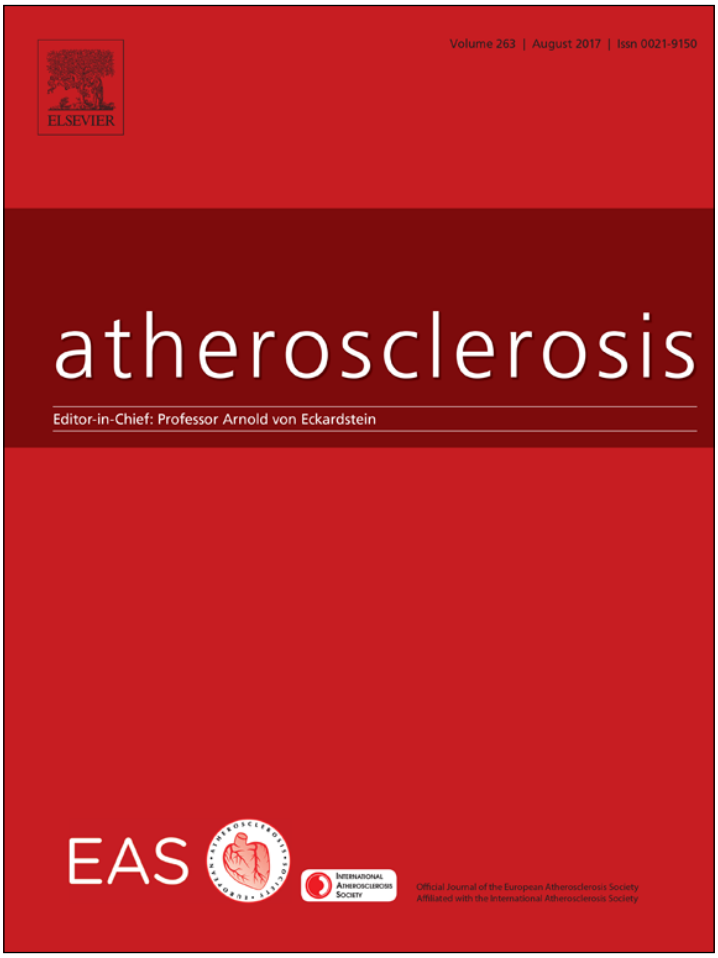

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Cardiovascular risk in advanced naïve HIV-infected patients starting antiretroviral therapy: Comparison of three different regimens - PREVALEAT II cohort

Paolo Maggi [a, *], Chiara Bellacosa [a], Armando Leone [a], Anna Volpe [a], Elena Delfina Ricci [b], Nicoletta Ladisa [a], Stefania Cicalini [c], Elisabetta Grilli [c], Rosaria Viglietti [d], Antonio Chirianni [d], Lara Ines Bellazzi [e], Renato Maserati [e], Canio Martinelli [f], Paola Corsi [f], Benedetto Maurizio Celesia [g], Federica Sozio [h], Gioacchino Angarano [a]

[a] Institute of Infectious Diseases, University of Bari, Policlinico Consorziale, Bari, Italy
[b] CISAI (Coordinamento Italiano per lo Studio dell'Allergia in Infezione da HIV), Italy
[c] INMI L. Spallanzani, IRCCS Roma2, Roma, Italy
[d] Department of Infectious Diseases, Ospedale Cotugno Napoli3, Napoli, Italy
[e] Institute of Infectious Diseases, Policlinico San Matteo, Pavia, Italy
[f] Institute of Infectious Diseases, Ospedale Careggi, Firenze, Italy
[g] Department of Infectious Diseases, Ospedale Garibaldi-Nesima, Catania, Italy
[h] Department of Infectious Diseases, Ospedale Civile Spirito Santo, Pescara, Italy

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ABSTRACT

Background and aims: PREVALEAT (PREmature VAscular LEsions and Antiretroviral Therapy) II is a multicenter, longitudinal cohort study aimed at the evaluation of cardiovascular risk among advanced HIV-positive, treatment-naïve patients starting their first therapy. We hypothesized that these patients, present a higher cardiovascular (CV) risk.

Methods: The study included all consecutive naïve patients with less than 200 CD4 cells/ml starting antiretroviral therapy. Our primary objective was to evaluate changes in carotid intima- media thickness (IMT). Secondary endpoints included changes in flow mediated vasodilation (FMD), inflammatory markers, triglycerides and cholesterol. Patients were evaluated at time 0, and after 3, 6 and 12 months.

