Subclinical atherosclerosis and endothelial dysfunction in patients with HIV infection: is there any new diagnostic test?

Francisco Arnaiz de las Revillas
Marqués de Valdecilla University Hospital
Vicente Gonzalez-Quintanilla
Marqués de Valdecilla University Hospital
Jose Antonio Parra
Marqués de Valdecilla University Hospital
Enrique Palacio
Marqués de Valdecilla University Hospital
Claudia Gonzalez-Rico
Marqués de Valdecilla University Hospital
Carlos Armiñanzas
Marqués de Valdecilla University Hospital
Manuel Gutierrez-Cuadra
Marqués de Valdecilla University Hospital
Agustin Oterino
Marqués de Valdecilla University Hospital
Concepcion Fariñas-Alvarez
Marqués de Valdecilla University Hospital
María Carmen Fariñas (mcarmen.farinas@scsalud.es)
Marqués de Valdecilla University Hospital

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Abstract

Introduction:

To analyze the association between human immunodeficiency virus (HIV) infection, and the presence of subclinical atherosclerosis and endothelial dysfunction.

Methods

Prospective cohort study of HIV positive patients who underwent to intimate thickness (IMT) determination and coronary artery calcium score to determine subclinical atherosclerosis. To detect endothelial dysfunction breath holding index, flow mediated dilation and concentration of endothelial progenitor cells (EPC) were measured.

Results

Patients with an IMT ≥ 0.9 mm had an average of 559.3 ± 283.34 CD4/µl and those with an IMT < 0.9 mm 715.4 ± 389.92 CD4/µl (p = 0.04). Patients with a low calcium score had a significantly higher average of CD4 cells value and lower zenith viral load than those with a higher score (707.7 ± 377.5 CD4/µl vs 477.23 ± 235.7 CD4/µl (p = 0.01)) and ($7x10^4$ ± $5x10^4$ c/ml vs 23.4 x 104 ± 19 x 104 c /ml (p = 0.02). Early EPCs concentration in patients with a CD4 nadir < 350/ul was lower than concentration among those presenting a CD4 nadir ≥ 350 (p = 0.03).

Conclusion

In HIV positive patients low CD4 cells levels and high viral load were associated to a higher risk of developing subclinical atherosclerosis.HIV patients with less CD4 cells may have fewer early EPCs.

Introduction

Nowadays due to the existence of high active antiretroviral treatment (HAART), patients with human immunodeficiency virus (HIV) infection have a life expectancy similar to the general population [1]. Non-acquired immunodeficiency syndrome (AIDS) events are globally more frequent than the classic AIDS events in developed countries [2] and cardiovascular disease acquires a great importance. Cardiovascular disease is the leading cause of death in the general world population [3], with a significantly higher risk in people with HIV infection [4]. Cardiovascular risk prediction functions used in the general population using classical cardiovascular risk factors may be inaccurate and underestimate the risk in HIV-infected patients [5].

New analytical and radiological markers are being investigated in order to achieve an earlier diagnosis of atherosclerosis that will allow a more accurate selection of patients who need to perform a primary cardiovascular prophylaxis [6]. Among radiological tests to determinate subclinical atherosclerosis, carotid

doppler ultrasound [7–10] for the measurement of mean intimate thickness (IMT) and coronary artery calcium score [11–13] are are the most studied ones in HIV- infected people.

Other radiodiagnosis tests for an early detection of endothelial dysfunction such as endothelial-dependent vasodilation or flow mediated dilation (FMD) of the brachial artery [14–15] and the breath holding index (BHI) [16–17] have inconclusive data in HIV-population.

Circulating endothelial progenitor cells (EPCs) are characterized by their ability to perform endothelial repair [18]. There are few studies that have evaluated EPCs in the context of HIV infection with divergent results. It may be related to the heterogeneity of the methodology of the studies, taking into account the differences between the characteristics of the populations [19–24].

