Drug and Alcohol REVIEW

APSAD

Drug and Alcohol Review (2016)
DOI: 10.1111/dar.12398

No significant effect of cannabis use on the count and percentage of circulating CD4 T-cells in HIV-HCV co-infected patients (ANRS CO13-HEPAVIH French cohort)

FABIENNE MARCELLIN[1,2,3], CAROLINE LIONS[1,2,3], ERIC ROSENTHAL[4,5], PERRINE ROUX[1,2,3], PHILIPPE SOGNI[6], LINDA WITTKOP[7,8], CAMELIA PROTOPOPESCU[1,2,3], BRUNO SPIRE[1,2,3], DOMINIQUE SALMON-CERON[9,10], FRANÇOIS DABIS[7,8], MARIA PATRIZIA CARRIERI[1,2,3] & HEPAVIH ANRS CO13 STUDY GROUP*

[1]*INSERM, UMR_S 912, Sciences Economiques & Sociales de la Santé et Traitement de l'Information Médicale (SESSTIM), F-13385, Marseille, France,* [2]*Aix Marseille Université, UMR_S 912, IRD, Marseille, F-13385, Marseille, France,* [3]*ORS PACA, Observatoire Régional de la Santé Provence-Alpes-Côte d'Azur, F-13006, Marseille, France,* [4]*Nice Sophia-Antipolis University, Nice, France,* [5]*Department of Internal Medicine, L'Archet Hospital, Nice, France,* [6]*Inserm U1016, Hepatology Unit, APHP, Hôpital Cochin, Paris Descartes University, Paris, France,* [7]*ISPED, Centre INSERM U897-Epidemiology-Biostatistics, Bordeaux University, Bordeaux, France,* [8]*ISPED, Centre INSERM U897-Epidemiology-Biostatistics, INSERM, Bordeaux, France,* [9]*Infectious and Tropical Diseases Service, Hôpital Cochin, AP-HP, Paris, France, and* [10]*Paris Descartes University, Paris, France*

Abstract

Introduction and Aims. *Despite cannabis use being very common in patients co-infected with HIV and hepatitis C virus (HCV), its effect on these patients' immune systems remains undocumented. Documenting the potential effect of cannabis use on HIV immunological markers would help caregivers make more targeted health recommendations to co-infected patients. We performed a longitudinal analysis of the relationship between cannabis use and peripheral blood CD4 T-cell measures in co-infected patients receiving antiretroviral therapy.* ***Design and Methods.*** *Cannabis use was assessed using annual self-administered questionnaires in 955 patients (2386 visits) enrolled in the ANRS CO13-HEPAVIH cohort. The effect of cannabis use on circulating CD4 T-cell count and percentage was estimated using multivariate linear regression models with generalised estimating equations. Sensitivity analyses were conducted after excluding visits where (i) tobacco use and (ii) smoking >=10 tobacco cigarettes/day were reported.* ***Results.*** *At the first visit, 48% of patients reported cannabis use during the previous four weeks, and 58% of these patients also smoked ≥10 tobacco cigarettes/day. After multiple adjustment, cannabis use was not significantly associated with either circulating CD4 T-cell count [model coefficient (95% confidence interval): 0.27 (−0.07; 0.62), P= 0.12] or percentage [−0.04 (−0.45; 0.36), P= 0.83]. Sensitivity analyses confirmed these results.* ***Discussion and Conclusions.*** *Findings show no evidence for a negative effect of cannabis use on circulating CD4 T-cell counts/percentages in HIV-HCV co-infected patients. In-depth immunological studies are needed to document whether cannabis has a harmful effect on CD4 levels in lungs and on cells' functional properties.* [Marcellin F, Lions C, Rosenthal E, Roux P, Sogni P, Wittkop L, Protopopescu C, Spire B, Salmon-Ceron D, Dabis F, Carrieri MP, HEPAVIH ANRS CO13 Study Group. No significant effect of cannabis use on the count and percentage of circulating CD4 T-cells in HIV-HCV co-infected patients (ANRSCO13-HEPAVIH French cohort). *Drug Alcohol Rev* 2016;xxx–xxx]

Key words: cannabis, CD4 T-cell, HCV, HIV, longitudinal analysis.

Marcellin Fabienne, PhD, Caroline Lions MSc, Statistician, Eric Rosenthal MD, Professor of Medicine, Perrine Roux PhD, Public Health Researcher, Philippe Sogni MD, Professor of Medicine, Linda Wittkop MD, PhD, Public Health Researcher, Camelia Protopopescu PhD, Research engineer, Bruno Spire MD, PhD, Public Health Researcher, Dominique Salmon-Ceron MD, Professor of Medicine, Public Health Researcher, François Dabis MD, Professor of Medicine, Public Health Researcher, Maria Patrizia Carrieri PhD, Public Health Researcher. Correspondence to Mrs Fabienne Marcellin, INSERM UMR912-SESSTIM-ORS PACA, Faculté de Médecine, 3e étage–Aile bleue, 27 boulevard Jean Moulin, 13385 Marseille Cedex 5, France. Tel: + 33 4 91 32 47 60; E-mail: fabienne.marcellin@inserm.fr

Received 22 June 2015; accepted for publication 25 January 2016.

