ORIGINAL ARTICLE

Atherosclerosis is associated with a higher risk of hepatic steatosis in HIV-infected patients

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Summary

Introduction. *Cardiovascular Diseases (CD) have emerged as a leading cause of morbidity and mortality in HIV population. Some studies have reported higher carotid Intima Media Thickness (c-IMT), a measure of subclinical atherosclerosis (AT), in this cohort of patients.*
Methods. *Here, we evaluate the role of Hepatic Steatosis (HS) as likely marker for AT in 128 HIV-infected patients without hepatitis C infection. c-IMT has been detected non-invasively by carotid ultrasonography to assess the progression of AT. HS has been evaluated using a process based on vibration-controlled transient elastography (Fibroscan) by a novel ultrasonic controlled attenuation parameter (CAP). The cut-off value for defining the presence of significant HS was CAP > 259 dBm^{-1}.*

Results. *AT has been detected in 26 patients (20.3%), whereas steatosis of grade 2 (S2) in 31 (24.2%). The variables statistically related to AT were age, obesity, diabetes, hypertension and S2. In the multivariate analysis, AT was only associated (p < 0.001) with age and S2. The optimal cut-off value indicated by ROC curve for predicting AT was CAP > 250 dB/m^{-1}.*
Discussion. *Our results highlight the presence of AT in HIV-infected persons and its association with fatty liver disease; therefore, HS assessment in HIV population results crucial to predict AT and CD.*

Introduction

Since the advent of highly active anti-retroviral therapy (HAART), the prognosis of HIV infection has been dramatically altered, transforming it from an inexorably fatal disease into a manageable chronic condition [1, 2].
In this context, cardiovascular diseases (CD) result an important cause of morbidity and mortality [3, 4]. Atherosclerosis (AT), a complex, active and progressive disease with inflammation involved at every stage, is closely correlated to an increased risk of heart diseases [5]. Epidemiological data show a relationship between HIV and AT, in fact in the last few years AT prevalence has increasingly been found in this cohort of patients [6].
Several factors may contribute to its development among HIV-infected individuals. First and foremost the role of inflammation has been recognized as the key pathologic process [7, 8]. Furthermore, other determinants including dyslipidemia, lipodystrophy, insulin resistance, tobacco abuse, and metabolic and mitochondrial dysfunctions may be involved in AT disease [9-13]. Previous researches have demonstrated an association between various components of HAART and the development of AT, as well [14, 15]. Finally, HIV infection itself may lead AT via monocyte or T-cell activation and likewise concomitant infections in the HIV-infected subjects could also promote this process [16, 17].

Epidemiological studies have showed strong association between AT and hepatic steatosis (HS) [18, 19]. The latter is a clinical condition associated with inflammation and hepatocyte changes [20]. The different grade of steatosis (S) is defined as S0 = 0-10%; S1 = 11-33%; S2 = 34-66% and S3 = 67-100% of hepatocytes that have a fatty accumulation [21]; it can also be a co-factor in many chronic liver diseases that can lead to fibrosis, cirrhosis and hepatocellular carcinoma, as well as the possibility of developing metabolic alterations that can lead to AT [22, 23].
Mounting evidence suggests that HS is common among HIV-infected individuals with or without HCV co-infection [24, 25], though data on factors associated with steatosis in HIV-mono-infected patients are scarce [26]. To date, liver biopsy is considered the gold standard for the assessment of HS [27]. But recently, ultrasound-based vibration-controlled transient elastography device has been developed to detect it [28] using controlled attenuation parameter (CAP). This tool is a non-invasive, quantitative, non-ionizing and inexpensive method that provides immediate results and it can be performed by an operator without specific radiological competence and individual interpretation. Furthermore, CAP is able to explore a liver volume ~ 100 times larger than liver biopsy [21].

Measurement of AT activity results fundamental for early detection of CD [29]. It can be detected non-invasively by carotid ultrasonography to assess carotid Intima Media Thickness (c-IMT) [30, 31]. IMT is a characteristic of arterial aging related to AT; the cellular and molecular alterations that underlie IMT are implicated in the development, progression or both of AT [32]. c-IMT is widely used as a validated marker for subclinical atherosclerosis in HIV-negative populations [33, 34]; moreover, its value greater than or equal to the 75th percentile for age, sex and ethnicity has been associated with an increased risk of CD [32]. For its low cost and the absence of exposure to radiation, this technology has also been used to identify the predictors of subclinical AT in HIV-infected individuals [10, 35, 36].