In this study, we aimed to analyze clinical (time of infection, treatment time) and analytical (current figure of CD4 cells; nadir number of CD4 cells and zenith viral load (VL)) characteristics of patients with HIV infection associated to endothelial dysfunction measured by FMD, BHI and EPCs and to the presence of subclinical atherosclerosis through the coronary artery calcium score and the determination of IMT.

Results

Screening was performed on 1332 patients. Fifty-one men and 26 women met the inclusion and exclusion criteria and agreed to enter the study. The patients included had a mean of 15.5±6.9 years of infection and 14.1±6.25 years of treatment. The average Charlson index [25] was significantly higher in men: 1.78 ± 0.4 vs 1.07 ± 0.3 ($p = 0.04$). All patients included were followed up until September 2020, 1 patient suffered an AMI and another a stroke. Two patients died from a neoformative process. No patient died from a cardiovascular event. Table 1 details the clinical and analytical characteristics of the included patients and their differences by sex.

The mean IMT in men was 0.79 ± 0.13mm and in women it was 0.68 ± 0.15mm ($p = 0.002$). A direct and moderate correlation was found between the IMT and the possibility of suffering a fatal cardiovascular event in the next 10 years through the SCORE index ($r = 0.423$ ($p <0.001$)). Thirty percent (23/77) of the patients presented a pathological IMT and in 19.5% (15/77) atheroma plaques were observed at the carotid level. Virological and immunological differences, as well as disparity in other inflammatory biomarkers (ultrasensitive PCR and D Dimer) between patients with IMT within normal limits and patients with pathological value are reflected in Table 2.

In 67.5% (52/77) of the patients calcified atheroma plaques were not found, in 5.2% (4/77) of the patients a minimal number of calcified plaques was found [0-10 AU], in 14% (11/77) a low amount [10-100 AU], in 9.1% (7/77) a moderate amount [100-400 AU] and 4% (3 / 77) of severe amount [> 400 AU]. The mean risk of suffering a fatal cardiovascular event in the next 10 years calculated using the SCORE system in patients without coronary calcification was 0.52 ± 0.1%, in patients with a coronary artery calcium score of [0-10 AU] 1.0 ± 0.7% was, in patients with [10-100 AU] it was 1.5±0.45%, with [100 -400 AU] it was 2.0 ± 1.15% and in those with the highest value [> 400 AU] 2.7 ± 0.6% ($p <0.001$).

In 88% of women and in 57% of men, coronary tomography did not show calcified plaques [0-10 AU]. Among all the patients in whom coronary calcium was detected, 91% (20/22) were men (p = 0.004).

Fifty percent (11/22) of the patients with a coronary calcium score > 10 AU had an IMT> 0.9 mm and 21 % (12/55) of the patients with <10 UA, had a IMT > 0.9mm (p = 0.015).

Table 2 shows the virological, immunological differences as well as disparity in other inflammatory biomarkers (ultrasensitive PCR and D Dimer) between patients with greater and lesser coronary calcification.

The average FMD Index of the 77 patients included was 13.02 ± 8.08%. Among the patients with IMT ≥ 0.9 the average of the FMD was 11.33 ± 6.39% and among those with a IMT <0.9 the average FMD was 13.24 ± 7.45% (p = 0.26). However, patients who presented a coronary calcium score <10 AU had an average of FMD of 13.53 ± 7.72%, significantly higher than those who presented a more severe coronary artery calcium score with a mean FMD of 10.5 ± 5.03% (p = 0.048).

Eighty six percent (66/77) of the patients included in the study completed the BHI test. Two patients did not complete it due to inability to complete 20 seconds of apnea and 9 patients due to poor transtemporal ultrasound window.

The average BHI of the population included was 0.89 ± 0.72%. Among the patients with IMT ≥ 0.9, the mean BHI was 0.63 ± 0.39% and among those with a IMT <0.9 the average BHI was 1.00 ± 0.62% (p = 0.006). Patients who presented a zero or minimal coronary artery calcium score [<10 AU] presented a BHI of 0.98 ± 0.62% and those who presented a more severe coronary calcium score had a mean BHI of 0.67 ± 0.44% (p = 0.029). Pearson´s between the BHI and the FMD was 0.134 (p = 0.262).

