HIV/AIDS

Distribution of Naïve (CD45RA+) and Memory (CD45RO+) T-Cells in HIV-Infected Puerto Rican Population

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HIV infection usually results in a gradual deterioration of the immune system. It is evident that early recognition of progression markers during HIV infection from asymptomatic to symptomatic state is needed. In the present cross-sectional study, peripheral blood lymphocytes from 63 HIV-infected Puerto Rican individuals were analyzed by two-color flow cytometry to study the co-expression CD45RA and CD45RO on both $CD4^+$ and $CD8^+$ T-cells and its correlation with age, gender, CD4 count, CD4:CD8 ratio, anti-retroviral therapy, clinical status, and viral load. Measurement of T-cell subsets in these patients showed an excessive increase of $CD3^+CD8^+$, $CD8^+CD45RA^+$, and $CD8^+CD45RO^+$ T-cells as disease progresses. In contrast, it was also observed a significant decrease in $CD3^+CD4^+$, $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ T-cells. The distribution of $CD8^+CD45RA^+$ T-cells did not change significantly between HIV and AIDS cases suggesting that this T-cell subset is not a good progression marker. Interestingly, $CD4^+CD45RA^+$ T-cells were significantly difference between genders, and $CD4^+CD45RA^+$ and $CD8^+CD45RO^+$ T-cells were influenced by age. In conclusion, the distribution of naïve/memory $CD4^+$ T-cells and memory $CD8^+$ T-cells significantly correlate with HIV infection in disease progression. It is also important to mention that these T-cell subpopulations may be influenced by both gender and age. Overall, these results suggest that a loss in the generation of new immune response and function may be occurring during disease progression. This study open new windows of understanding that will be beneficial for future studies on immunopathogenesis, diagnosis, prognosis, and treatment monitoring for HIV/AIDS.

Key words: T-cells, Naive, Memory, Gender, Age, HIV, Disease progression

Puerto Rico has become the sixth highest area of incidence among the United States and its territories. The latest statistics indicate that the incidences of HIV cases are increasing (1). According with the Department of Health of Puerto Rico, on February 2001, there have been 26,662 AIDS cases reported in Puerto Rico. The highest proportion of the individuals became infected via injecting drug use (58%) followed by heterosexual (23%) and homosexual contact (17%) (2). The majority of the AIDS cases fall between 20 and 49 years old and among the gender there is approximately 77% men and 23% females.

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In a recent work by our Retrovirus Research Center showed that age, gender, and the use of illicit drug influence the survival of HIV-infected individuals (3). It is evident that early recognition of the progression of HIV infection from asymptomatic to symptomatic state is of major public health importance. This could lead to earlier medical intervention, better diagnosis, and new treatment modalities. The HIV infection severely and progressively disrupts the immune system (4). It is associated with gradual continuous depletion of T cells and loss of immune function (5). The T-cells are responsible for the specific immune recognition of pathogens and initiate the immune response (6). It is important to evaluate the markers that indicate immunological failure and disease progression. The $CD4^+$ cell count was accepted as the indicator of HIV progression and was adopted as guideline to initiate HIV antiretroviral prophylactic therapy. The HIV viral load quantification also influences the initiation and modification of antiretroviral regimens (7). The HIV-1 viral load determination is currently used in a complementary fashion to $CD4^+$ cell count and clinical manifestation to construct HIV management strategies (8).

By using a combination of new progression markers with the existing markers $CD4^+$ cell count and viral load will provide powerful prognostic information for progression to AIDS (9). One of the potential markers is the leukocyte common antigen (CD45) that is a transmembrane protein tyrosine phosphatase expressed in lymphocytes and it is known to be important in lymphocyte activation (10) and plays a crucial role in the maturation and differentiation of lymphocytes (11). CD45 exists as a number of different isoforms generated by alternative splicing of mRNA (12). CD45RA high-molecule weight isoform has been associated with the naïve T-cell phenotype, and the low-molecule weight isoform, CD45RO, has been taken to represent an activated or memory phenotype (13).