Results: We enrolled 119 patients, stratified into three different groups: patients receiving atazanavir/ritonavir boosted (ATV/r) based regimens, efavirenz (EFV) based regimens and darunavir/ritonavir boosted (DRV/r) based regimens. At baseline, advanced naïve patients showed a relevant deterioration of CV conditions in terms of traditional CV risk factors, endothelial dysfunction and serum biomarkers. During the 12-month follow up period, mean blood lipids significantly increased: total cholesterol from 159 to 190 mg/dL, HDL-C from 31 to 41 mg/dL, and LDL-C from 99 to 117 mg/dL. D-dimers steadily decreased (median level 624 at baseline and 214 at T3), whereas ICAM and VCAM consistently raised. DRV/r and ATV/r determined a more marked decrease of D-dimers as compared to EFV. Regarding the epi-aortic changes (IMT >1 mm or presence of atherosclerotic plaques), patients in the DRV/r group were at risk of developing pathological IMT during the study (OR 6.0, 95% CI 0.9–36.9), as compared to EFV ones.

Conclusions: CV risk was elevated in advanced naïve patients and tended to remain high in the first year of therapy.

1. Introduction

The introduction of effective antiretroviral (ARV) regimens has produced a deep impact on the natural history of HIV infection, leading to a dramatic decrease in its mortality and a considerable

* Corresponding author. Institute of Infectious Diseases University of Bari, Policlinico Consorziale, Piazza Giulio Cesare 11, 70124 Bari, Italy.
E-mail address: p_maggi@yahoo.com (P. Maggi).

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increase in the life expectancy of HIV-infected persons. Nevertheless, these patients still appear to be at higher risk of a number of co-morbidities, such as cardiovascular disease (CVD), than the general population [1–3]. The etiology of the increased risk remains not completely understood, however, endothelial activation due to chronic inflammation may play a pivotal role in CVD events [4]. Patients with low $CD4^+$ cell count, not in treatment with antiretroviral therapy (ART), could be at higher CVD risk because of their inflammatory condition [5–7]. PREVALEAT (PREmature VAscular LEsions and Antiretroviral Therapy) was a multicenter, longitudinal cohort study aimed at evaluating the cardiovascular (CV) risk in HIV-infected patients since 1998 [8–12]. PREVALEAT II project aimed at evaluating CVD risk among advanced HIV-positive, treatment-naïve patients starting their first ARV therapy. Our working hypothesis was that these patients may present a higher degree of inflammation and, consequently, a higher CVD risk.

2. Patients and methods

This is a multicenter, longitudinal cohort study. It includes all consecutive naive patients with less than 200 CD4 cells/ml starting any ritonavir-boosted protease inhibitor (PI/r)-based or non-nucleoside reverse-transcriptase inhibitors (NNRTI)-based plus two nucleoside/nucleotide reverse transcriptase T-inhibitor (NRTIs) regimen. Our primary endpoint was the evaluation of carotid intima-media thickness (IMT) changes. Secondary endpoints included: a) the evaluation of changes in brachial artery flow mediated vasodilation (FMD) among study groups; b) changes in circulating endothelial inflammatory markers; c) changes from baseline in serum triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc).

The study has been conducted in seven Italian centers for a total of 119 enrolled patients. The principal investigator selected the participating centers, selecting centers in which the ultrasonographic examination were performed by specifically trained physicians during a Continuing Medical Education stage previously organized by the coordinating center. We considered all consecutive patients that had started a regimen including a PI/r-based or NNRTI-based + 2 NRTIs therapy during the 12 months enrolment period and that met the inclusion criteria described in the protocol. The type of therapy and the choice of drugs included in the scheme were at physician's discretion, according to standard international guidelines and producer indication [13–15].

Written informed consent was obtained from each patient. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study protocol has been priorly approved by the Institution's ethics committee on research on humans.

The enrolment started on March 2012 and was closed on March 2014. Follow up was completed on March 2015.