Introduction

In France, cannabis is considered an illicit drug [1]. The oral spray Sativex® is the only tetrahydrocannabinol-containing therapeutic drug currently authorised with medical prescription in the country. However, its use remains rare and restricted to patients with multiple sclerosis. Along with increased supply, cannabis use has increased in recent years among people aged 18–64 years in the French general population, with 8% of users in 2010 and 11% in 2014. As in Canada [2], cannabis use is frequent in patients co-infected with HIV and hepatitis C virus (HCV) in France. In a survey conducted in 2011 in French hospitals among a nationally representative sample of people living with HIV, 49% of HIV-HCV co-infected patients reported cannabis use [3]. As cannabinoids have immunomodulatory effects, cannabis use may influence T-lymphocyte subpopulations. In this study, we focused our attention on the possible effect of cannabis use on peripheral blood CD4 T-cell measures (i.e. CD4 T-cell counts and percentages in the total lymphocyte count), which are key markers of HIV infection progression. Several studies addressing this issue have been conducted in different populations, with mixed results [4–9]. However, no data are currently available in the context of HIV-HCV co-infection.

We estimated the effect of cannabis use on CD4 T-cell measures using longitudinal data collected among co-infected patients receiving antiretroviral therapy (ART) enrolled in an observational cohort. Given the high rate of tobacco smokers among co-infected patients in France [3], our study took into account the potential concomitant (and possibly opposite) immune effects of tobacco smoking. Indeed, higher CD4 counts have been observed in tobacco smokers compared with non-smokers both in the general population [10] and in HIV-infected and uninfected men [5,11,12].

Patients and methods

Study sample

The sample for this study comprised patients participating in the ANRS CO13-HEPAVIH cohort [13,14] with cannabis use data available at least once during the first 5 years of follow-up, i.e. until month 60 (M60). A description of the cohort methodology can be found in the Supporting Information. Analyses included data from all annual visits associated with a self-administered questionnaire. For the purpose of sample homogeneity regarding HIV treatment status, the data corresponding to visits where ART was interrupted were excluded from the analyses.

Variables

Use of cannabis and other illicit drugs. In the self-administered questionnaire, patients were asked the following question concerning their consumption of cannabis and other illicit drugs (including cocaine, heroin, crack, ecstasy, street buprenorphine, amphetamines, LSD and other hallucinogens): 'During the last four weeks, have you used one or more of the following drugs?'. Possible answers were 'never', 'sometimes', 'regularly' and 'every day' in relation to each drug type. This question has been previously used to assess drug consumption in the French general population [15] and in HIV-infected patients [16]. Patients who answered 'never' to this question for cannabis but who reported daily cannabis use during face-to-face interviews by their physician were classified as daily cannabis users.

Other behavioural variables. During a face-to-face medical interview, patients were asked about their current and past tobacco use practices, as well as the number of tobacco cigarettes they smoked per day. A cut-off of 10 tobacco cigarettes/day was used to distinguish moderate smokers from non-moderate smokers [17–19]. Data about patients' alcohol consumption (during the previous six months) and adherence to ART (during the previous four days) were collected in the self-administered questionnaire. In addition, history and current presence of alcohol-related problems were documented by physicians. With regard to alcohol consumption, patients were classified into one of the following three categories [20]: (i) current alcohol-related problems (physician's report); (ii) hazardous drinking or binge drinking without alcohol-related problems, defined as an Alcohol Use Disorders Identification Test–Consumption (AUDIT-C) score $\geq$4 (for men) or $\geq$3 (for women) (with a time frame for this scale fixed at six months in our study), or consumption of $\geq$6 drinks on any one occasion during the previous six months; and (iii) non-hazardous drinking, defined as an AUDIT-C score $<$4 (for men), $<$3 (for women) [21]. Patients were also classified into the categories 'high', 'medium' or 'low' adherence to ART, according to both the rate of prescribed doses of ART they actually took during the previous four days (100%, between 80% and 99.9%, $<$80%) and their compliance with drugs schedule [22,23].

Assessment of CD4 T-cell counts and percentages. CD4 T-cell numbers and percentages were determined using flow cytometry, following the World Health Organization laboratory guidelines for enumerating CD4 T lymphocytes in the context of HIV/AIDS [24]. CD4 T-cell measures were performed by the laboratory of each given hospital participating in the cohort.

Biomedical and clinical variables. Analyses of circulating CD4 T-cell counts and percentages were adjusted for significant correlates from a list, including gender, age, body mass index, and HIV- and HCV-related characteristics (time since HCV diagnosis, HCV status at enrolment in the cohort, F3–F4 fibrosis stage defined as a FIB-4 index > 3.25 [25,26], HCV treatment status, time since HIV diagnosis, time since ART initiation, undetectable HIV viral load defined as a viral load below the limit of detection of the given hospital's laboratory test, Centers for Disease Control and Prevention clinical stage and type of ART drugs received).

