Original Paper

Markers of subclinical atherosclerosis and cardiovascular risk in young adults horizontally infected with HIV-1 during infancy

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ABSTRACT: Objectives: to evaluate the markers of subclinical atherosclerosis and to identify a potential cardiovascular risk (CVR) profile in young adults horizontally infected with HIV-1 during infancy. Methods: prospective randomized study, carried out between 01 January 2010 and 30 June 2010, on 56 HIV infected persons (PIH) parenterally infected with HIV-1 during 1988-1990, following antiretroviral treatment (ART), under surveillance of Craiova HIV/AIDS Regional Center, Romania. Variables followed: personal history and clinical data, traditional and additional CVR factors, metabolic, immunological and virological parameters, inflammation markers (high-sensitivity C reactive proteine- hs-CRP), ultrasound data regarding carotid intima-media thickness (IMT). Twenty-six HIV-seronegative young adults were assigned as control group (CG) for metabolic parameters, hs-CRP and IMT. Results: general characteristics of the group: average age = 20.82 ± 1.1 years, equal gender distribution, 47 PIH (83.93%) classified as clinical and/or immunological AIDS, 26 PIH (46.43%) with CD4>500/mm3, 40 PIH (71.43%) with undetectable RNA-HIV when evaluated, average ART duration = 9.09 ± 3.2 years, average number of ART regimens = 3.2 ± 1.63, 40 PIH (71.43%) experienced to protease inhibitors (PIs). In PIH – hs-CRP = 2.17 mg/l, equivalent with a moderate CVR, statistically different compared with CG (p<0.0001); IMT = 0.76 ± 0.12 mm in PIH vs 0.6 ± 0.11 mm in CG. From the traditional CVR factors dyslipidemia levels were higher in the PIH group vs CG (p<0.0001). In PIH, linear analysis of the evaluated parameters identified direct correlations between hs-CRP- erythrocytes sedimentation rate (ESR) (p=0.04), number of ART regimens and PIs exposure (p=0.007), IMT (p=0.000) and HIV-RNA (p=0.000) and also between IMT–triglycerides (p=0.004), PIs exposure (p=0.004), CD8+ (p=0.0000) and HIV-RNA (p=0.001). Conclusions: young PIH have had an average value of hs-CRP suggestive for a moderate CVR. Elevated triglycerides, ESR, CD8+, HIV-RNA values and long period of PIs exposure should be components of a CVR profile in young adult infected with HIV-1 during infancy.

***KEYWORDS**: HIV, young, atherosclerosis, cardiovascular risk*

Introduction

The introduction of combined ART (cART) marked the course and prognostic of HIV infection, decreased opportunistic infections, increased survival in PIH, but has brought the toxic side effects and also the emergence of chronic non-infectious impairments, such as cardiac and neurological involvement. The assessment of CVR by scores based on traditional risk factors (e.g. Framingham score, PROCAM, SCORE) proved to be insufficient for certain categories of population, such as youth and women [1]. Furthermore, in HIV-infected young people, there is an issue to diagnose subclinical atherosclerosis and to indentify PIH with increased risk of cardiovascular events [2]. Efforts are required to establish effective strategies for primary prevention. Therefore, scientists are looking for the most eloquent factors that can be used as markers of atherosclerosis in its early stages, before clinical manifestations. Among biochemical markers of atherosclerosis, hs-CRP has been the most studied and carotid intima-media thickness is a proposed independent marker for early diagnosis of atherosclerosis. There are under the study models for evaluating of the CVR in the PIH, taking into account that PIH are generally younger than general population from whom traditional scores were validated and to quantify the additional role of ART [3].

Objectives

The aim of this study is to evaluate the markers of subclinical atherosclerosis and to identify a potential CVR profile in young people following ART, parenterally infected with HIV-1 during infancy.