Few data are available on the relationship between AT and HS in HIV subjects and since fatty liver diseases are common among this population [37], determining the link with AT may be useful for predicting CD risk [38, 39].

Therefore, objective of our study is to evaluate this relationship in a cohort of HIV patients without concomitant hepatitis C virus (HCV) infection.

Methods

PATIENTS

We performed a cross-sectional study by consecutively enrolling HIV-infected outpatients from January to June 2012. All patients approached for the study gave consent to participate.

Exclusion criteria were: age less than 18 years, HCV infection, liver cirrhosis, active psychiatric disorders, alcoholism and drug abuse.

At enrolment time the following demographic, clinical and laboratory variables were collected for each subject through a patient interview (using a predefined form) and a chart review: gender, age, ethnicity, duration of HIV infection, CD4 cell count, nadir CD4, HIV viral load, therapy, CD risk factors (current smoking habit; obesity, defined as a body mass index above or equal to 30 kg/m^2; diabetes, defined as fasting plasma glucose (FPG) levels above 126 mg/dl; hypertension, defined as blood pressure (BP) above 140/90 mmHg and/or treatment with antihypertensive medications; dyslipidemia, characterized by increased plasma concentration of triglycerides, reduced high-density lipoprotein cholesterol and increased numbers of small, dense low-density lipoprotein particles).

ULTRASONOGRAPHY EXAMINATION

A Logiq 5 ultrasound scanner (General Electric Medical Systems, Wallingford, Connecticut, USA) was used to determine AT defined as c-IMT > 0.9 mm. The c-IMT was defined as the distance between media-adventitia and lumen-intima interfaces and it was measured at about 1 cm proximal to the bifurcation of the common carotid artery using a 7.5 MHz linear probe. The probe was placed so that the near and far walls were parallel to it and lumen diameter was maximized in the longitudinal plane. Mean common c-IMT was defined as mean IMT of the right and left common carotid arteries, calculated after 3 measurements on each side.

The patients were placed in the supine position with their head in the midline position, tilted slightly upwards and the heart in systole. Sonographic evaluations were performed by a single trained sonographer blinded to the patients' data [40].

FIBROSCAN EXAMINATION

In all patients the liver steatosis was evaluated by CAP measuring ultrasonic attenuation in the liver at 3.5 MHz using signals acquired by the FibroScan M probe (Fibroscan 502, Echosens, Paris, France) based on vibra-

Tab. I. Baseline characteristics: Demographics, HIV factors, clinical characteristics of HIV patients (N = 128).

Variable	n (%)	Range
Male sex	92 (71.9)	
Age, years*	44	37-49
Ethnicity, Caucasian	123 (96.0)	
Smoke use	52 (40.6)	
Duration of HIV infection, years *	11	5-16
Current CD4, cells/mm^3*	543	360-700
Nadir CD4, cells/mm^3*	233	112-313
HIV RNA <20 cp/ml	107 (83.5)	
Current cART	128 (93.7)	
Duration of cART, years*	8	4-13
Obesity (BMI≥30 kg/m^2)	8 (6.2)	
Diabetes (FPG>126 mg/dl)	7 (5.4)	
Hypertension (BP>140/90 mmHg)	23 (17.9)	
Dyslipidemia	101 (78.9)	
Atherosclerosis (IMT > 0.9 mm)	26 (20.3)	
S2	31 (24.2)	

*median (interquartile range); BMI, body mass index

tion-controlled transient elastography. The principles have been described elsewhere [28]. An elastographic evaluation has been performed by a single trained sonographer blinded to patients' data. S2 was defined as CAP > 259 dBm^{-1} according to previously published data demonstrating that this cut-off was the most discriminating value [21].