Table 3 shows the relationship between BHI and FMD values and Nadir of CD4 cells; Zenith VL, the time since diagnosis and the duration of HAART.

The average concentration of CD34 + mononuclear cells was 34.45 ± 65.72 c / µl, the average concentration of early EPCs was 0.252 ± 0.848 c / µl, and concentration of very early EPCs was 0.456 ± 1.15 c / µl.

Among the patients with a nadir of CD4 T lymphocytes <350 / µl (n = 60) a concentration of CD34 + 309 + 133 + cells of 0.334 ± 0.606 c / µl was observed and in patients with a nadir ≥ 350 CD4 (n = 16) of 0.913 ± 2.21 c/µl (p = 0.07). Regarding the concentration of CD34 + 309 + 133- cells in patients with a CD4 nadir <350c / µl was 0.144 ± 0.218 c / µl and among those presenting a CD4 nadir ≥ 350 µl the concentration was 0.654 ± 1.78 c / µl (p = 0.03). Table 4.

The relationship between the concentration of the EPCs and the chronology of the infection is showed in Table 4.

Discussion

HIV infection is associated with an increased cardiovascular risk t therefore new tests are needed to allow an early diagnosis. Our study is the first study, to our knowledge, which evaluates endothelial dysfunction markers such as EPCs, BHI and FMD and subclinical atherosclerosis markers such as IMT and coronary

calcium score in the same patients with HIV infection and studies their relationship with HIV related parameters such as viral load and CD4 cells.

In this study, as shown in other publications, IMT in patients with HIV infection is associated with classic cardiovascular factors such as age and sex as in general population [7]. Regarding the relationship of the IMT and the immunological situation o, we found a tendency to a lower ratio of CD4/CD8 cells among patients with pathological IMT although without statistical significance. Similar findings have been published [8]. In addition, in this study it was observed that patients who presented a pathological IMT had a lower value of CD4 cells at the time of study inclusion. Previous studies have observed that patients with a < 200 CD4 cells have a higher IMT and the progression over time of IMT is faster than in those with normal immunological situation [9, 26].

Regarding the Coronary Calcium Score, in this study the patients whom the calcium score was > 100 AU not only had a lower CD4 cells value at the moment of the study inclusion and a lower nadir of CD4cells, but also had a higher zenith VL in a significant way.

The information obtained from the literature about the association between the quantification of coronary calcium and the parameters related to HIV is controversial. In other study of similar methodologic characteristics, no association was observed between lymphocyte subpopulations and VL with coronary calcium score in patients with HIV infection. This fact is probably related to greater prevalence of classic vascular risk factors in the included population, since the patients were older and a large percentage of them were smokers (63%) [11]. However, in other studies with a lower percentage of smokers, whom people included is more similar to our population, the progression over time of coronary calcium score was related to the viral load of the patients the coronary and to the number of CD4 cells [12–13].

We did not find a significant association between FMD and the different parameters related to HIV infection, although there was a tendency towards a greater FMD among patients with a worse immunological and virological status and longer infection time. We found a study whose methodology for the measurement of FMD is the same as the technique performed in our study and no differences were found in FMD in the different subgroups of patients with HIV infection, in the same way as in our study [14].

However, other studies have found an association between FMD and HIV related parameters and globally the FMD values were overall lower than in our study [27]. This may be related to the fact that the patients with HIV infection included in that study did not receive HAART and that we do not know if the methodology for measuring FMD was the same as in our study. Another study compared FMD of patients with HIV infection receiving HAART and naive patients, in the naive subgroup the vascular reactivity was greater than in the group of patients receiving treatment, contrary to the tendence observed in our study [15]. This data may be related to a poorer lipidic control and a higher percentage of smokers among patients already receiving HAART.

In our study, patients who presented a higher zenith VL had a lower cerebral vascular reactivity close to statistical significance. We did not find an association with CD4 cells value. In addition, patients longer since

HIV diagnosis and those who did not received treatment during long periods of time had also a worse BHI. Patients with a pathological IMT and a calcium score > 100 UA had a lower BHI.