Material and methods

We carried out a prospective study, randomized 3:1, from 01 January 2010 and 30 June 2010, including 56 PIH parenterally infected in early childhood, following ART,

which were evaluated for predictive CVR markers: hs-CRP and IMT carotid arteries . The control group included young people without HIV infection, having similar age to those in the PIH group, also assessed for metabolic parameters, hs-CRP and IMT measurement. Exclusion criteria: presence of any acute sufferings when evaluated. The following variables were collected: demographic data, personal and family history, traditional and additional CVR factors (smoking, exercise, obesity, stress), complete physical examination, presence or absence of symptomatic cardiovascular disease, data on ART (regimens, duration), 12-lead resting ECG, laboratory data: CD4 cell count, CD8 lyT (flow cytometry, FACS flowcytometry-COUNT); HIV viral load (determined by Polymerase Chain Reaction, Roche Amplicor device with a limit of detection of 50 copies/mL), total cholesterol, triglycerides, HDL-cholesterol, blood glucose (enzymatic colorimetric method-analyzer HITACHI902), markers of inflammation: ESR, hs-CRP (latex immunoturbidimetric method), serological markers for co-infections: hepatitis B, C, cytomegalovirus (CMV), toxoplasmosis.

Carotid ultrasound examinations were performed on Aloka machine at the Emergency Hospital from Craiova, aiming to measure the IMT and to reveal atherosclerotic lesions. IMT measurement was performed for the posterior wall of the common carotid artery. We have measured the linear thickness of the echo (which represents the intima of the artery) and the anechogenic area between the intima and the vascular adventitia (which approximates the media of the vessel). For each carotid artery we used averages of five measurements determined at equal intervals along the length of the artery. Calculations were performed using average values obtained for both common carotid.

Statistical analysis used Data Analysis-Descriptive Statistics module of Microsoft Excel XP. The univariate analysis used Chi square Cramer, Mann-Whithey-Wilcoxon tests. To identify linear correlations between different parameters, we used the Pearson correlation coefficient ($r \in [-1, 1]$, negative values signifies anti-correlation, positive values signifies correlation, 0 indicates no correlation); p-value< 0.05 has been considered significant for statistical differences.

Results

Characteristics of the groups**:** we have enrolled 56 PIH, 28 M/ 28 F (50%/50%) with an average age of 20.82±1.10 years, nosocomially infected with HIV in infancy, during 1987-1990. The control group included 26 HIV negative young people, with an average age of 21.68±1.36 years.

According to CDC classification (Atlanta 1993), 47 PIH (83.93%) of the study group were in clinical and/or immunological AIDS stage at the time of evaluation.

The average value of the CD4+ cell count was 514.70 ± 299.69 cells/ mm ^{3}, with an average plasmatic HIV viral load of 9616 ± 34751.05 copies/ml. Almost 70% of the studied group had PIH undetectable viral load at the time of evaluation, however a good immune reconstruction reconstitution (CD4 + cell count> 500 cells/mm^3) was registered only in 26 PIH (46.43%). Most of PIH already had more than 3 ART regimens, with an average total ART duration 9.09 ± 3.20 years; 40 PIH (71.43%) were exposed to PIs.

Table 1. Metabolic parameters and inflammatory markers

	Average – study group	**SD***	**CV(%)**	**Average - control**	**SD***	**CV(%)**	**P value M-W test****
CT*** (mg/dl)	188.89	43.04	22.79	178.52	15.23	16.22	0.532
HDL (mg/dl)	45.23	15.28	33.79	57.43	1.62	2.88	<0.0001
LDL (mg/dl)	105.82	37.22	35.17	108.36	11.87	11.23	0.06
TG** (mg/dl)	209.23	122.60	58.60	98.62	16.98	17.34	<0.0001
Glucose (mg/dl)	104.84	106.95	102.01	78.11	6.78	8.91	<0.0001
	Average - study group	**SD***	**CV(%)**	**Average - control**	**SD***	**CV(%)**	**P value M-W test****
ESR (1h. mm)	27.38	21.50	78.53	7.79	2.89	37.17	<0.0001
ESR (2h, mm)	43.73	25.26	57.76	15.75	5.68	36.11	<0.0001
hs- CRP (mg/l)	2.17	1.47	67.75	0.78	0.11	58.89	<0.0001

*SD=standard deviation; **MW=Mann-Whitney test; ***CT=total cholesterol; ****TG= triglycerides

The average values of metabolic parameters were outside the normal range for TG, HDLc, with highly statistically significant differences compared with the values recorded in the control group for TG, HDLc, glucose (table I).

Considering inflammation markers we found that ESR values were above normal in 43 PIH (76.7%) at 1h and in 45 PIH after 2h, while the hs-CRP level was equivalent with moderate CVR. There were highly statistically significant differences compared with controls (table I).