Statistical analyses

The main characteristics of the study sample at first visit with available self-administered questionnaire were compared between patients who reported cannabis use and those who did not (Student's *t*-test or Mann–Whitney test for continuous variables, χ^2 test for categorical variables). Linear regression models using generalised estimating equations [27] were used on repeated measures of the count and percentage of circulating CD4 T-cells. The distribution of circulating CD4 T-cell counts was normalised using a square root transformation. Six time-dependent variables assessing cannabis use were tested for a possible relationship with CD4 measures. A dichotomous variable (not using cannabis vs. using cannabis) was first tested. Three variables exploring the frequency of cannabis use were then tested, including a four-category variable (no use; occasional use; regular use; daily use), and two dichotomous variables for 'daily use' (yes vs. no) and 'regular or daily use' (yes vs. no), respectively. Finally, in order to take into account potential interactions between cannabis use and tobacco use, two variables cross-referencing these behaviours were then tested, including a four-category variable (no regular or daily cannabis use and history of tobacco use; no regular or daily cannabis use and smoking < 20 tobacco cigarettes (one pack)/day; no regular or daily cannabis use and smoking > one pack/day; regular or daily cannabis use), and a three-category variable (cannabis use; cannabis use and smoking < 10 tobacco cigarettes/day; cannabis use and smoking ≥ 10 tobacco cigarettes/day). Variables with a P value <0.25 in the univariate analyses were considered eligible for multivariate analysis. Multivariate models were adjusted for significant biomedical, clinical and behavioural variables ($P < 0.05$, backward selection procedure). The variable 'Current tobacco use' was systematically entered in the multivariate models and retained when significant ($P < 0.05$) or when modifying substantially the model coefficients. In order to minimise potential confounding between the effect of cannabis use and that of tobacco use, two sensitivity analyses were conducted after exclusion of data corresponding to: (i) visits with self-reports of tobacco use; and (ii) visits with self-reports of tobacco use exceeding 10 tobacco cigarettes/day. Finally, in order to account more directly for the issue of varying ART use and adherence to ART, the stability of results from the main analysis was checked in an additional analysis restricted to data collected during follow-up visits with suppressed plasma HIV viral load. All tests were two-sided, with a significance threshold fixed at $\alpha = 0.05$. SAS version 9.3 for Windows was used for the analyses.

Results

Among the 1246 participants with available records in the HEPAVIH socio-behavioural database as of 21 November 2014, the 955 patients (reflecting 2386 visits) who had cannabis use data available at least once until M60 constituted the study sample. A total of 78 patients (8% of the study sample) were not receiving ART at enrolment in the cohort. The proportion of patients with undetectable HIV viral load at enrolment was significantly higher in the study sample compared with the remaining cohort participants (i.e. patients without available cannabis use data) (74% vs. 59%; $P < 0.001$). Further, median (interquartile range) percentage of circulating CD4 T-cells was significantly lower [26 (20; 34) vs. 28 (20; 36); $P = 0.02$]. No significant difference was detected between patients in the study sample and the remaining cohort participants with respect to gender, age, circulating CD4 T-cells count, HCV status and fibrosis stage at enrolment (data not shown).

Characteristics of the study sample at first visit

In the study sample, median (interquartile range) count and percentage of circulating CD4 T-cells were 454 (309; 652) cells/mm^3 and 26% (20%; 34%), respectively, with no significant difference between patients who reported cannabis use at their first visit and those who did not (Table 1). Patients who reported cannabis use at their first visit were significantly younger, and more often underweight than those who did not. They also reported higher rates of tobacco, alcohol and illicit drugs consumption, and they were diagnosed with HIV for a longer time.

The vast majority of patients (124 out of 132) who initiated HCV treatment during follow-up received pegylated interferon and ribavirin. Mean (standard deviation) duration of follow-up was 31 (24) months.

Table 1. *Characteristics of the study sample (n = 955) at first visit with available self-administered questionnaire: data according to cannabis use status (ANRS CO13-HEPAVIH cohort, France)*