Inclusion criteria were: age more than 18 years; documented HIV infection for at least 3 months; naive for antiretroviral therapy; advanced infection with CD4 cell counts less than 200 cells per ml; willingness to provide informed consent; barrier contraception for women in fertile age. Exclusion criteria were: presence of severe cardiovascular diseases (past history for myocardial infarction, *angina pectoris*, cerebral stroke, transient ischemic attacks in the previous 12 months); insulin-dependent diabetes diagnosed in the previous 12 months; documented acute HIV infection; presence of decompensated cirrhosis; estimated Glomerular Filtration Rate (eGFR) inferior to 60 ml/min; presence of an active opportunistic infection (i.e. patient receiving therapy for opportunistic infection); actual or programmed pregnancy.

The following parameters were evaluated at time 0 (T0), after 3 (T1), 6 (T2) and 12 months (T3):

(1) IMT of common and internal carotid for both left and right sides: ultrasonography of the epi-aortic vessels were performed using a power colour-Doppler instrument with 7.5 MHz probes. We evaluated the characteristics of the intima, together with the pulsation index, the resistance index, the minimal speed, the peak speed and mean speed. A minimum of three measurements were requested: on the common carotid artery: 1 cm before the carotid bifurcation and at carotid bifurcation; on the internal carotid: 1 cm after the carotid bifurcation and 2 cm after the carotid bifurcation. An intima media thickness (IMT) of >1 mm was considered to be pathological. Atherosclerotic plaques, if present, were described. All images were photographed and properly archived.

(2) FMD: brachial artery FMD was calculated as the percentage increase in brachial artery diameter with induced hyperemia relative to the resting brachial artery diameter.

(3) Inflammatory markers: soluble intracellular adhesion molecule-1 (ICAM-1); soluble vascular cell adhesion molecule-1 (VCAM-1); interleukin-6 (IL-6); high-sensitivity C-reactive protein (hs-CRP); D-dimers. Plasma D-dimers were quantified by means of an enzyme-linked fluorescent assay (Vidas D-Dimer Exclusion II, Biomerieux) at the Central Laboratory of the University-Policlinico Hospital of Bari (normal values: 0–500 ng/ml). Hs-CRP, IL-6, ICAM-1 and VCAM-1 were measured at the Infectious Diseases Laboratory by commercially available assays (for hs-CRP: Biokit Quantex CRP, normal values: 0–300 ng/ml; for IL-6: Boster Biological Immunoleader, China, detection limit: 0.3 pg/ml, assay range: 4.69–300 pg/ml; for ICAM-1 and VCAM-1, Boster Biological Immunoleader, China, detection limit: 10 pg/ml, assay range: 156-10,000 pg/ml).

Data regarding independent risk factors for CVD (family history, smoke, active drug addiction, alcohol consumption) were collected at baseline (T0). Viral load, $CD4^+$ cell counts, serum total cholesterol, LDLc, HDLc, glycemia, triglycerides, BMI were recorded at every control. Moreover, during the study, periodical meetings were hold using filmed reports and/or images in order to obtain comparison and standardization of the techniques.

Ordinal and categorical variables were described as frequency (%) and compared using the heterogeneity $\chi 2$ test (or the Mantel-Hanszel $\chi 2$ test as appropriate) to assess the association between groups and categorical variables. Continuous variables were described as means (standards deviation, SD) or medians (interquartile range, IQR) as appropriate, and then compared among ART groups using analysis of variance or Mann-Whitney test.

Changes from baseline were evaluated using the McNemar test for categorical variables (main comparison T3 *vs.* T0). Over time, continuous variables were analysed using the analysis of variance for repeated measures: normally distributed variables were analysed as such, whereas not normally distributed ones were transformed into square roots, to reach an approximately normal distribution. Comparing treatment groups, multivariate analysis of variance for repeated measures was performed including sex, age and AIDS at presentation as covariates.

Odds ratios (ORs) and the corresponding 95% confidence intervals (CI) were used to evaluate the association between occurrence of pathologic IMT among subjects with normal IMT at baseline and patients' characteristics and clinical variables. We ran multivariate analyses including potential confounders in the equation, as specified in the text. All analyses were based on an

intention-to-treat approach and conducted using SAS for Windows 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

A total of 140 consecutive patients were screened. 119 met all entry criteria, whereas 21 patients were excluded as the therapeutic regimen did not include the study drugs. 49 patients were on atazanavir/ritonavir boosted (ATV/r) based regimens, 31 on efavirenz (EFV) based regimens and 39 patients on darunavir 800 mg/ritonavir boosted (DRV/r) based regimens. Median age was 46 years (range 36–51); 78.2% of the patients were males, 87.4% Caucasians, 34.5% smokers, 2.5% had a family history of CVD; 6% were HCV co-infected. Regarding the risk factor for HIV acquisition, 79.0% was sexual, 14.3% intravenous drug use; 22.7% had AIDS at presentation.

