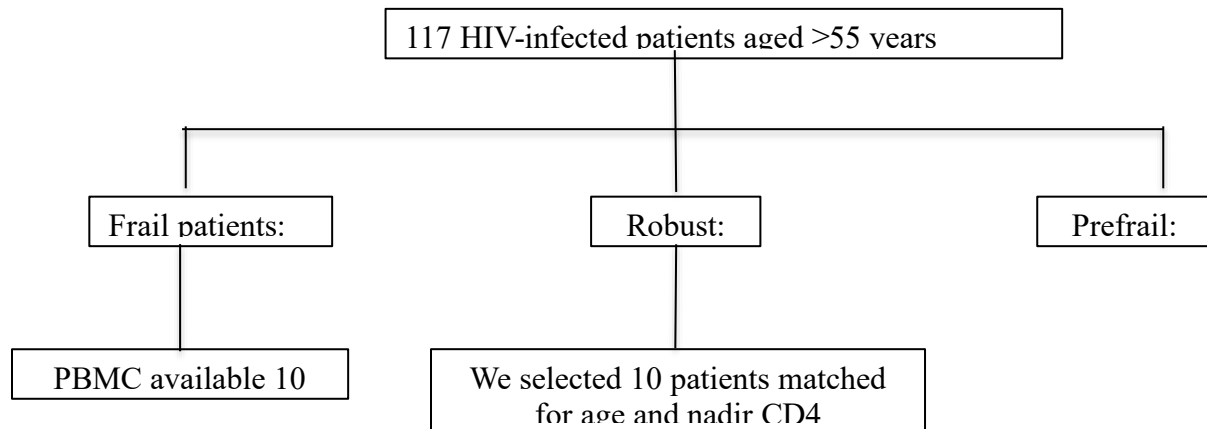
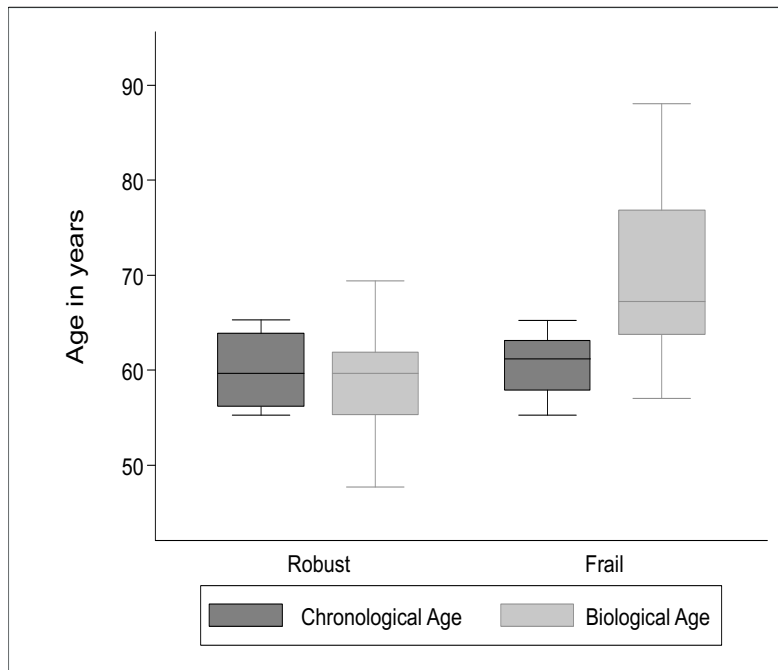


Figure 2. Chronological and biological predicted age in frail and robust patients.



HIV-infected frail patients had significantly higher biological predicted age than chronological age (mean age acceleration: 10.3 years; $p = 0.012$).