Characteristics	Overall	Cannabis use		*P*-value[a]
		Yes (48%)	No (51%)	
	No. (%) of patients, *mean* (*standard deviation*) or median [interquartile range]			
Male gender	671 (70)	321 (73)	322 (69)	0.18
Age, years	*45.6* (*6.0*)	*45.1* (*5.0*)	*46.0* (*6.3*)	0.01
Behavioural characteristics				
Frequency of cannabis use (*n* = 905)				
- never	466 (51)	—	466 (100.0)	—
- sometimes	195 (22)	195 (44)	—	
- regularly	109 (12)	109 (25)	—	
- everyday	135 (15)	135 (31)	—	
History of tobacco use (*n* = 924)	137 (15)	25 (6)	98 (22)	<0.0001
Current tobacco use (*n* = 927)	678 (73)	386 (91)	272 (60)	<0.0001
Cannabis use according to past and current tobacco use (*n* = 857)				<0.0001
- no regular or daily cannabis use, history of tobacco use	112 (13)	14 (3)	98 (22)	
- no regular or daily cannabis use, smoking <one 20-pack/day	306 (36)	94 (22)	212 (49)	
- no regular or daily cannabis use, smoking >one 20-pack/day	195 (23)	71 (17)	124 (29)	
- regular or daily cannabis use	244 (28)	244 (58)	0	
Cannabis use according to the no. of daily tobacco cigarettes used (*n* = 842)				
- no cannabis use	434 (52)	—	434 (100)	—
- cannabis use and <10 tobacco cigarettes/day	113 (13)	113 (28)	—	
- cannabis use and ≥10 tobacco cigarettes/day	295 (35)	295 (72)	—	
Use of at least one illicit drug[b]	108 (11)	73 (17)	35 (7)	<0.0001
Alcohol use category[c]				
- non-hazardous drinking	575 (60)	236 (54)	304 (65)	0.002
- hazardous or binge drinking	259 (27)	135 (31)	112 (24)	
- alcohol-related problems	121 (13)	68 (15)	50 (11)	
Adherence to ART[d] (*n* = 941)				
- low	72 (7)	37 (8)	31 (7)	0.30
- medium	223 (24)	111 (26)	104 (23)	
- high	646 (69)	286 (66)	324 (70)	
Biomedical and clinical characteristics				
Body mass index[e] (*n* = 931)				
- underweight	112 (12)	72 (17)	37 (8)	
- normal	655 (71)	312 (73)	314 (69)	
- overweight	141 (15)	34 (8)	92 (20)	
- obese	23 (2)	8 (2)	13 (3)	<0.0001
Time since HCV diagnosis—in years (*n* = 947)	*10.2* (*5.5*)	*10.3* (*5.3*)	*10.1* (*5.6*)	*0.56*
HCV status at enrolment				
- chronically infected patient	846 (89)	395 (90)	408 (88)	0.25
- treatment-induced HCV-cleared patient	109 (11)	44 (10)	58 (12)	
F3–F4 fibrosis stage[f] (*n* = 939)	147 (16)	66 (15)	74 (16)	0.65
Time since HIV diagnosis, years (*n* = 952)	*16.2* (*5.5*)	*17.0* (*5.0*)	*15.8* (*5.7*)	0.001
Time since ART initiation, years	*10.3* (*4.6*)	*10.5* (*4.4*)	*10.4* (*4.7*)	0.82
CD4 T-cell count◆, cells/mm^3 (*n* = 941)	454 [309; 652]	458 [311; 649]	455 [313; 660]	0.88
CD4 T-cell percentage◆ (*n* = 942)	26 [20; 34]	26 [19; 33]	27 [20; 34]	0.30
Undetectable HIV viral load[g] (*n* = 940)	715 (76)	330 (76)	349 (76)	0.95
CDC stage (*n* = 948)				
- A	429 (45)	184 (42)	222 (48)	0.09
- B	248 (26)	128 (29)	108 (23)	
- C	271 (29)	125 (29)	132 (29)	
Receiving a protease inhibitor-containing ART regimen	694 (73)	315 (72)	337 (72)	0.85
Receiving a non-nucleoside reverse transcriptase inhibitor-containing ART regimen	221 (23)	101 (23)	111 (24)	0.77
	885 (93)	413 (94)	426 (91)	0.12

(*Continues*)

Table 1. (Continued)

Characteristics	Overall	Cannabis use		*P*-value[a]
		Yes (48%)	No (51%)	
	No. (%) of patients, *mean* (*standard deviation*) or median [interquartile range]			
Receiving a nucleoside reverse transcriptase inhibitor-containing ART regimen				
Receiving an integrase inhibitor-containing ART regimen	34 (4)	14 (3)	20 (4)	0.38
Receiving a fusion inhibitor-containing ART regimen	17 (2)	10 (2)	6 (1)	0.26
Receiving a nucleoside and non-nucleoside reverse transcriptase inhibitors combined ART regimen	2 (0.2)	1 (0.2)	1 (0.2)	0.97