At baseline, no statistically significant difference was observed among groups (Table 1). Overall, 45.4% of patients had an epi-aortic vessels lesion (IMT and/or plaque) and 51.3% showed a pathological FMD. The baseline characteristics of the general population and by treatment groups are summarized in Table 1.

Metabolic and cardiovascular changes during follow-up in the overall population are summarized in Table 2. Immune-virological outcomes were similar in groups of ARV treatment: at T3, 78% of patients were in control, and 77% had reached a CD4 level >200 cells/mm^3, with better although not statistically significant results in the EFV group. As compared to T0, during follow-up the percentage of patients with pathologic D-dimer or hs-CRP declined, although not consistently. The same pattern was observed when comparing these factors among groups: no statistically significant difference emerged at T1, T2 and T3.

The curves of mean FMD, ICAM-1, VCAM-1, and median IL-6, D-dimers and hs-CRP, in the general population and in the three groups during the treatment period, are illustrated in Fig. 1.

Analyses for repeated measures showed that FMD and IL6 did not significantly vary over time, and no difference was observed among treatment groups. Conversely, ICAM and VCAM tended to increase over time ($p = 0.02$ and 0.01 respectively), and more markedly in patients presenting with AIDS (ICAM: 16500 to 17600 in non-AIDS presenters *vs.* 17800 to 22240 in AIDS presenters; VCAM: 17500 to 17700 in non-AIDS presenters *vs.* 20000 to 21600 in AIDS presenters). However, no difference emerged among ART groups. D-dimer significantly and consistently decreased in all groups, whereas hs-RCP showed a median increase at T2 in patients on EFV.

In Fig. 2, we reported the metabolic changes by treatment group. Total cholesterol (Fig. 2A), as well as HDL- (Fig. 2B) and LDL- (Fig. 2C) cholesterol, significantly increased during the observation, without marked differences among ART groups. On the contrary, triglycerides levels (Fig. 2D) did not show significant modification over time even if they increased.

All analyses were run again, including potentially confounding factors (sex, age, AIDS at presentation) in the repeated measure analysis model: univariate analyses results were confirmed.

Sixty patients had no pathologic IMT at baseline. Among these, 18 (30%) showed a pathologic IMT during the follow-up: 2/15 (13.3%) on EFV, 6/25 (24.0%) on ATV/R and 10/20 (50.0%) on DRV/R ($p = 0.045$). Using EFV as reference, the OR for IMT was 2.1 (95% CI 0.4–11.8) in subjects on ATV/R and 6.5 (95% CI 1.2–36.6) in those on DRV/R. When we included in the equation baseline characteristics as sex, age, total cholesterol, BMI, and AIDS at presentation, the estimates remained similar (OR 1.7, 95% CI 0.3–12.2 for ATV/r and OR 6.0, 95% CI 0.9–36.9 for DRV/r), although not statistically significant because of the small sample.

Table 1
Baseline characteristics of 119 patients: treatment groups and overall population.

