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Abstract

Background: Adults aging with HIV are at greater risk for several comorbidities. The CD4+ cell count and CD4+/CD8+ ratio often fail to normalize in elderly patients despite prolonged antiretroviral therapy; this has been associated with concomitant diseases and poor prognosis.

Methods: A cross-sectional analysis in antiretroviral-treated HIV-positive patients aged ≥65 years. Aim of the study was to describe the predictors of normalized T cell subsets ("nT", CD4+/CD8+ ratio ≥1 and CD4+ ≥500 cells/uL) in a cohort of geriatric HIV-positive patients and its association with HIV-associated non-AIDS conditions (HANA).

Results: 1092 patients were included: nT was observed in 340 patients (31.1%). Multivariate binary logistic analysis showed that plasma HIV RNA <50 copies/mL (p=0.004), female gender (p=0.002) and nadir CD4+ cell count (p<0.001) were independent predictors of nT. Age and gender-adjusted prevalence of hypertension (p=0.037), lipid abnormalities (p=0.040) and multimorbidity (MM, p=0.034) was higher in subjects with nT while chronic obstructive pulmonary disease (COPD) and cancer were lower (respectively p=0.028 and p=0.005). Multivariate analysis showed that HIV duration was an independent predictor of several comorbidities while nT was protective for cancer and COPD. HIV duration and nT were simultaneously predictors of MM.

Conclusions: Normalized T cell subsets were observed in approximately one third of geriatric HIV-positive subjects and they were predicted by female gender and immunovirological features. HANA conditions were more prevalent in patients with longer HIV duration while nT represented a protective factor for cancer and COPD.

Key words: CD4/CD8 ratio; aging; multimorbidity; non AIDS co-morbidities, COPD.

Introduction

The introduction of combination Antiretroviral Treatment (cART) profoundly changed the life expectancy of HIV-positive patients. By transforming a deadly infection into a chronic disease the number of elderly patients increased significantly: models estimate that the proportion of patients aged 60 years or older will increase from 8% to 39% in 2030.(1) Aging with HIV is seldom healthy and patients have to face the inevitable passage of time with additional burdens.(2)(3)(4) HIV-infected patients age with several years of immune deficiency, chronic inflammation, co-infections and drug-associated toxicities: the longer the infection the higher the prevalence of comorbid conditions. The aging immune system faces several changes (including the reduction in CD4-T cell reserves, in naïve T cell anliad telomere shortening) that eventually lead to immune senescence and low grade inflammation (the so-called inflammaging).(5)(6) In HIV-uninfected subjects the ratio between CD4+ and CD8+ T lymphocytes (CD4+/CD8+ ratio) represents a marker of T-cell function and an "inverted" value (<1) has been identified as a predictor of unfavourable outcomes in elderly individuals.(7) In 85-90% of virologically suppressed cART-treated patients the CD4+/CD8+ ratio fails to normalize: low nadir CD4+ cell counts and advanced age have been identified as risk factors for incomplete immunogical recovery.(8) Several pieces of evidence confirm that a persistently low CD4+/CD8+ ratio during effective cART is associated with increased innate and adaptive immune activation, an immunosenescent phenotype and higher risk of morbidity/mortality in this group of individuals.(9)(10) Aim of this study was to describe the predictors of normalized T cell subsets ("n-T" *i.e.* CD4+/CD8+ ratio >=1 and CD4+ cell count>=500/uL) and its association with HIV-associated non AIDS conditions (HANA) in a cohort of HIV-positive patients above the age of 65.

Methods

The GEPPO cohort (GEriatric Patients living with HIV/AIDS: a Prospective Multidimensional cOhort) is a multi-centric ongoing study in HIV-positive geriatric patients (≥65 years old) enrolled in ten Italian institutions. This study is a cross-sectional analysis of the entire GEPPO cohort (n=1679) at initial visit (performed between June 2015 and May 2016) including patients with available CD4+ and CD8+ T-cell count. Inclusion criteria were: age older than 65 years, confirmed HIV-positivity, being on cART (combination AntiRetroviral Treatment) for at least 6 months. Ethics Committee approval was obtained from Research Ethics Board of each individual centers belonging to the GEPPO cohort. All participants provided written consent at their initial clinical visit.