◆In the peripheral blood (i.e. circulating cells). [a]Comparison between patients reporting cannabis use and those not reporting cannabis use (Student's *t*-test or Mann–Whitney test for continuous variables, χ^2 test for categorical variables). [b]Defined as reporting to have used at least one of the following drugs during the previous four weeks: cocaine, heroin, crack, ecstasy, street buprenorphine, amphetamines, LSD or other hallucinogens. [c]Variable using the following three-category classification for alcohol consumption [20]: (i) current alcohol-related problems (physician's report); (ii) hazardous or binge drinking without alcohol-related problems, defined as an Alcohol Use Disorders Identification Test—Consumption (AUDIT-C) score ≥4 (for men) or ≥3 (for women), reporting consumption ≥6 drinks on any one occasion during the previous 6 months; and (iii) non-hazardous drinking, defined as an AUDIT-C score <4 (for men), <3 (for women) [21]. [d]Patients were classified into the categories 'high', 'medium' or 'low' adherence to ART according to both the rate of prescribed doses of ART they actually took during the previous four days (100%, between 80% and 99.9%, < 80%) and their compliance with their drugs schedule [22,23]. [e]The four classifications for body mass index were defined as follows [28]: 'underweight' for values <18.5, 'normal' for values between 18.5 and 24.99, 'overweight' for values between 25 and 30, 'obese' for values ≥30. [f]Defined as an FIB-4 index > 3.25 [25,26]. [g]Defined as a plasma HIV viral load below the limit of detection of the given hospital's laboratory test. ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HCV, hepatitis C virus.

Rates of cannabis use during follow-up

The percentage of patients reporting cannabis use varied between 40% and 48% during follow-up. Occasional users (i.e. patients reporting that they used cannabis 'sometimes') comprised the largest proportion of cannabis users (Figure 1a). Non-moderate tobacco users (≥10 tobacco cigarettes/day) accounted for 58% to 72% of cannabis users (Figure 1b).

Analyses of circulating CD4 T-cells counts and percentages during the follow-up

The counts and percentages of circulating CD4 T-cells significantly increased with time ($P < 0.0001$). No systematic difference was observed for the distributions of these two variables between patients who reported cannabis use and those who did not (Figures 2 and 3).

Univariate analyses. In the univariate analyses, the significant correlates of circulating CD4 T-cell count or percentage included CD4 levels at first visit, gender, age, time since HIV and HCV diagnosis, duration and type of ART, HCV treatment status, clinical parameters (HIV viral load, Centers for Disease Control and Prevention clinical stage, fibrosis stage) and alcohol-related problems (Table 2).

Current tobacco use was not significantly associated with the count ($P = 0.24$) or percentage ($P = 0.33$) of circulating CD4 T-cells.

Cannabis use, whether combined or not with current or past tobacco use, was not significantly associated with the count or percentage of circulating CD4 T-cells (Table 3).

Cannabis use overall and according to the number of tobacco cigarettes smoked, i.e. <10 versus ≥10 tobacco cigarettes/day, were both eligible to enter the multivariate model for circulating CD4 T-cell count ($P < 0.25$).

Multivariate analyses. In the multivariate analyses, cannabis use and cannabis use according to the number of tobacco cigarettes smoked (<10 vs. ≥10 tobacco cigarettes/day) were not significantly associated with either count or percentage of circulating CD4 T-cells, after adjustment for baseline CD4 value and time-dependent HIV- and HCV-related clinical variables (Table 3). The variable 'Current tobacco use' was not retained in the multivariate models, as it was not significant at the 0.05 level and was not a substantial effect modifier.

Sensitivity analyses. After exclusion of the 1553 visits with self-reported tobacco use, cannabis use was significantly associated with a lower percentage of circulating CD4 T-cells ($P = 0.049$) in the univariate analyses.