	EFV N = 31		ATV/R N = 49		DRV/R N = 39		Total N = 119		*p*
	N or mean or median	% or SD or IQR	N or mean or median	% or SD or IQR	N or mean or median	% or SD or IQR	N or mean or median	% or SD or IQR	
Age (median, IQR)	48	35–55	46	41–50	50	43–57	46	36–51	0.46
Males (n, %)	25	80.6	35	71.4	33	84.6	93	78.2	0.30
Caucasian (n, %)	25	80.6	43	87.8	36	92.3	104	87.4	0.34
RF for HIV acquisition (n, %)									
IDU	4	12.9	7	14.3	6	15.4	17	14.3	
Sexual	24	77.4	41	83.7	29	74.4	94	79.0	0.55
Current alcohol consumption (n, %)	5	16.1	6	12.2	6	15.4	17	14.3	0.86
Current smoker (n, %)	10	32.3	17	34.7	14	35.9	41	34.5	0.90
BMI (mean, SD)	23.7	3.3	23.1	3.2	24.0	3.6	23.5	3.4	0.39
Family history of CVD (n, %)	1	3.2	1	2.0	1	2.6	3	2.5	0.99
AIDS at presentation (n, %)	8	25.8	11	22.4	8	20.5	27	22.7	0.87
CD4$^+$ cell/mm^3 (median, IQR)	89	56–164	106	36–145	94	30–158	101	41–154	0.80
HIV-RNA <25 cp/mm^3 (n, %)	0	100	0	100	0	100	0	100	1.0
Total cholesterol (mean, SD)	173	40	157	44	150	45	159	44	0.09
HDL (mean, SD)	34	16	30	10	32	11	31	12	0.40
Triglycerides (median, IQR)	141	91–175	154	102–204	115	106–162	131	99–189	0.23
LDL (mean, SD)	104	20	99	36	93	33	99	31	0.39
Glycemia (mean, SD)	91	12	86	11	87	14	88	12	0.18
IMT and/or plaques (n, %)	14	45.2	24	49.0	16	41.0	54	45.4	0.76
Pathologic D-dimer >500 (n, %)	14	45.2	25	51.0	21	53.8	60	50.4	0.85
Pathologic hsCRP >300 (n, %)	5	16.1	19	38.8	15	38.5	39	32.8	0.08
IL6 (median, IQR)	10	4–13	10	5–17	6	4–13	10	4–15	0.10
Hs-RCP (median, IQR)	0	0–174	226	0–432	261	0–450	175	0–410	0.02
D-dimer (median, IQR)	486	360–1107	614	312–1363	669	280–1136	624	312–1181	0.93
ICAM (mean, SD)	16447	7436	18326	11432	15267	7356	16819	9280	0.32
VCAM (mean, SD)	18852	6445	17540	7236	18324	7305	18138	7025	0.73
FMD (mean, SD)	10.9	11.8	13.2	15.8	18.8	14.0	14.7	14.5	0.08

SD, standard deviation; IQR, interquartile range.

Table 2
Metabolic and cardiovascular changes during follow-up: overall population.

	T1		T2		T3	
	N or mean or median	% or SD or IQR	N or mean or median	% or SD or IQR	N or mean or median	% or SD or IQR
BMI (mean, SD)	24.1	3.2	24.5	3.5	24.9	3.7
IMT and/or claque (n, %)	41	34.5	44	37.0	46	38.7
Total cholesterol (mean, SD)	183	43	187	38	190	39
HDL (mean, SD)	40	18	39	13	41	13
Triglycerides (median, IQR)	154	104–198	153	107–215	151	94–206
LDL (mean, SD)	114	31	118	31	117	31
Glycemia (mean, SD)	89	15	88	13	87	11
$CD4^+$ cell/mm^3 (median, IQR)	193	115–267	243	153–316	327	220–445
HIV-RNA<25 cp/mm^3 (n, %)	**36**	**30.2**	**64**	**53.8**	**77**	**64.7**
Pathologic D-dimer (n, %)	**27**	**22.7**	**14**	**11.8**	**15**	**12.6**
Pathologic hsCRP (n, %)	**15**	**12.6**	**16**	**13.4**	**12**	**10.1**
IL6 (median, IQR)	8.0	0.3–14.3	6.7	0.8–10.0	8.0	4.6–15.0
Hs-RCP (median, IQR)	0	0–236	0	0–245	0	0–176
D-dimer (median, IQR)	368	231–609	259	169–392	214	158–443
ICAM (mean, SD)	17020	7556	17117	6328	18607	8471
VCAM (mean, SD)	17670	4930	17670	4930	18574	4865
FMD (mean, SD)	18.5	14.9	20.2	14.0	17.6	12.2

* Bold: McNemar test (T3 *vs.* T0): $p < 0.05$.
SD, standard deviation; IQR, interquartile range.

4. Discussion

As seen previously, HIV-infected individuals appear to be at higher risk of CVD than the general population. In HIV patients, chronic inflammatory processes are activated and atherosclerosis is accelerated. Consequently, cardiovascular disease is one of the most common non-AIDS events with overall increased morbidity and mortality. Although the mechanisms involved remain elusive, endothelial activation due to the chronic inflammation may play a pivotal role [16].