The prevalence of co-infections in young people from study group was as follows: VHB-15 PIH (26.78%), T.gondii-15 PIH (26.78%), CMV-15 PIH (23.21%). On ultrasound examination, atherosclerotic lesions were not detected in any of the PIH, however the average value of IMT was 0.76 ± 0.12 mm (CV = 15.73%), which is above the normal range recorded in adults without atherosclerotic lesions. The average value of IMT in the control group was 0.60 ± 0.11 mm.

Correlations hs-CRP-traditional CVR factors-ART-immune activation-chronic inflammation. Patients without traditional CVR factors-smoking, hypertension, obesity, sedentary life, generally had low levels of hs-CRP, but we have not found significant differences between increased hs-CRP levels and the presence of these CVR factors (smoking, p=0.30; hypertension, p=0.07; obesity, p=0.5; sedentary, p=0.82). Within the same category of traditional CVR factors, we have separately analyzed the correlation between dyslipidemia and the hs-CRP level: there were highly statistically significant correlations between the increased values of hs-CRP level and TG (p Chi ^{2} = 0.0007), as well as between increased level of hs-CRP and the presence of mixed dyslipidemia (p Chi ^{2} = 0.0059) (table no. II)

Table 2. Correlations between hs-CRP and dyslipidemia

hs-CRP	**CT<200 No.**	**CT>200 No.**	**Total No.**	**CT<200 %**	**CT>200 %**	**Total %**		
hs-CRP ↑	9	9	18	16.07	16.07	32.14	Chi² 4.995	
hs-CRP ⇔	9	7	16	16.07	12.50	28.57		
hs-CRP ↓	18	4	22	32.14	7.14	39.29	p Chi² 0.082	NS*
Total	36	20	56	64.29	35.71	100.0	Cramer 0.298	
hs-CRP	**TG<150**	**TG>150**	**Total**	**TG<150**	**TG>150**	**Total**		
hs-CRP ↑	8	10	18	14.29	17.86	32.14		
hs-CRP ⇔	1	15	16	1.79	26.79	28.57	Chi² 14.535	
hs-CRP ↓	15	7	22	26.79	12.50	39.29	p Chi² 0.0007	HS
Total	24	32	56	42.86	57.14	100.00	Cramer 0.509	
hs-CRP	**CT↑↑ , TG↑↑↑**	**CT↑↑ +TG↑↑**	**Total**	**CT↑↑, TG↑↑**	**CT↑↑ +TG↑**	**Total**		
hs-CRP ↑	10	8	18	17.86	14.29	32.14	Chi² 10.251	
hs-CRP ⇔	9	7	16	16.07	12.50	28.57		
hs-CRP ↓	21	1	22	37.50	1.79	39.29	p Chi² 0.005	S
Total	40	16	56	71.43	28.57	100.00	Cramer 0.427	

hs-CRP ↓ = hs-CRP<1mg/l – low CVR; hs-CRP ⇔= hs-CRP=1-3mg/l – moderate CVR;
hs- CRP ↑ = hs-CRP=3-10 mg/l – high CVR; HS=high significance

Most patients has been exposed to major classes of ARV. There was a statistically significant correlation between exposure to PIs and high levels of hs-CRP (p Chi ^{2} = 0.007); of all PIs, ritonavir (RTV) was particularly associated with increased levels of hs-CRP (p Chi ^{2} = 0.007), followed by lopinavir (LPV/r). PIH exposed to stavudine (d4T) had higher levels of hs-CRP, but without statistical significance (p Chi ^{2} = 0.5073) compared with those who had not taken this drug. Correlations between hs-CRP levels and ART may overlap, at least in part, with those for hs-CRP and dyslipidemia.

Patients with good immune reconstitution (increased CD4/CD8 ratio) had low levels of hs-CRP, and there was a highly statistically significant correlation between low hs-CRP levels, low plasma viral load, and good immune status (Table III).