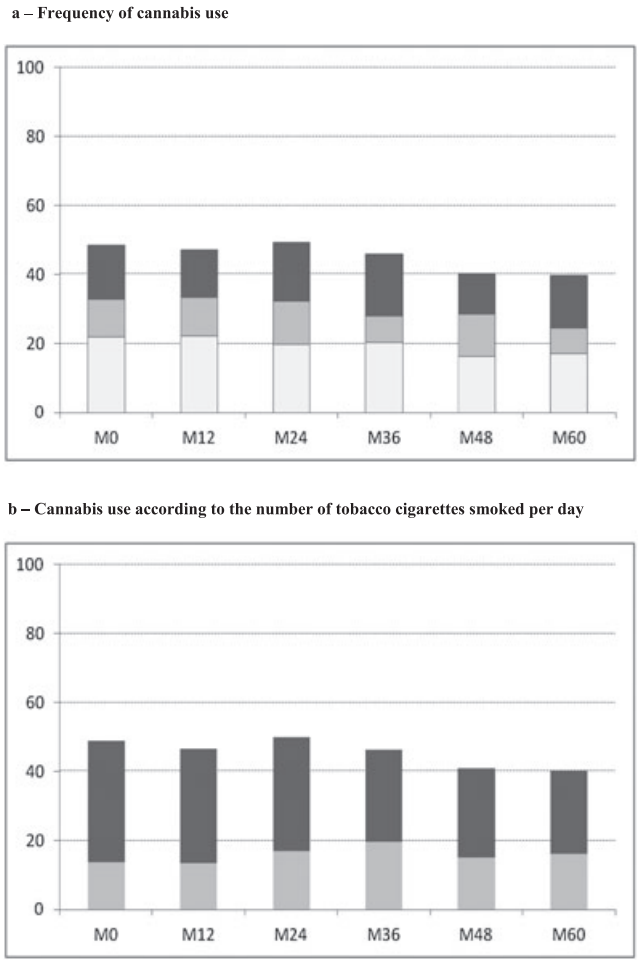

Figure 1. *Percentage of patients reporting cannabis use in the study sample: data per visit (ANRS CO13-HEPAVIH cohort, France). 1a—Frequency of cannabis use. Horizontal axis: months of follow-up; vertical axis: percentage of patients in each category of cannabis users (light gray: patients who reported using cannabis 'sometimes' (occasional use); medium gray: patients who reported using cannabis 'regularly'; dark gray: patients who reported using cannabis 'daily'). 1b—Cannabis use according to the number of tobacco cigarettes smoked per day. Horizontal axis: months of follow-up; vertical axis: percentage of patients in each category of cannabis users (medium gray: patients who reported using cannabis and less than 10 tobacco cigarettes/day; dark gray: patients who reported using cannabis and at least 10 tobacco cigarettes/day).*

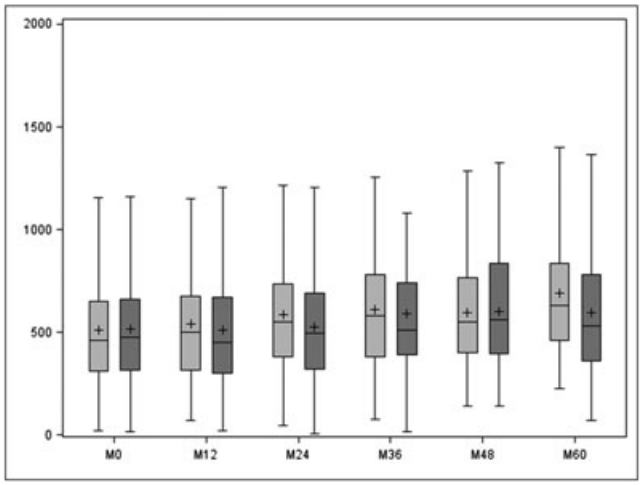

Figure 2. *Distribution of circulating CD4 T-cell count in the study sample in terms of cannabis use or not, as a function of follow-up visit (ANRS CO13-HEPAVIH cohort, France). Horizontal axis: follow-up visit (months); vertical axis: circulating CD4 T-cell count (cells/mm^3); light grey = cannabis use; dark grey = no cannabis use. The boxplots present median values and interquartile ranges (box), lines (whiskers) include all points within 1.5 interquartile range of the nearest quartile. The '+' sign locates the mean.*

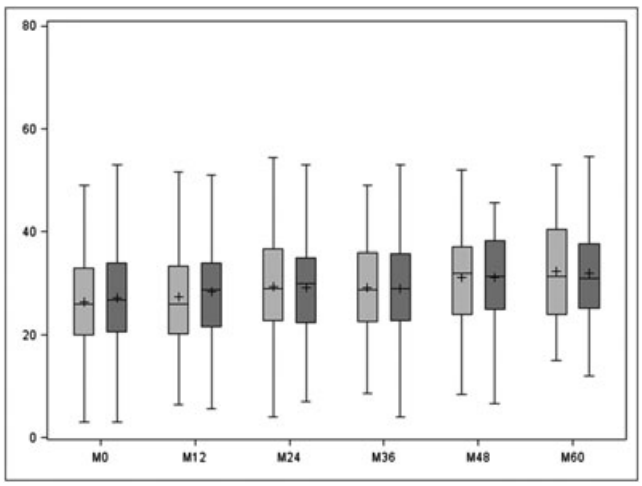

Figure 3. *Distribution of circulating CD4 T-cell percentage in the study sample in terms of cannabis use or not, as a function of follow-up visit (ANRS CO13-HEPAVIH cohort, France). Horizontal axis: follow-up visit (months); vertical axis: circulating CD4 T-cell percentage (in the total lymphocyte count); light grey = cannabis use; dark grey = no cannabis use. The boxplots present median values and interquartile ranges (box), lines (whiskers) include all points within 1.5 interquartile range of the nearest quartile. The '+' sign locates the mean.*

However, this relationship was no longer significant in the multivariate analysis.

After exclusion of the 1144 visits with self-reported tobacco use exceeding 10 tobacco cigarettes/day, cannabis use was not significantly associated with the count or percentage of circulating CD4 T-cells in the univariate analyses, irrespective of whether or not it was combined with current or past tobacco use (data not shown).

Analyses restricted to follow-up visits with suppressed plasma HIV viral load. `The results of this additional analysis (including 877 patients, for a total of 1943 visits) confirmed that cannabis use has no significant effect on the count or percentage of circulating CD4 T-cells (data not shown).