Measurement of carotid IMT with color-Doppler ultrasonography is a non-invasive, sensitive and highly reproducible technique for identifying and quantifying atherosclerotic lesions, even at a very premature stage. It is a well-validated research tool and is widely used in clinical practice. The American Heart Association (AHA) and NCEP-ATP III have endorsed the use of carotid IMT for CV risk assessment [17]. In preventive medicine, IMT measurement is especially important for subjects with an intermediate CV risk, i.e. for subjects with a 10-year risk of CV disease between 6% and 20% [18]. Moreover, pathological findings with IMT have consistently been related to future CV events [19,20].

Endothelial dysfunction is considered an early marker of atherosclerosis that can be assessed by ultrasound assessment of brachial or radial artery FMD. Changes in FMD are correlated with the severity and extent of coronary sclerosis, and are able to predict future cardiovascular events. FMD is a non-invasive indicator of endothelial dysfunction and is associated with elevated cardiovascular risk in the general population [21]. Impaired FMD is also widely reported in HIV-infected populations and appears to be associated with both viral RNA levels [22,23] and HAART [24–27].

Enhanced endothelial activation, inflammation, and increased carotid IMT can occur in HIV-infected patients despite antiretroviral therapy. Inflammatory markers, in particular hsCRP and IL-6 and sVCAM-1, are related to an increased cardiovascular risk and mortality in HIV-positive patients [5]. Moreover, the same inflammatory markers are associated with endothelial activation and both are associated with internal carotid artery IMT, supporting a potential role of inflammation in endothelial activation and cardiovascular disease in HIV infection [28].

Our initial hypothesis was that advanced HIV-positive, treatment-naïve patients, presented a high degree of inflammation and thus a high CVD risk. Our results seemed to confirm the working hypothesis. In fact, considering the entire cohort of patients at baseline, we found a relevant deterioration of CV conditions in terms of traditional CV risk factors, endothelial dysfunction and serum biomarkers (Table 1).

During the 12-month follow up period, the FMD, a sensitive marker of endothelial function, tended to improve between T1 and T2, but decreased again at T3 to a level similar to baseline. BMI and total cholesterol tended to further increase, thus adding important factors to the global CVD risk of these patients. Regarding the cytokines, we observed that ARV treatment seemed beneficial on D-dimers and hs-CRP, notwithstanding the latter showed a median increase at T2 in patients on EFV. The markers of endothelial activations (ICAM-1, VCAM-1 and IL6) tended to increase among AIDS presenters, documenting an immune activation in this category of patients.

Among the three therapeutic regimens groups, we could observe some differences, though they were not statistically significant. In particular, as compared to EFV, the two regimens including PIs (ATV/r and DRV/r) had initial higher and final lower levels of both D-dimers and hs-CRP. Despite being two non-specific and poorly sensitive inflammation biomarkers, this finding suggests a benefit of these drugs on the inflammatory cascade. However, the improvement in these two parameters seemed not to influence the epi-aortic changes. In fact, while in all three groups we observed a 30% of patients that worsened, EFV showed a better outcome: considering the patients with a normal IMT at baseline, 13.3% in the EFV group worsened, *versus* 24.0% in the ATV/r group and 50.0% in the DRV/r group. Finally, while the BMI tends to increase in all groups, in the DRV/r group we observed the worst outcome.

Regarding the percentage of patients reaching HIV RNA below undetectable levels and $CD4^+$ cell count over 200 cell/mm^3, no significant differences were observed among the three groups.

As said before, the role of chronic inflammation is the main determinant of the CVD risk in HIV patients. We hypothesize that CV risk is elevated in advanced naïve patients because of the inflammation tied to the immune deficiency. This CV risk tends to remain high, especially in the first year of therapy, probably as consequence of the immune reconstitution events that characterize the first period of the ARV treatment. In particular, we observed an increase in VCAM and ICAM especially among AIDS presenters.

One possible limitation of our study is the 12-month follow up.