Table 3. Correlations between hs-CRP and the immune status, respectively RNA- HIV (VL)

hs-CRP	**CD4<200 No.**	**200-500 No.**	**>500 No.**	**Total No.**	**CD4 <200 %**	**200-500 %**	**>500 %**	**Total %**		
hs-CRP ↑	7	9	2	18	12.50	16.07	3.57	32.14	Chi² 21.065	
hs-CRP ⇔	1	7	8	16	1.79	12.50	14.29	28.57		
hs-CRP ↓	0	6	16	22	0.00	10.71	28.57	39.29	p Chi² 0.0003	HS
Total	8	22	26	56	14.29	39.29	46.43	100.	Cramer 0.433	
hs-CRP	**CD4/CD8 ↓ No.**	**CD4/CD8 ↑ No.**	**Total No.**	**CD4/CD8 ↓ %**		**CD4/CD8 ↑ %**		**Total %**		
hs-CRP ↑	15	3	18	26.79		5.36		32.14	Chi² 22.341	
hs-CRP ⇔	8	8	16	14.29		14.29		28.57		
hs-CRP ↓	2	20	22	3.57		35.71		39.29	p Chi²0.001	HS
Total m	25	31	56	44.64		55.36		100.	Cramer 0.632	
hs-CRP	**VL <50 No.**	**50-10^4 No.**	**>10^4 No.**	**Total No.**	**VL <50 %**	**50-10^4 %**	**>10^4 %**	**Total %**		
hs-CRP ↑	6	5	7	18	10.71%	8.93%	12.50%	32.14%		
hs-CRP ⇔	13	2	1	16	23.21%	3.57%	1.79%	28.57%	Chi² 20.934	
hs-CRP ↓	21	1	0	22	37.50%	1.79%	0.00%	39.29%	p Chi² 0.000	HS
Total	40	8	8	56	71.43%	14.29%	14.29%	100.00%	Cramer 0.432	

Young people with elevated ESR, both at 1 h and at 2 h, have had increased value of hs-CRP, with a highly significant statistical correlation (p Chi²=0.00413, 0.0379 respectively). PIH with HIV-VHB coinfection had higher values of hs-CRP (p Chi ² = 0.0380).

Considering direct linear correlation: there are some interesting connections between CVR, immune activation, chronic inflammation in HIV infection:

- the number of ARV regimens correlates with the tryglicerides values and also with the time elapsed from the first ARV regimen initiation, which highlights the influence of ART on lipid parameters and, furthermore, the cumulative impact of ART.

- hs-CRP correlated directly the ESR 1h, ESR 2h, the number of ARV therapy regimens, IMT and VL. Thus, it appears that elevated ESR in PIH without acute conditions may be surrogate markers for inflammatory CVR. CVR is directly related to the longer duration of ART and the number of ART regimens (also reflecting duration of ART exposure), proatherosclerotic response (by increasing IMT) and high HIV plasma viremia levels.

- IMT value is directly correlated to the TG level, the number of regimens and CD8+ cell count. An elevated TG level and immune activation of CD8+ lymphocytes may be associated with atherosclerotic profile in young PIH with a history of ART.

- CD8+ count, TG level and glucose - probably reflects the effect of chronic immune activation on levels of glucose and lipids.

Indirect linear correlations**:**

- the CD4 + cell count is inversely correlated with ESR 1h, ESR 2h and hs-CRP, VL; we estimate that the PIH from the study group have mainly a classical immune-virological response, meaning a good immune reconstitution associated with low plasma viremia. Also, a good immune reconstruction is associated with a low level of chronic inflammation and reduced CVR.

- CD4/CD8 ratio is indirectly correlated with ESR (at 1h and 2h), hs-CRP, IMT and viral load; CD8 lymphocytes activation is directly correlated with the chronic inflammation, increased viral load and subclinical atherosclerosis.

Discussion

Studies of large groups in the general population have shown that elevated levels of biochemical markers of atherosclerosis, IL-6, VCAM-1, selectines, fibrinogen, D-dimer and especially CRP may predict the occurrence of a cardiovascular event in the future. Some markers were correlated with cardiovascular events and death in the SMART study and other studies with PIH [4].

HIV-infected persons, even those with undetectable viremia, present most common signs of endothelial dysfunction and subclinical

atherosclerosis compared to HIV-negative individuals.