Discussion

In this longitudinal analysis based on five-year follow-up data, we found no significant effect of cannabis

Table 2. *Main correlates of the count and percentage of circulating CD4 T-cells in the study sample: univariate linear regression models with generalised estimating equations (ANRS CO13-HEPAVIH cohort, France)*

	Model outcome = CD4 T-cell count⋆	Model outcome = CD4 T-cell percentage⋆
Variables	Coefficient [95% confidence interval] (*P-value*)	
Male gender	−1.63 [−2.46; −0.80] (*P < 0.0001*)⋆	−3.89 [−5.19; −2.60] (*P < 0.0001*)⋆
Age, years	0.12 [0.07; 0.17] (*P < 0.0001*)⋆	0.21 [0.12; 0.30] (*P < 0.0001*)⋆
Behavioural characteristics		
History of tobacco use	−0.25 [−1.02; 0.51] (*P = 0.52*)	0.08 [−1.01; 1.18] (*P = 0.88*)
Current tobacco use	0.39 [−0.26; 1.05] (*P = 0.24*)⋆	−0.48 [−1.44; 0.48] (*P = 0.33*)
Use of at least one illicit drug during the previous 4 weeks	0.40 [−0.46; 1.25] (*P = 0.37*)	−0.48 [−1.43; 0.46] (*P = 0.31*)
Alcohol use category (ref. = non-hazardous)	(*P = 0.02*)⋆	(*P = 0.02*)⋆
- hazardous or binge drinking	0.52 [−0.10; 1.04]	−0.50 [−1.24; 0.25]
- alcohol-related problems	−0.96 [−2.04; 0.11]	−2.77 [−4.71; −0.82]
Adherence to ART (ref. = low) - medium or high	−0.18 [−0.72; 0.36] (*P = 0.52*)	−0.20 [−0.91; 0.50] (*P = 0.58*)
Clinical characteristics		
Body mass index (ref. = underweight)	(*P = 0.10*) ⋆	(*P = 0.28*)
- normal	0.15 [−0.67; 0.98]	−0.97 [−2.11; 0.18]
- overweight	0.89 [−0.17; 1.94]	−0.95 [−2.43; 0.53]
- obese	1.64 [0.09; 3.19]	−0.08 [−2.17; 2.01]
Time since HCV diagnosis, years	0.12 [0.07; 0.18] (*P < 0.0001*)⋆	0.28 [0.18; 0.37] (*P < 0.0001*)⋆
HCV status at enrolment (ref. = chronically infected patient)		
- treatment-induced HCV-cleared patient	0.96 [−0.03; 1.96] (*P = 0.06*)⋆	1.05 [−0.74; 2.84] (*P = 0.25*)
Receiving HCV treatment	−3.77 [−4.46; −3.09] (*P < 0.0001*)⋆	5.00 [3.74; 6.25] (*P < 0.0001*)⋆
F3–F4 fibrosis stage	−3.12 [−3.73; −2.51] (*P < 0.0001*)⋆	−0.38 [−1.48; 0.71] (*P = 0.49*)
Time since HIV diagnosis, years	0.13 [0.07; 0.18] (*P < 0.0001*)⋆	0.25 [0.17; 0.34] (*P < 0.0001*)⋆
Time since ART initiation, years	0.17 [0.11; 0.23] (*P < 0.0001*)⋆	0.35 [0.25; 0.45] (*P < 0.0001*)⋆
CD4 T-cell count◆ at first visit, cells/mm^3	0.88 [0.85; 0.91] (*P < 0.0001*)⋆	—
CD4 T-cell percentage◆ at first visit	—	0.90 [0.87; 0.94] (*P < 0.0001*)⋆
Undetectable HIV viral load	1.42 [0.90; 1.95] (*P < 0.0001*)⋆	2.97 [2.35; 3.60] (*P < 0.0001*)⋆
CDC stage (ref. = A)	(*P = 0.003*) ⋆	(*P = 0.0002*) ⋆
- B	−0.35 [−1.25; 0.54]	−0.12 [−1.60; 1.36]
- C	−1.49 [−2.37; −0.62]	−2.74 [−4.11; −1.36]
Receiving a protease inhibitor-containing ART regimen	−0.74 [−1.32; −0.16] (*P = 0.01*)⋆	−1.76 [−2.64; −0.88] (*P < 0.0001*)⋆
Receiving a non-nucleoside reverse transcriptase inhibitor-containing ART regimen	0.87 [0.27; 1.46] (*P = 0.005*)⋆	1.45 [0.52; 2.38] (*P = 0.002*)⋆
Receiving a nucleoside reverse transcriptase inhibitor-containing ART regimen	−0.10 [−0.84; 0.64] (*P = 0.79*)	0.61 [−0.53; 1.75] (*P = 0.29*)
Receiving an integrase inhibitor-containing ART regimen	0.11 [−0.52; 0.75] (*P = 0.73*)	0.66 [−0.14; 1.47] (*P = 0.11*)⋆
Receiving a fusion inhibitor-containing ART regimen	−0.38 [−1.68; 0.92] (*P = 0.56*)	−3.09 [−4.73; −1.45] (*P = 0.0009*)⋆
Receiving a nucleoside and non-nucleoside reverse transcriptase inhibitors combined ART regimen	2.27 [1.33; 3.20] (*P < 0.0001*)⋆	4.82 [2.92; 6.71] (*P < 0.0001*)⋆