HIV infection has been associated with lower cerebral reactivity without relationship between BHI and CD4 cells [16] and with a trend toward higher cerebral vasoreactivity for each additional year of viral suppression [17]. This data is also in accordance with our findings, as patients with a high VL had a worse BHI.

In our cohort patients with a lower CD4 cells nadir and with a higher zenith VL had a lower blood concentration of early and very early EPCs although without statistically significant differences. Patients with longer time of HAART, more time of infection and more time without treatment had a minor concentration of EPCs. There are few studies that have determined EPCs in patients with HIV infection. In the Seang et al study [19] EPCs were measured in 57 HIV positive men and the concentration was lower than in our study with more patients with no cells detected. This fact may be related to a higher prevalence or classic cardiovascular risk factors in their patients, which were older, and dyslipidemia and diabetes were more frequent. No No association was found between EPCs and HIV related parameters.

Papassavas et al [20] determining the same type of EPCs as ourselves, and a direct association between early EPCs and CD4 cells was observed. However, in the rest of the studies, this relationship was not found neither with VL [23–24]. In all the studies that have determined EPCs in patients with HIV infection except in the Costinuk et al [21], included smokers patients, and it is difficult to draw conclusions if we take into account that nicotine alters the proliferation of EPCs.

The lack of a consensus definition in other articles of the EPCs [22–24] and the heterogeneity of classic cardiovascular risk factors of their populations makes it difficult to obtain conclusions from them.

Our study has several limitations. Firstly, the lack of a control group (patients without HIV infection) since it does not allow us to know the values of the endothelial function tests and subclinical atherosclerosis in relation to the virological status of the patient. However, it is known that patients with HIV infection are related to a greater vascular risk and our objective was to know the HIV related parameters that guide us to carry out diagnostic tests for early detection of atherosclerosis.

Secondly the small sample size decreased our power to detect relationships between diagnostic tests and HIV related parameters. However, the careful selection of patients without cardiovascular risk factors and non-smoking patients made it possible to eliminate confounding factors and to relate VL and CD4 cells with some of the selected tests.

Conclusions

In HIV-positive patients, low CD4 cells levels and high VL are associated with an increased risk of subclinical atherosclerosis despite having a low SCORE index. We did not find a significant association between endothelial function and parameters related to HIV infection such as CD4 cells or VL. HIV patients with lower CD4 cells may have fewer early EPCs. In HIV-infected patients despite a low SCORE index, if they had a high zenith VL and a low current nadir or CD4 cells concentration, diagnostic tests (IMT or coronary artery calcium score) could be indicated for the diagnosis of subclinical atherosclerosis and that would allow primary

prevention. Further studies are needed to introduce new techniques for the diagnosis of subclinical cardiovascular disease and endothelial dysfunction into clinical practice, but probably the patients who benefit most from them are those with the worst virological and immunological history.

Patients And Methods

Prospective cohort study carried out at the Marques de Valdecilla University Hospital from June 1, 2015 to May 31st, 2018, which includes patients with HIV infection with more than 5 years of HAART and low cardiovascular risk according to systematic coronary risk estimation (SCORE) index and without previous cardiovascular events. Exclusion criteria were considered: patients not virally suppressed (>20 c/ml), smokers or former smokers for the 15 years prior to inclusion, patients who had less than 5 years of HAART and patients suffering from systemic inflammatory diseases (connective diseases, vasculitis, inflammatory bowel diseases or others other than the study groups). Arterial hypertension or dyslipidemia were not considered exclusion criteria when there was not if there was no damage of the target organ.

Data were collected by a direct interview with the patient and a review of their medical records. Data were registered a database designed specifically for this purpose were: CD4 cells concentration, nadir CD4 cells and zenith VL. Also were registered D Dimer, erythrocyte sedimentation rate, ultrasensitive C-reactive protein, and the lipid profile (total cholesterol, cholesterol bound to high density lipoproteins (HDLc), cholesterol bound to low density lipoproteins (LDLc), triglycerides and complete blood count. All studies were performed after an overnight fast (>8 hours) free of exposure to vasoactive medications and caffeine intake at least 24 hours before and were performed in a temperature-controlled room (22º C). Measurement of blood pressure and the drawing of blood were performed at the moment of ultrasonographic study.