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Study endpoints were the prevalence and the predictors of nT; secondary endpoint was the association of nT with comorbidities, multimorbidity (MM) and polypharmacy (PP). MM was defined as the presence of 3 or more HANA while PP as the regular consumption of 5 or more drugs beyond ARVs as previously described.(10) Duration of HIV infection was calculated as the time between HIV diagnosis and last study visit. This variable was stratified into <10, 10-20 and >20 years duration groups. Laboratory results were included if within 6 months of the study visit.
Data are expressed as number (prevalence) or average (± standard deviation). Multivariate binary logistic regression was used for obtaining age and gender-adjusted associations between nT and HANA conditions. Stepwise multivariate binary logistic regression models were tested using clinically relevant and significant variable at bivariate parametric analysis ($p<0.10$).

Results

A total of 1092 patients were included: their clinical and epidemiological features are described in Table 1 (and stratified by gender in supplementary Table). Their median age was 71.3 years (±4.9, range 65-91). nT was observed in 340 patients (31.1%): patients not presenting nT (nnT) were more likely to be male ($p=0.01$), with lower nadir and current CD4+ cell count, higher CD8+ cell count and not virologically suppressed. No other statistically significant difference emerged between the two groups. No association between the number of ARV drugs (triple vs. dual vs. mono therapies) or the features of third drugs [protease inhibitors (PIs) vs. non nucleoside reverse transcriptase inhibitors (NNRTIs) vs. integrase strand transfer inhibitors (INSTIs)] and the prevalence of nT was observed. When we stratified patients for HIV duration we observed that 305 subjects (28.3%) were infected for >20 years, 511 (47.4%) between 10 and 20 years while 261 (24.2%) for <10 years. Current CD4+ cell count was higher in patients with longer infection [655 (>20years) vs. 631 (10-20 years) vs. 579 (<10 years), ANOVA $p=0.005$)]; nadir CD4+ cell count was lower in those with HIV duration between 10 and 20 years [197 cells/uL ±165 vs. 225 cells/uL ±171 (<10 years) and 244 cells/uL ±202 (>20 years), ANOVA $p=0.002$]. CD4+/CD8+ ratio did not differ among HIV duration strata. The prevalence of nT was not higher in subjects with longer HIV duration [31.4% (<10) vs. 32.1% (10-20) vs. 29.8% (>20), $p=0.796$)]. At multivariate binary logistic analysis (including HIV duration and age) only plasma HIV RNA <50 copies/mL ($p=0.004$, Beta 3.77, 95%CI 1.53-9.26), female gender ($p=0.002$, Beta 1.75, 95%CI 1.22-2.51) and nadir CD4+ cell count (per 100 cells/uL increase $p<0.001$, Beta 1.50, 95%CI 1.36-1.66) were independent predictors of nT.

The prevalence of specific HANA conditions, MM and PP is described in table 1. The prevalence of hypertension [172 (69.1%) vs. 336 (61.7%), p=0.037], lipid abnormalities [189 (75%) vs. 370 (68%), p=0.040] and MM [105 (55.6%) vs. 202 (46.7%), p=0.034] were significantly higher (age and gender-adjusted) in subjects with nT compared to nnT patients. Conversely the prevalence of COPD [8 (3.4%) vs. 43 (8.5%), p=0.028] and cancer [35 (15.5%) vs. 27 (25.2%), p=0.005) were significantly lower in nT patients (Table 1). When stratified for HIV duration the prevalence of HANA conditions was higher in patients with more than 20 years of infection: such a difference was statistically significant for bone disease (39.8% vs. 27.6% vs. 2.6%, p=0.001), cardiovascular disease (25.7% vs. 18% vs. 16.7%, p=0.048), lipid abnormalities (74.6% vs. 74.4% vs. 59.8%, p<0.001), diabetes (39.9% vs. 28.2% vs. 20%, p<0.001), cirrhosis (11.7% vs. 4.5% vs. 1%, p<0.001), COPD (11.1% vs. 4.6% vs. 6.4%, p=0.015) and multimorbidity (65.3% vs. 48.6% vs. 34.2%, p<0.001).

To explore whether nT was associated with HANA conditions or MM independently of HIV duration bi-variate (Fig.1) and multivariate binary logistic regression analysis were carried out. After correcting for age, gender, body mass index and n-T, HIV duration was an independent predictor of chronic kidney impairment, bone disease, lipid abnormalities, hypertension, diabetes and cirrhosis. n-T was independently protective for cancer and COPD. HIV duration and n-T were simultaneous predictors of multimorbidity.