In 2010, Hsue et al. reported that in the HIV positive cohort of SCOPE study atherosclerosis progressed much faster than HIV-negative patients in control group, within two years, as evidenced by IMT measurements at the bifurcation of the carotid artery. This was observed including in PIH with undetectable viral load under ART, even in elite controllers. IMT progression was associated with inflammation, highlighting the correlation between IMT and elevated CRP levels [5].

Thickening of carotid intima-media complex is one of the proposed independent markers for early diagnosis of atherosclerosis. Using the high-resolution ultrasound examinations it was demonstrated the correlation between carotid wall thickness and the presence of atherosclerotic lesions at both the carotid and the abdominal aorta, peripheral arteries, and coronary arteries [6, 7]. It turned out that diffuse thickening of the carotid wall is associated with an increased prevalence of cardiovascular disease even in younger age groups of the general population [8, 9].

A study published in 2010 by Arsenescu et al., in a group of HIV-negative patients with angina pectoris, with an average age of 59.9 ± 8.6 years, confirmed the association between IMT progression and the presence of coronary lesions. It was also demonstrated a correlation between IMT progression and extension level of coronary atherosclerotic lesions, the measured thickness increasing from 0.82 ± 0.30 mm in patients with single-vessel lesions, to 0.95 ± 0.20 mm in those with double vessels lesions, and up to 1.02 ± 0.10 mm in patients with coronary lesions three. Intima-media complex thickness was greater in patients with angina pectoris (0.9 ± 0.2 mm) than the control group (0.6 ± 0.1 mm), $p < 0.0001$ [10].

Another prospective cohort (SUN Study), conducted on 424 PIH, showed a modest IMT progression after 2 years (0.013 mm) in the common carotid. After adjusting for traditional risk factors for CVD, PIH with HIV RNA <400 c/ml had significantly lower IMT progression compared to those with HIV RNA>400c/ml (0.011mm vs. 0.019 mm, $p < 0.001$). NNRTI therapy was significantly associated ($p = 0.01$) with a slower IMT progression than treatment with PIs [11].

Robert Kaplan et al. reported at CROI in 2010 that at carotid artery IMT progression and reduced artery extensibility correlated with increased activation of CD4 + and CD8 + and T-cell senescence in PIH [12].

Regarding cardiovascular clinical events, Triant et al. concluded by retrospective analysis performed at more hospitals, that both HIV infection and CRP double the risk for myocardial infarction (MI). These factors had an additive effect; PIH with elevated CRP had a four times higher risk of MI compared to HIV negative with normal CRP [13].

In the present study, HIV+ youth had an hs-CRP level equivalent to a moderate CVR. Despite of their age (20-22 years old) they have a cardiovascular risk similar to that of adult PIH.

Comparing the data that we have obtained with those from medical literature, we found that IMT value for our group of PIH was similar to that found in the adult population; IMT was higher in those with severe immunosuppression, higher CD8 + and increased viremia.

A statistically significant correlation was detected between IMT and exposure to PIs, especially to RTV.

Elevated levels of hs-CRP and IMT were correlated with high ESR, hypertriglyceridemia, mixed dyslipidemia, and co-infection with HBV. Highest correlation with statistical significance was recorded for hs-CRP and IMT.

Study limitations: although the study group is representative for the cohort nosocomially infected during 1987-1990, the number of PIH evaluated is small and further studies are needed on larger groups. In order not to influence the hs-CRP, we chose PIH assessment at a time where there hadn't any acute conditions, but we could not quantify the impact of past opportunistic infections on atherosclerosis. IMT measurement was not performed dynamically, this goal remaining to be considered for further studies to assess IMT in this particular category of PIH.

Conclusions

1. We found in some young HIV infected during early childhood, long exposed to cART, subclinical cardiovascular impairments expressed by moderate increasing of biochemical markers of atherosclerosis (hs-CRP) and echo changes (measurement of carotid IMT suggests "aging" of the vessel by approx. two decades, highly suggestive for a probable major cardiovascular events in the near future).

2. Among young PIH, we identified a group which coagulates more factors associated with CVR, similar to HIV negative adults with CVR (increased levels of TG, elevated ESR, elevated

CD8, increased HIV plasma viremia, long time ART, exposure to PIs, especially exposure to RTV).

3. Considering the fact that HIV-associated cardiovascular impairment is frequently underdiagnosed, at least in young adult, we strongly support the screening, monitoring and management of early signs of cardiovascular diseases.

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