T-lymphocyte cell surface isoforms, $CD45RA^+$ (naïve) and $CD45RO^+$ (memory) represent functionally different subsets (14). Both, $CD4^+$ (helper) and $CD8^+$ (cytotoxic) cells can be divided into a naïve ($CD45RA^+$) and memory ($CD45RO^+$) phenotype (15). $CD45RA^+$ that is thought to indicate cells that have not encountered sufficient stimulatory signals, and $CD45RO^+$, is found on cells thought to have previously responded to antigenic stimulation (16). T-cell expressing $CD45RA^+$ respond well to mitogenic stimulation and differentiate into $CD45RO^+$ cell when activated (17). Discrepancy in the involvement of different T-cell maturational stages is also related to disease progression and apparent characterized asymptomatic and advance HIV infection (18).

In this cross-sectional study, we attempted to characterize the T-cell immunophenotypic profile using flow cytometry to directly identify the phenotypic expression of naïve and memory T-lymphocytes subset and correlated it with HIV disease progression. In order to examine alterations in the naïve and memory T-cell population, we used monoclonal antibodies (mAbs) to $CD45RA^+$ and $CD45RO^+$ on both $CD4^+$ and $CD8^+$ T lymphocyte in HIV infected patients. Our study demonstrated that both gender and age influenced both naïve and memory T-cell subsets. These variations may impact the disease progression to AIDS. This study provides a possible understanding of HIV immunopathogenesis and shows the importance of studying disease progression makers.

Materials and Methods

This work is based on a cross-sectional study of HIV infected patients that were recruited through the HIV Registry of the Universidad Central del Caribe/Hospital Universitario Ramón Ruiz Arnau at Bayamón, Puerto Rico. The study's population consists of sixty-three HIV-infected patients both genders between 18 and 55 years old. The infection status of each individual was confirmed by the presence of anti-HIV antibodies by ELISA (Organon Teknika Corp., Durham, NC) and Western blot techniques (Biotech-Dupont, Billerica, MA). This study was performed following an IRB approved protocol.

Description of the study group. Table 1 contains the description of the study group, gender, age, and viral load. Anti-retroviral therapy (ARVT) category was stratified into HIV-infected individuals who were receiving (yes) or not receiving (no) any anti-retroviral therapy at the time of the blood sample. The clinical status of the HIV-infected individual was categorized into two groups: AIDS patients who are HIV-infected patients who have developed at least one of the following criteria: $CD4 < 200$, or $CD4 < 14\%$ with opportunistic infection (OI), and HIV-infected patients with $CD4 \geq 200$, $CD4 \geq 14\%$ without OI (CDC, 1993).

Table 1. Description of the distinct categories in study (n=63)

Variables	n	Percent
Gender		
Female	20	32%
Male	43	68%
Clinical status		
AIDS	31	49%
HIV+	32	51%
Viral load		
≤400	8	14%
400-20,000	15	26%
≥21,000-750,00	34	60%
ARVT		
No	24	38%
Yes	39	62%
Age (years)		
18-30	6	10%
31-40	31	50%
41-50	17	27%
>50	8	13%

AIDS =<200 cells/ml or <14% CD4+ T-cells
Viral load RNA copies/ml
ARVT = Antiretroviral therapy

Cell surface staining and flow cytometry analysis. Two-color flow cytometric analysis was performed on peripheral blood. Samples were collected in presence of EDTA and prepared labeling 100 ml of whole blood with fluorochrome–conjugated monoclonal antibodies followed by lysis of erythrocytes. The samples were incubated with matching

combinations of monoclonal antibodies conjugated with fluorochromes: fluorescein isothiocyanate (FITC) and phycoerythrin (PE) (BD/Pharmingen, San Diego, CA) and were analyzed in a Becton Dickinson FACScan flow Cytometer (Benton Dickinson Immunocytometry Systems, San Jose, CA). The panel that was used included the following markers: CD3-FITC, CD4-PE, CD4-FITC, CD8-PE, CD8-FITC, CD45RA-FITC, and CD45RO-PE. For each sample 10,000 events were collected and analyzed. The analysis and calculations were performed by Simulset V 3.1 software package (Benton Dickinson).

Viral load. Plasma from each subject was stored at –80°C until used. HIV-1 RNA was determined using the Roche Amplicor Monitor Standard Assay (Roche diagnostics, Indianapolis, IN). The lowest limit of quantification was 400 copies/ml with 0.1 ml of sample.