Discussion

We observed that approximately one third (31.1%) of geriatric HIV-positive patients obtained normalized T cell subsets; female gender, a higher pre-cART CD4+ cell count and control of plasma viral load were associated with such immunological goal. The nT prevalence is not different from what observed in other studies where it ranged from 19 to 26% in younger subjects; using a 0.7 threshold this further increased to 37%.(8)·(11) While CD4+ cell count nadir has been linked to nT in almost all studies, other predictors have been variably associated, such as treatment with non-nucleoside reverse transcriptase inhibitors, age and negative cytomegalovirus IgG serological results.(12)·(13) Male and female seem to suffer the same age-related changes in the immune system; however males usually display a more rapid immune senescence and more commonly an inverted CD4+/CD8+ ratio.(14)·(15) In our analysis female subjects, despite being only 17.5% of the cohort, had a significantly higher prevalence of nT than males (39.3% vs. 29.4%; p=0.010) and of normalized CD8+ cell percentage (73.3% vs. 60.6%, p=0.001). Our initial hypothesis was that the age of HIV acquisition might be a determinant for insufficient T cell normalization as it is relevant for the prevalence of several comorbidities.(9) We observed no effect of longer HIV

duration on the probability of reaching normalized T cell subsets, though our results may be biased by a lower CD4+ cell count at nadir in the intermediate HIV duration range. The strongest predictor of a nT was the presence of controlled plasma HIV viral load. Persistent low level viremia has been associated with microbial translocation and inflammation.(16) Furthermore lifelong cumulative HIV viremia has been associated with the CD4+/CD8+ ratio recovery in a cohort of patients followed since primary infection and persistent low level viremia (measured as viremia-copy-years) has been associated with a high risk of all-cause mortality in HIV-infected patients.(17),(18) These pieces of evidence highlight the need for optimal viral control (potentially including other chronic infections such as cytomegalovirus) to improve the function of a senescent immune system.

In this study we also observed a very high prevalence of comorbidities; almost half of the included patients fulfilled the multimorbidity criteria. This is in line with the observed and projected data in HIV-positive subjects and shows a higher prevalence than in age-matched negative individuals.(10),(1) In younger HIV-positive subjects the association of an inverted CD4+/CD8+ ratio with several comorbidities has been reported. Nonetheless data in elderly individuals are limited and affected by a relevant survival bias. In our study we observed a significantly higher prevalence of multimorbidity, independently from traditional risk factors, in individuals with normalized T cell subsets; these surprising results might be explained by several years of cART-associated toxicities (and confirmed by the higher prevalence in patients with longer HIV duration) and they may represent a survivor effect where other factors (including health conditions, body mass index and genetics) have affected the outcome of major cardiovascular disorders. On the other hand normalized T cell subsets were protective for COPD and non-AIDS cancers independently from the duration of HIV infection and antiretroviral treatment. Both diseases, COPD and non AIDS-cancer, require immune vigilance and it seems reasonable that a normalized T cell count might be associated with a lower prevalence of such conditions. Recent evidence confirms our results since low CD4/CD8 ratio has been associated with non AIDS-defining cancers in patient on cART and a ratio <0.5 could identify patients who require a more intensive strategy for cancer prevention and screening.(19) Other data, also, showed that HIV suppression normalizes peripheral and lung immunity in smokers reducing the risk of COPD.(20) The association between a normalized CD4+/CD8+ ratio and a lower prevalence of comorbidities and cancers is still uncertain: the reduction in PD1 expression on CD8+ cells in patients with high CD4+/CD8+ ratios may determine a better immunological effectiveness as well as an improved response to vaccines.(9),(21)

We should acknowledge a few limitations of this study: the cross-sectional design, the lack of cytomegalovirus serology and titer, the lack of specific T cell subpopulations. The single-point

evaluation and the lack of blood pressure measurements in all patients warrant caution in interpreting the observed association with hypertension.

In conclusion 31.1% of geriatric HIV-positive subjects have normalized T cell subsets; higher CD4+ nadir, female gender and plasma HIV RNA undetectability were independently associated with this endpoint. n-T was independently associated with hypertension and protective for cancer and COPD. HANA conditions were more common in patients with more than 20 years of infection that represents an independent predictor of chronic kidney impairment, bone disease, lipid abnormalities, hypertension, diabetes and cirrhosis. Both HIV duration and nT were simultaneous predictors of MM. According to these results the normalization of T-cell subset is uncommon in geriatric HIV-positive patients but it is protective for certain comorbidities. Beside earlier cART initiation specific strategies for improving the immune system in elderly HIV-positive subjects are needed.

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Compliance with Ethical Standards

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