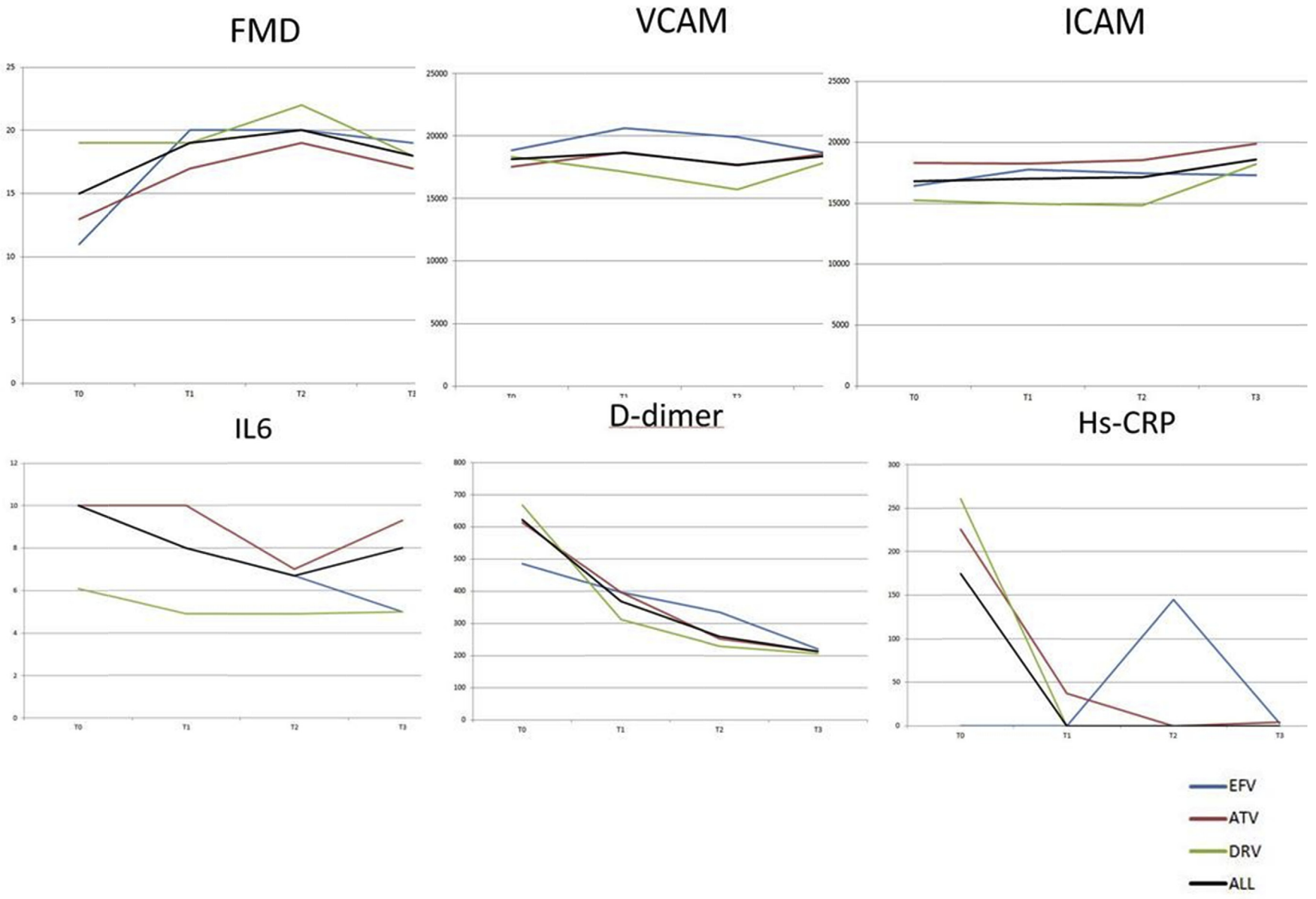

Fig. 1. FMD, VCAM, ICAM, IL6, D-dimers and Hs-CRP in the general population and in the three treatment groups during the treatment period. FMD, VCAM and ICAM are showed as means, IL6, D-dimers and Hs-CRP as medians.