Statistical analysis. Analysis of clinical and immunovirological variables among the sample was analyzed, and age and gender comparison was made. Analysis of variables was performed utilizing: frequencies, percent, mean, median and standard deviation. Student's t-test was used to determine significant differences between group means. Spearman correlation coefficient was calculated to test significant correlation between ordinals and continues variables. The Wilcoxon rank sum test was used for comparisons of continuous variables, and the Chi-square test was used to compare categorical variables. All significant levels were defined two-tailed with $p \leq 0.05$. The statistical analysis was performed using SPSS version 9.0 software package (SPSS, Chicago, IL).

Table 2. Correlation analyses of variables and immunophenotypes under study in our HIV /infected study group

A. Variable	AGE	CD4 count	CD4:CD8 Ratio	Viral load
AGE	1.00	-0.102	-0.193	-0.224
CD4: count	-0.102	1.00	+0.801†	-0.616†
CD4:CD8 Ratio	-0193	+0.801†	1.00	-0.505†
Viral load	+0.224	-0.616†	-0.505†	1.00
B. Variable	**CD4+CD3+**	**CD4+CD45RA+**	**CD4+CD45RO+**	
AGE	-0.185	-0.250	-0.103	
CD4 count	-0.954†	+0.900†	+0.968†	
CD4:CD8 Ratio	-0.943†	+0.790†	+0.857†	
Viral load	-0.539†	-0.555†	-0.621†	
C. Variable	**CD8+CD3+**	**CD8+CD45RA+**	**CD8+CD45RO+**	
AGE	-0.212	-0.184	+0.268*	
CD4 count	-0.698†	-0.170	-0.418*	
CD4:CD8 Ratio	-0.587†	-0.181	-0.307	
Viral load	+0.384†	+0.067	+0.255*	

Significance difference (two-tailed) at the 0.05 * and 0.01 † level

Results

Spearman correlations were utilized to determine the relation between the co-expression of CD45RA and CD45RO on both $CD4^+$ and $CD8^+$ T-cell. Table 2a showed that CD4:CD8 ratio, CD4 count, and viral load were not influenced by age. However, among the studied immunophenotypes both $CD4^+CD45RA^+$ and show a variation as age increases. On Table 2b showed that $CD4^+CD45RA^+$ correlate negatively with the age, whereas on Table 2c showed that $CD8^+CD45RO^+$ correlate positively with the age.

Table 2A shows that both CD4:CD8 ratio and CD4 absolute number correlate negatively with the viral load ($p \leq 0.01$). We observed a significant reduction in $CD3^+CD4^+$, $CD4^+CD45RA^+$ and $CD4^+CD45RO^+$ T-cells with the reduction in CD4:CD8 ratio and CD4 absolute number and an increase in viral load (Table 2B). However, table 2C shows that $CD3^+CD8^+$ and $CD8^+CD45RO^+$ T-cells increase according to the reduction in CD4:CD8 ratio and CD4 absolute number and increase in viral load.

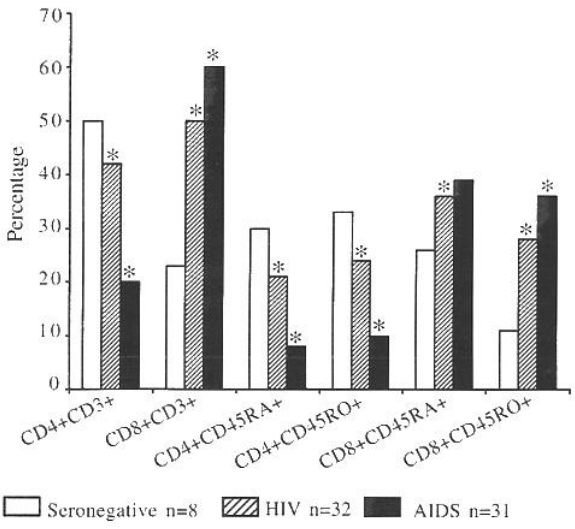

Figure 1. Median of percentages of $CD4^+CD3^+CD4^+CD45RA^+$, $CD4^+CD45RO^+$, $CD8^+CD3^+$, $CD8^+CD45RA^+$, and $CD8^+CD45RO^+$.T-cells in HIV and AIDS group are shown. Asterisks indicate significant differences between the three groups ($p<0.05$).