Definitions and methodology of diagnostic tests:

For the measurement of the IMT, the carotid territory was explored in a standardized way. The patient was placed supine and using high-resolution B-mode ultrasound with a 10 MHz linear transducer the IMT was measured at a distance of 1 cm below the carotid bulb in the far wall of the vessel. In addition, the presence or the absence of atheroma plaques was collected [7].

All patients underwent computed tomography imaging of coronary arteries using a 32-slice multidetector scanner to determine the coronay calcium score. This is the sum of the calcium scores (measured as Agatston scores of all calcifications) in the left main coronary artery, left anterior descending artery, left circumflex coronary artery, right coronary artery, and posterior descending artery. Patients were stratified into 4 groups: 0 (normal), 1–100 (low-to-moderate cardiovascular risk), 101–400 (moderate-to-high cardiovascular risk), and >400 (high cardiovascular risk) [28].

The recommendations of the International Brachial Artery Reactivity Task Force [29] were followed to calculate FMD. The image of the brachial artery was obtained above the antecubital fossa in the longitudinal plane. To create a flow stimulus a blood pressure cuff was inflated on the proximal forearm, approximately 50 mmHg above systolic blood pressure of the patient for 5 minutes. Approximately 60 seconds after withdrawal of the cuff, measurements of the artery diameter were made again.

FMD = [(post occlusion diameter - basal diameter) / basal diameter] x 100.

Middle cerebral artery was located around 50 mm deeply from the surface through the transtemporal window. Once the basal average velocity was obtained, the patient was asked to hold their breath for 30 seconds obtaining a new velocity record (Vbh). With the data obtained, the BHI was calculate with the following formula: *BHI = [(Vbh - Vb) / Vb) x Duration of apnea (s)] x100* [30].

Number of EPCs were measured using flow cytometry on whole blood samples. Blood was collected in two sodium heparin tubes in the fasting state. The peripheral blood EPCs were quantified using a protocol previously described [18]. The antibodies CD-34 PE, KDR-APC, CD62E-FITC and CD34-FITC, KDR-APC, CD 133-PE were added in two different tubes. The populations under study were separated in a BD FACS Aria 1 model flow cytometer, and the results obtained were analyzed with the FACSDiva Software V5.03 ModFit V3.0. The populations identified for each of the samples were:

stem heamatopoietic cells: CD34 +; Very early EPCs: CD34 + KDR +CD133 + and early EPCs: CD34 + KDR + CD 133-.

Statistical analysis:

Quantitative variables were expressed as mean and standard deviation (SD); qualitative variables were expressed as frequency and percentage. Statistical analysis was performed using a two-tailed $\chi 2$ test and a Fisher's exact test, or an analysis of variance test (ANOVA), as appropriate in each case. The association between continuous variables was assessed using the Pearson correlation coefficient (r) and a linear regression analysis. A two-tailed $p<0.05$ was considered statistically significant. Data were analysed using SPSS package v19.0 (SPSS Inc., Chicago, IL) and Stata statistical software (Release 11.0, Stata Corporation, College Station, TX).

Ethical approval and informed consent.

The study was performed in accordance with the Declaration of Helsinki. The protocol was reviewed and approved by the Clinical Research Ethics Committee of Cantabria (Ref: 2015.09), according to local standards. Informed consent was obtained from each patient.

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Declarations

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Authors' contribution: F.A., C.F.A., A.O. and M.C.F conceived and designed the study. C.G.R performed sample processing, J.A.P., V.G.Q. and E.P. carried out radiological diagnostic tests. F.A. and C.F.A. analyzed the data. C.A. and M.G.C. contribute to the data analysis and interpretation of the results. F.A. wrote the article. M.C.F. guided and reviewed the research. F. A., C. A., C.F.A. and M.C.F contributed equally to this manuscript.