STATISTICAL ANALYSES

The factors associated with AT were identified by logistic univariate regression analysis. The variables showing a p-value < 0.100 in the univariate analysis were evaluated in a multivariable analysis afterwards. The ROC (Receiver Operating Characteristic) curve and corresponding AUC (Area Under Curve) were calculated in order to evaluate the CAP cut-off value able to predict AT.
A two-tailed p-value < 0.05 was considered statistically significant. Statistical calculations were performed with MedCalc software, version 11.6.0.0.

Results

In total, 128 patients [71.9% males, median age 44 years (IQR 37-49), median CD4 543 cells/µl, (IQR 360-700), 83.5% with HIVRNA < 20 cp/ml] were enrolled.
At enrollment time, 93.7% of patients were receiving ART and were using it for an average of 8 years (IQR 4-13). Obesity was diagnosed in 8 patients (6.2%), diabetes mellitus in 7 (5.4%) and hypertension in 23 (17.9%). Instead, dyslipidemia was detected in a large proportion of patients (78.9%) (Tab. I).
26 (20.3%) patients had AT while 31 (24.2%) subjects had liver S2 (Table I). HS was observed in 12 patients (11.8%) considering the subjects (n = 102) without AT, whereas among those (n = 26) with AT, 19 patients had also S2 (73.1%). Subjects with AT showed a higher significant difference (p < 0.001) of S2 than those without AT.
In univariate analysis, age (5.80, CI 1.8-19.5), obesity (15.0, CI 2.8-79.7), diabetes (6.0, CI 1.3-28.7), hypertension (3.90, CI 1.5-5.3), and liver S2 (20.3, CI 7.0-

58.4) were significantly associated with subclinical AT (Tab. II).
In the multivariate model, subclinical AT was associated with age [odds ratio (OR) 5.8; p < 0.001] and liver S2 (OR 20.3; p < 0.001). ROC curve indicated that the most discriminant CAP value for predicting AT was > 250 dB/m^{-1} (AUC = 0.92, sensitivity 92.31%, specificity 81.37%, p < 0.0001) (Fig. 1).

Discussion

Several reports have shown that CD and in particular AT are common among HIV infected patients [29, 41, 42].
In this cross-sectional study, conducted in a cohort of HIV-infected subjects receiving care at the Infectious Disease Department in a tertiary hospital in southern Italy, 20.3% of the study population was affected by AT. These results, similar to those of other studies, underline the importance of CD in this group, also with regard to

Fig. 1. ROC curve.
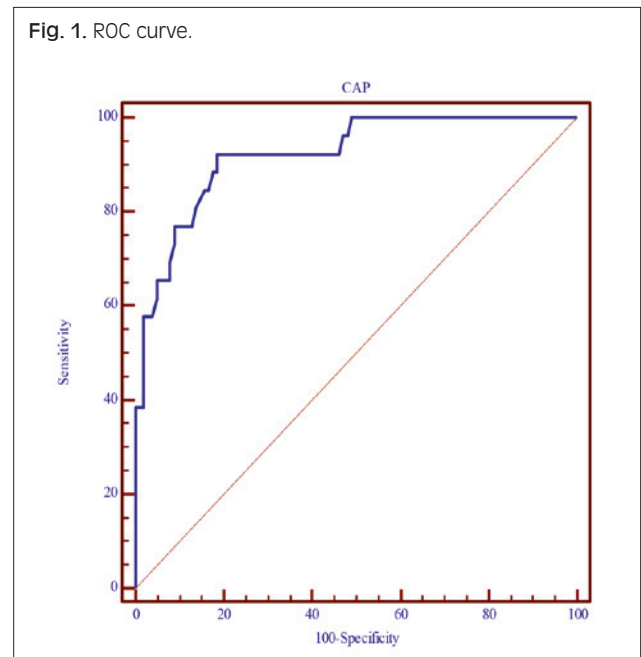
Tab. II. Factors associated with atherosclerosis.