Naïve and memory CD8⁺ T-cells were augmented in AIDS patients. In Figure 1, HIV-infected individuals were categorized in AIDS and HIV groups including control subjects. As expected, CD4⁺CD3⁺ T-cells were significantly reduced in the AIDS group as compared with the HIV group and both naïve and memory CD4⁺ T-cells were also reduced in this group. This decline was accompanied by a significant increase in CD8⁺CD3⁺, CD8⁺CD45RA⁺ and CD8⁺CD45RO⁺T-cells as compare with seronegative control subjects. However, CD8⁺CD45RA⁺ T-cells show no significant difference between HIV and AIDS patients.

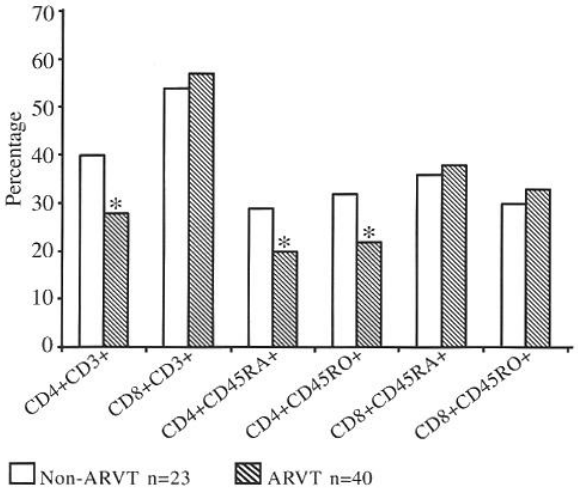

Figure 2. Median of percentages of CD4⁺CD3⁺CD4⁺CD45RA⁺, CD4⁺CD45RO⁺, CD8⁺CD3⁺, CD8⁺CD45RA⁺, and CD8⁺CD45RO⁺.T-cells in ARVT and non-ARVT group are shown. Asterisks indicate significant differences between the three groups ($p<0.05$).

Table 3. Comparison between ARVT, gender , and clinical status in HIV-infected individuals

	Clinical status		
A.			
ARVT	HIV+	AIDS	p
No	15	8	0.05*
Yes	17	23	
GENDER			
Males	20	23	0.32
Females	12	8	
B.	**Gender**		
ARVT	Males	Females	p
No	14	9	0.34
Yes	29	11	

Significance difference (two-tailed) $p \leq 0.05$*

Table 4. Comparison between viral load, gender, amd clinical status om HIV-infected patients

A. Viral load (RNA copies/ml)	Gender			ARVT			Clinical status		
	F	M	p	No	Yes	p	HIV	AIDS	p
≤400	4	4	0.32	3	5	0.31	6	2	0.02*
400-20,000	5	10		7	8		10	5	
↑↓									
≥21,000-750,000	10	24		10	24		12	22	

B. Age (Years)	Gender			ARVT			Clinical status		
	M	F	p	No	Yes	p	HIV	AIDS	p
18-30	3	4	0.43	2	5	0.88	5	2	0.74
31-40	23	8		12	19		15	16	
41-50	12	5		7	10		8	9	
>51	5	3		2	6		4	4	

Significance difference (two-tailed) ≤ 0.05*;slightly significant p=0.05-0.10

ARVT significantly influenced both naïve and memory CD4⁺ T-cell subsets but not CD8⁺ T-cell subsets. Figure 2 shows that CD4⁺CD3⁺, CD4⁺CD45RA⁺, and CD4⁺CD45RO⁺ T-cells were reduced in HIV-infected patients who were undergoing ARVT. However, CD8⁺ T-cell subsets were not influenced by ARVT. In Table 3a, the clinical status shows a significant correlation with ARVT (p=0.05), but not with gender (p=0.32). In addition, in Table 3b ARVT

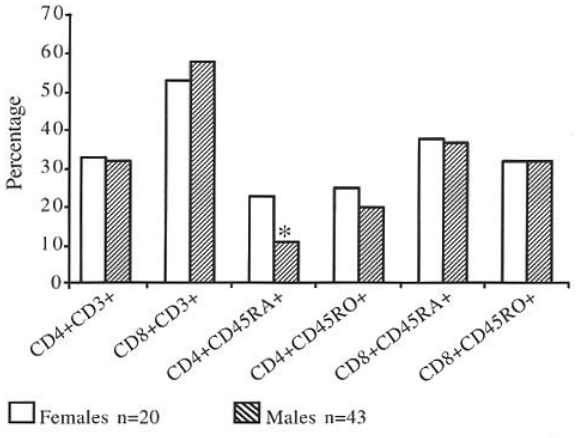

Figure 3. Median of percentages of CD4⁺CD3⁺CD4⁺CD45RA⁺, CD4⁺CD45RO⁺, CD8⁺CD3⁺, CD8⁺CD45RA⁺, and CD8⁺ CD45RO⁺.T-cells in HIV-infected men and women are shown. Asterisks indicate significant differences between the three groups ($p<0.05$).