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Tables

Table 1. Patient characteristics stratified by gender.

	Male Mean (SD) (n=51)	Female Mean (SD) (n=26)	*P value*
Age (years)	51.8±11.9	47.04 ±7.8	0.06
Weight (kg)	81.6±11.6	64.8±12.24	<0.001
BMI (kg/m^2)	27±3.59	25±4.72	0.07
Abdominal perimeter (cm)	99.59±9.9	96.2±23.2	0.62
Leucocytes (cells/ml)	7472±11158	5892.3±1833.4	0.31
Hb (g/dl)	14.9±1.5	13.1±1.5	<0.001
Limphocytes (cells/ml)	2039.7±738.6	2123.6±762.7	0.63
Zenith VL (cells/ml)	167953±196611	348236±1167861	0.22
Lymphocytes T CD4 (cells/ µl)	906.7±441.9	831.4±538.5	0.53
CD4%	30.3±10.5	33.4±9.7	0.24
CD4/CD8	0.83±0.44	0.94±0.48	0.36
Months since diagnosis HIV	197.7+80.8	237.2+84.7	0.06
HAART (months)	166 +77.6	178+70.8	0.51
T Col (mg/dl)	174.31±47.16	193.38±54.47	0.13
HDLCol (mg/dl)	46.61±31.40	59.81±17.29	0.002
LDLCol (mg/dl)	105.39±31.40	114.42±37.02	0.29
Triglycerides (mg/dl)	149.92±106.28	140.5 ±135.09	0.73
SBP (mmHg)	132.49±15.21	122±17.8	0.01
MBP (mmHg)	105.5±11.3	98.6±13.73	0.03

SD, standard deviation; VL, viral load; HIV, human immunodeficiency virus; HAART, highly active antiretroviral therapy; T Col, total cholesterol; HDLCol, cholesterol bound to high density lipoproteins; LDLCol, cholesterol bound to low density lipoproteins; SBP, systolic blood pressure; MBP, mean blood pressure.

Table 2. Analytical characteristics of patients in relation to IMT and CACS

Characteristics of patients n (%) or mean±SD	IMT			CACS		
	<0,9 mm (*n*=54)	≥0,9 mm (n=23)	*P* value	0-100 UA (n=64)	>100 UA (n=13)	*P* value
Male, n (%)	31 (57.4)	20 (87.0)	0.01	40/64 (63.0)	11/13 (84.6)11/13)	0.12
Age (years)	47.8±10.4	55.8±9.98	0.02	48.09±9.93	60.7±9.87	0.01
BMI (Kg/m^2)	25.89±3.76	27.37±4.68	0.14	26.02±3.87	27.85±4.88	0.14
CD4 (cells/ml)	715.4±389.92	559.3±283.34	0.04	707.7±377.5	477.23±235.7	0.01
CD4%	33.2±9.47	26.9±10.99	0.02	32.0±9.5	28.3±10.6	0.25
CD4/CD8	0.85±0.41	0.74±0.3	0.06	0.88±0.47	0.79±0.4	0.54
uCRP (mg/dl)	0.13±0.14	0.18±0.19	0.29	0.14±0.15	0.20±0.19	0.17
D-Dimer (mg/ml)	241.4±136.29	858.1±1195.8	0.02	324.4±391.0	749.7±1275.6	0.15
Zenith VL (cells/ml)	15x10^4±17x10^4	25.9x10^4±8x10^4	0.36	7x10^4 ± 5x10^4	23.4x10^4±19x10^4	0.02
Nadir CD4 (cells/µl)	269.5±172.5	236.9±168.2	0.45	345.4± 182.9	207.7±148.1	0.04
TCol (mg/dl)	185.69±59.7	178.64±46.1	0.57	183.11±52.7	169.15±34.87	0.36
HDLCol (mg/dl)	55.04±21.76	49.37±16.18	0.21	51.03±18.2	51.23±18.08	0.90
LDLCol (mg/dl)	107.68±33.28	110.22±34.5	0.76	110.92±33.91	96.30±29.14	0.15
Tryglicerides (mg/dl)	153.51±54.9	130.82±54.9	0.44	154.18±124.12	110.08±50.73	0.21
MBP (mmHg)	101.56±12.07	106.95±13.03	0.09	103.10±12.8	103.53±11.5	0.91