Variable	Univariate analysis OR (95% CI)	p	Multivariate analysis OR (95% CI)	p
Male sex	1.78 (0.68-4.70)	0.23		
Age	5.80 (1.8-19.5)	0.004	5.8 (2.2-24.5)	< 0.001
CD4 at nadir (per 100 cells increase)	1.08 (0.90-1.42)	0.29		
CD4 cells count (per 100 cells increase)	0.99 (0.78-1.13)	0.66		
HIVRNA < 20 cp/ml	0.44 (0.05-1.67)	0.26		
Current smoking	1.25 (0.5-13)	0.62		
Obesity (BMI≥30 kg/m^2)	15.0 (2.8-79.7)	0.005	5.82 (0.6-39.5)	0.14
Diabetes (FPG > 126 mg/dl)	6.0 (1.3-28.7)	0.02	2.5 (2.3-20.7)	0.30
Hypertension (BP > 140/90 mmHg)	3.90 (1.5-5.3)	0.005	2.14 (0.53-8.6)	0.28
Dyslipidemia	3.80 (0.9-17.6)	0.07	4.39 (0.45-42.7)	0.20
S2	20.3 (7.0-58.4)	<0.001	20.3 (4.5-60.1)	< 0.001

age; therefore, to address this issue is important for normalizing the life expectancy of HIV population [43, 44]. In our study, we have found that 24.2% of HIV-infected subjects had S2, 78.9% dyslipidemia and 17.9% were affected by hypertension. Our results regarding the univariate analysis have shown that age, diabetes, hypertension, obesity and S2 were associated with AT. This correlation did not remain significant in multivariate analysis unless age and S2.

The elevated prevalence of CD and its correlation with age suggest that HIV patients may present accelerated vascular aging [44]. Nevertheless further studies are needed to clarify the mechanisms unrelated to natural aging process. Furthermore, the study has shown an association between carotid AT disease and S2, and the CAP value > 250 dB/m^{-1} discriminant in predicting AT has been detected. Thus, in patients with a value higher than the discriminant CAP value, an early evaluation of c-IMT may be strongly recommended.

Previous studies on the correlation between fatty liver disease and CD have displayed a prevalence of fatty liver of 37% and 13% in population with coronary artery disease [44, 45], *versus* 73.1% founded in our study.

The cause-effect relationship between these two factors still remains confusing, but the inflammatory state may be the common risk factor for AT [46].

The ultrasonographic system used to measure c-IMT seems a well-established method for assessing subclinical AT in the HIV-negative population and it is the only non-invasive imaging recommended by the American Heart Association for risk assessment for CD [47]. Moreover, c-IMT may be a useful methodology to evaluate the intima-media thickening implicated in the development and progression of atherosclerotic disease in HIV patients, as well [40, 48].

Our study have several strengths, in fact to the best of our knowledge, it is one of the few studies that examines the association between fatty liver disease and AT in HIV-infected persons.

Furthermore, the results of this paper may have important clinical implications. The elastography, used to detect HS, is a useful tool not dependent on operator skill and does not expose the patient to ionizing radiation. Other advantages of CAP include its simplicity, accurate quantification, inexpensive and sensitivity to lesser degrees of HS [49]. Therefore, its use in the normal clinical routine could be strongly recommended.

Weaknesses of the research include the cross-sectional design, therefore prospective studies might be appropriate to evaluate the progression of fatty liver disease in CD. Moreover, the sample size was limited to arrive at definite conclusions, thus the study needs to be confirmed in a larger population. The assessment of inflammatory markers to explain the association between HIV serostatus and c-IMT should be included in future studies also consequently to conflicting evidences founded [50].

In summary, HIV-infected populations have a high prevalence of subclinical AT. Moreover, AT is associated with fatty liver disease and HS assessment results crucial both in clinical practice for management of patients with chronic liver disease and for reducing risk factors, and in clinical research for epidemiological and therapeutic studies.

Early identification of CD risk in HIV patients could permit to modify lifestyle and to take therapeutic measures in order to prevent or delay the onset of chronic diseases difficult to manage.

Acknowledgments

Authors' contributions

AZ, MG, PG conceived, designed and coordinated the research. GP performed ultrasonography and fibroscan examination. AZ, MRT and DDA performed the data quality control. MG and FB optimized the informatics database. MG performed the statistical analyses. AZ, MG, MRT, DDA and PG evaluated the results. AZ, MG, and MRT wrote the manuscript. All Authors revised the manuscript and gave their contribution to improve the paper. All authors read and approved the final manuscript.

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