did not show a significant correlation with gender (p=0.34) and Table 4a showed that HIV-infected patients with higher viral load had AIDS (p=0.02) as compared with the HIV group. Either gender or ARVT did not influence the viral load (p=0.32) and age did not correlate with gender, ARVT, and clinical status (Table 4b). A low proportion of $CD4^+CD45^+$ T-cells were found in HIV-infected males as compared with HIV-infected females. However, the other studied T-cell subsets did not show any significant difference between genders.

Discussion

Previous studies have shown disturbance in the naive and memory T-cell population during HIV infection. These abnormalities could be relevant to disease progression and better prognosis of HIV-infected individuals. Early asymptomatic HIV infection is characterized by selective depletion of memory $CD4^+CD45RO^+$T-cells, contrasting with eventual predominant loss of naive $CD4^+CD45RA^+$ T-cells during the later stages of HIV infection. (8).

In this study, we demonstrated that the surface molecules CD45RA and CD45RO on $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T-cells were affected during HIV infection. We observed that $CD4^+CD45RA^+$ (naïve) T-cells were decreased with age whereas $CD8^+CD45RO^+$ (memory) T-cells were increased (Table 2). In some accordance with our observations, in a previous study was also demonstrated that far advance age was characterized by a profound reduction of naïve T-cells, suggesting a loss of adaptive immunity against new pathogenic infections (19). The most profound age-related change in the T-cells compartment was the loss of naive $CD8^+$ T-cells. In contrast to our results, we showed no significant age difference in naïve $CD8^+$ T-cells, but instead we observed a significant increase in memory $CD8^+$ T-cells with age. This latter is an observation that was not explored by those previous investigators. The decrease in naïve $CD4^+$ T-cells and increase in memory $CD8^+$ T-cells may represent a secondary biological clock related to the human life span. We suggest that these two subsets could be used as indicators or predictor markers for evaluating the risk of immune system failure in older people.

In the present study was also demonstrated that $CD4^+$ T-cell count and CD4:CD8 ratio inversely correlated with an increase in memory $CD8^+CD45RO^+$ T-cells and as expected in viral load and not with naïve $CD8^+CD45RA^+$ T-cells (Table 2). This suggests that the progressive expansion of memory $CD8^+$ T-cells could be a particular event that occurs in both HIV infection and advanced age. It could be possible that HIV chronic infection could be accelerating the age-related immunoregulatory process. This also suggests that memory $CD8^+CD45RO^+$ T-cells could be used as a biomarker for not only aging but also HIV disease progression.

We also showed that both naïve and memory $CD8^+$ T-cells were significantly augmented in AIDS patients as compared to seronegative controls (Figure 1). Although naïve $CD8^+$ T-cells were increased in HIV-infected patients, there was not significant difference between the HIV group and the AIDS group. However, memory $CD8^+$ T-cells showed a significant difference amount the HIV and the AIDS groups suggesting that this $CD8^+$ T-cell subset is a good HIV disease progression marker.

HIV-infected patients undergoing ARVT had significantly lower proportions of both naïve and memory $CD4^+$ T-cell subsets but not $CD8^+$ T-cell subsets as compared with non-ARVT HIV-infected patients (Figure 2). In order to understand this phenomenon, we compared ARVT with gender, age, and viral load and there was not a significant difference (Table 3 and 4); however, when ARTV was compared with clinical status, it showed that the majority of the HIV-infected patients undergoing ARVT were in the AIDS group as compared with the HIV group (p=0.05). This suggested that the reason why the patients not undergoing ARTV had higher proportion of $CD4^+$ T-cells is because in most cases the majority of the HIV-infected patients start using ARTV when they start developing AIDS. This effect is more pronounced in the naïve and memory $CD4^+$ T-cell subsets than in the naïve and memory $CD8^+$ T-cell subsets since most physicians use only CD4 count as a progression marker and not CD8 count. We are suggesting that more physicians include CD8 count as a monitoring tool.