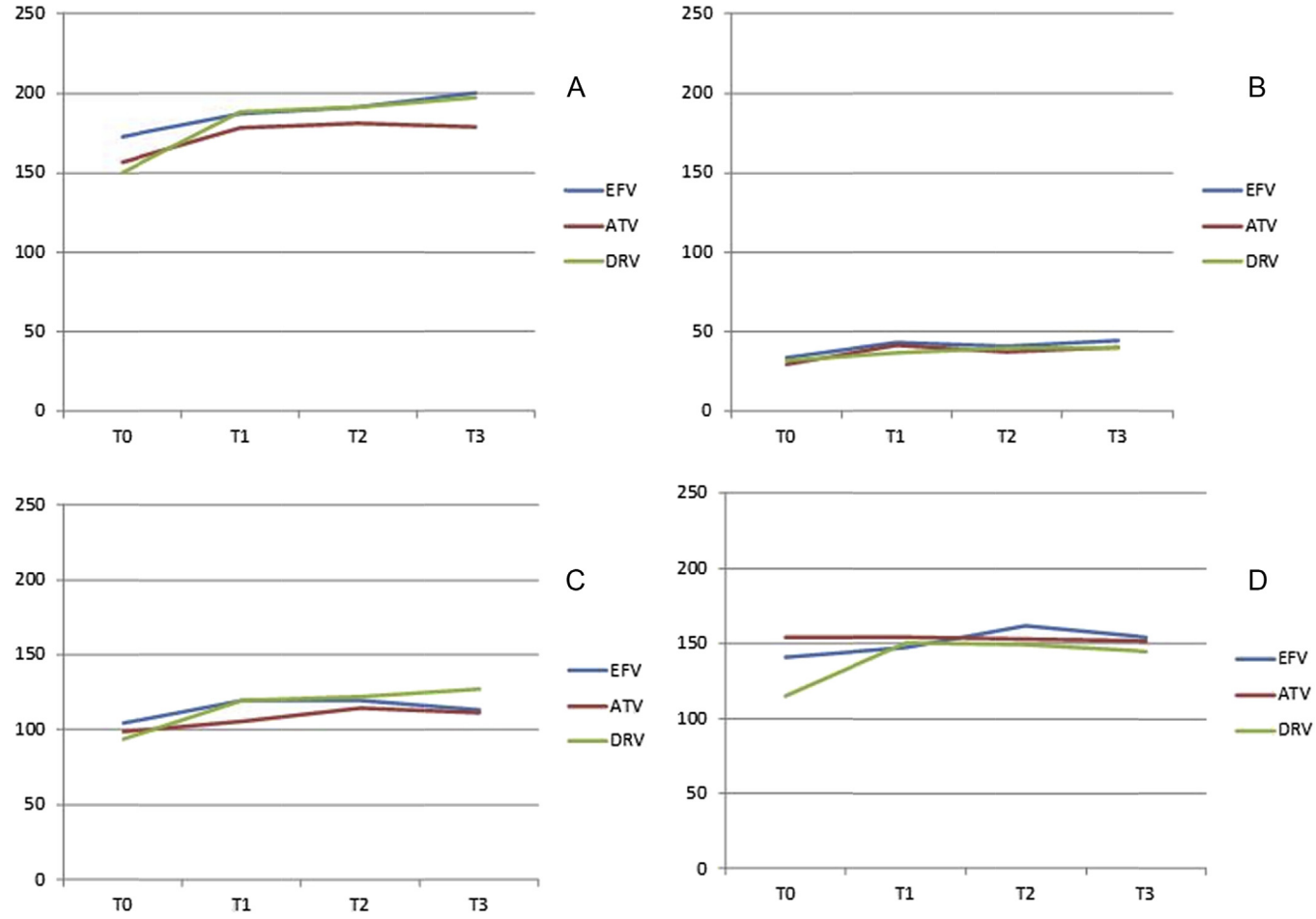

Fig. 2. Metabolic changes by treatment group.
(A) Mean total cholesterol; (B) mean HDL-c; (C) mean LDL-c; (D) median triglycerides.

Undoubtedly, a longer follow-up may have provided other useful data. However, we hypothesized that, after stabilization of the immuno-virologic condition the inflammation would decrease, modifying the cytokines pattern.

Our results reinforce the need to start the ARV treatment as early as possible to avoid the dangerous interplay between the inflammatory effects of the virus and the immune reconstitution events.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

PM designed the study, analyzed, interpreted the data and wrote the manuscript; CB analyzed and interpreted the data; AV performed the centralized laboratory analysis, EDR and AL performed the statistical analysis; NL, SC, EG, RV, LIB, CM, PC, BMC and FS collected the data from the participant centers; GA, RM and AC acquired the data and reviewed the manuscript.

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