MT, intima media thickness; CACS, coronary artery calcium score; SD, standard deviation; u-CRP, ultrasensitive -reactive protein; VL, viral load; TCol, total cholesterol; HDLCol, cholesterol bound to high density lipoproteins; DLCol, cholesterol bound to low density lipoproteins; Tg, triglycerides; MBP, mean blood pressure.

Table 3. HBI and FMD values in relation to CD4 cell Nadir, Zenith VL, time since diagnosis, duration of HAART, IMT and CCAS

		FMD (n=77)			BHI (n=66)		
		n	mean ±SD	*P value*	n	mean ±SD	*P value*
Nadir CD4 (cells/ µl)	<200	33	12.21% ± 7.34%		29	0.89% ± 0.57 %	
	≥200	44	13.63% ± 8.63 %	0.44	37	0, 90% ± 0.61%	0.09
Zenith VL(cells/ml)	<200000	58	13.17% ± 7.51%		55	0.91% ± 0.58%	
	≥200000	19	11.12% ± 5.91%	0.52	11	0.64% ± 0. 42%	0.06
Time of infection (years)	<20	42	13.63% ± 6.48%		42	0.98% ± 0.66%	
	≥20	35	11.16% ± 7.99%	0.16	24	0.75% ± 0.42%	0.09
Time of HAART (months)	<200	32	13.45% ± 6.42%		28	0.93% ± 0.68%	
	≥200	45	11.56% ± 8.06 %	0.18	38	0.83% ± 0.43%	0.51
>1 year from the diagnosis to HAART	Yes	41	11.60% ± 6.76%		34	0.76% ± 0.45%	
	No	36	14.53% ± 8.03%	0.12	32	0.98 %± 0.65%	0.11
IMT	<0,9	54	13.24% ± 7.45%		46	1.00% ± 0.62%	
	≥0,9	23	11.33% ± 6.39%	0.26	20	0.63% ± 0.39%	0.006
CACS	<10 AU	56	13.53% ± 7.72%		44	0.98% ± 0.62%	
	≥10AU	21	10.51% ± 5.03%	0.04	22	0.67% ± 0.44%	0.002

HI, breath holding index; FMD, flow mediated dilation; VL, viral load; HAART, highly active antiretroviral herapy; IMT, intima media thickness; CACS, coronary artery calcium score; SD, standard deviation.

Table 4. EPCs values in relation to CD4 Nadir, Zenith VL, time since diagnosis and duration of HAART.

			Very Early EPCs CD34+309+133+		Early EPCs CD34+309+133-	
		n	cells/µl (mean±SD)	*P* value	cells/µl (mean±SD)	*P* value
Nadir CD4 (cells/ µl)	<350	61	0.335± 0.606		0.144± 0.218	
	≥350	16	0.913± 2.214	0.07	0.654± 1.786	0.03
Zenith VL (cells/ml)	<200000	42	0.554±1.30		0.313±0.963	
	≥200000	35	0.123± 0.17	0.20	0.059±0.076	0.31
Time of infection (years)	<20	32	0.318± 0.578		0.156± 0.245	
	≥20	45	0.666± 0.245	0.28	0.397±0.284	0.33
Time of HAART (months)	<200	41	0.291± 0.396		0.152± 0.220	
	≥200	36	0.682±1.711	0.21	0.381±1.281	0.34
>1 year from diagnosis to HAART	Yes	41	0.308±0.598		0.373±1.239	
	No	36	0.639±1.583	0.254	0.153±0.237	0.31

EPCs, endothelial progenitor cells; VL, viral load dl; HAART, highly active antiretroviral therapy; SD, standard deviation.