Finally, we observed that HIV-infected men had a significant decrease in $CD4^+CD45RA^+$ naïve T-cell subset as compared with HIV-infected women (Figure 3). In a previous study, $CD4^+CD45RA^+$ naïve T-cells demonstrated to be an excellent predicted progression marker. The progressive loss of these cells may cause a decrease capability to eliminate HIV-related pathogens. Gender difference in this immunophenotype suggests that HIV-infected women may have an advantage over HIV-infected men with regard to generate new adaptive immunity against opportunistic infections and consequently a different clinical outcomes. In accordance with our observations, previous publications have shown that HIV-infected women had a higher CD4 counts than men (20). We demonstrated that most of those $CD4^+$ T-cells are naïve cells. It has also been demonstrated that HIV-infected women had an apparently slighter faster rate of achieving virologic suppression (21); however, in our study, we did not observe a gender difference in viral load (Table 4a). This gender difference could be due to the hormonal status

of women and men (22). It is well known that sex hormones are able to control the immune system and perhaps female hormones may slow down the disease progression to AIDS. For instance, it has been shown that estrogen was able to decrease immune activation and viral replication by inhibiting tumor necrosis factor –alpha (TNF-a) (23). In a recent paper from our HIV registry, we demonstrated that HIV-infected men had a lower survival as compared with HIV-infected women (3). We suggest further investigations attempting to understand the role of female hormones in HIV infection.

This is one of the first study that directly compare a Puerto Rican population of HIV-infected patients to detect differences in their clinical status, gender, age, viral load and both naive and memory $CD4^+$ and $CD8^+$ T-cells. The distribution of naïve/memory $CD4^+$ T-cells and memory $CD8^+$ T-cells significantly correlate with HIV infection in disease progression. In addition, these T-cell subpopulations may be influenced by both gender and age. Therefore, these findings indicate that gender and age may play an important role in the pathogenesis of HIV infection and this may lead to new treatment modalities. Moreover, this study provides new information for further studies of gender differences and their implications in terms of recommendations for and provision of clinical care for both HIV-infected men and women.

Resumen

La infección del virus de inmunodeficiencia humana (VIH) deteriora gradualmente el sistema inmune. Es evidente que el reconocimiento temprano de los marcadores de progresión durante la infección del VIH se hace necesario para estadíos síntomaticos y asíntomaticos. En este estudio transversal, los línfocitos en sangre de 63 individuos infectados con el virus se analizaron por citometría de flujo de dos colores para observar la coexpresión de $CD45RA^+$ y $CD45RO^+$ en las células T $CD4^+$ y $CD8^+$ y su correlación con la edad, sexo, la razón CD4:CD8, terapia de antiretrovirales, estadío clínico y carga viral. Se midieron las subpoblaciones de células T de esos pacientes demostrando un aumento en $CD3^+$ $CD8^+$, $CD45RA^+$, $CD8^+$ $CD45RO^+$ según progresaba la enfermedad. Además se observó una disminución significativa de $CD3^+$ $CD4^+$, $CD45RA^+$ y $CD45RO^+$. La distribución de las células T $CD8^+$ $CD45RA^+$ no demostró cambio significativo entre los casos de VIH y SIDA, sugiriendo que esa subpoblación de celulas T es un buen marcador de progresión de la enfermedad. Interesantemente el $CD4^+CD45RA^+$ demostró una diferencia significativa entre géneros y el $CD4^+CD45RA^+$ y el $CD8^+$ $CD45RO^+$ se vieron influenciados por la edad del paciente. En conclusión la distribución de células T $CD4^+$ vírgenes/memoria y las $CD8^+$ memoria correlacionaron significativamente con la progresión de la enfermedad del VIH. Es importante saber que esas subpoblaciones de células T son influenciadas por el género y la edad del paciente. Estos resultados sugieren que durante la progresión de la enfermedad podría haber una pérdida en función y generación de nuevas respuestas inmune especialmente en hombres infectados con VIH. Este estudio ayuda en el entendimiento y será beneficioso para estudios futuros en inmunopatología, diagnóstico y tratamiento en el seguimiento del VIH/SIDA.

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