◆In the peripheral blood (i.e. circulating cells). [a]A square-root transformation was applied to normalise CD4 T-cell counts. ⋆Variable eligible for multivariate analysis ($P < 0.25$). ART, antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HCV, hepatitis C virus.

use on CD4 T-cells measures in the peripheral blood of 955 ART-treated HIV-HCV co-infected patients. This result confirms those of previous studies conducted in HIV-infected and uninfected patients [5,7]. However, given the deleterious effects of the smoking route of administration, studies considering other routes of administration for cannabis (such as oral sprays) may give different results. In addition, the immune effects of cannabinoids are highly dependent on the type, concentration and context (named the 'entourage effect') of administered cannabinoids. The findings of this study thus suggest that the cannabinoids contained in cannabis—irrespective of their types and ratios in the product used—do not influence circulating CD4 T-cell

Table 3. *Effect of cannabis use on the count and percentage of circulating CD4 T-cells in the study sample (linear regression models with generalised estimating equations, data from the ANRS CO13-HEPAVIH cohort, France)*

	Model outcome = CD4 T-cell count◆[a]		Model outcome = CD4 T-cell percentage◆	
	Univariate analysis	Multivariate analysis[b]	Univariate analysis	Multivariate analysis[b]
	Coefficient [95% CI] (*P-value*)			
Cannabis use (ref. = no)	0.48 [−0.11; 1.08] (*P = 0.11*)⋆	0.27 [−0.07; 0.62] (*P = 0.12*)	−0.31 [−1.11; 0.48] (*P = 0.48*)	−0.04 [−0.45; 0 .36] (*P = 0.83*)
Frequency of cannabis use (ref. = never)	(*P = 0.36*)	(*P = 0.41*)	(*P = 0.50*)	(*P = 0.32*)
- sometimes	0.55 [−0.13; 1.23]	0.38 [−0.06; 0.82]	−0.09 [−0.94; 0.76]	0.17 [−0.31; 0.65]
- regularly	0.57 [−0.21; 1.36]	0.11 [−0.38; 0.60]	−0.70 [−1.77; 0.38]	−0.30 [−0.86; 0.26]
- everyday	0.22 [−0.61; 1.05]	0.18 [−0.29; 0.64]	−0.72 [−1.83; 0.39]	−0.26 [−0.83; 0.30]
Regular or daily cannabis use	−0.14 [−0.46; 0.75] (*p = 0.65*)	−0.07 [−0.41; 0.26] (*p = 0.66*)	−0.66 [−1.50; 0.18] (*p = 0.13*)	−0.36 [−0.77; 0.05] (*p = 0.09*)
Daily cannabis use	−0.16 [−0.88; 0.55] (*P = 0.65*)	0.03 [−0.42; 0.48] (*P = 0.89*)	−0.37 [−1.23; 0.48] (*P = 0.39*)	−0.20 [−0.70; 0.31] (*P = 0.45*)
Cannabis use according to past and current tobacco use (ref. = no regular or daily cannabis use, smoking <one 20-pack/day)	(*P = 0.49*)	(*P = 0.49*)	(*P = 0.37*)	(*P = 0.26*)
- no regular or daily cannabis use, history of tobacco use	−0.43 [−1.32; 0.46]	−0.43 [−1.32; 0.46]	0.01[−1.49; 1.52]	−0.44 [−1.21; 0.33]
- no regular or daily cannabis use, smoking >one 20-pack/day	0.34 [−0.47; 1.16]	0.34 [−0.47; 1.16]	0.32 [−0.95; 1.58]	−0.12 [−0.71; 0.47]
- regular or daily cannabis use	0.18 [−0.51; 0.87]	0.18 [−0.51; 0.87]	−0.67 [−1.68; 0.33]	−0.47 [−0.97; 0.04]
Cannabis use according to the no. of tobacco cigarettes smoked (ref. = no cannabis use)	(*P = 0.17*)⋆	(*P = 0.22*)	(*P = 0.61*)	(*P = 0.89*)
- Cannabis use and <10 tobacco cigarettes/day	0.62 [−0.16; 1.40]	0.25 [−0.22; 0.72]	−0.31 [−1.35; 0.73]	0.02 [−0.56; 0.61]
- Cannabis use and ≥10 tobacco cigarettes/day	0.60 [−0.09; 1.28]	0.34 [−0.06; 0.75]	−0.47 [−1.41; 0.47]	−0.09 [−0.54; 0.36]

◆In the peripheral blood (i.e. circulating cells). ⋆Variable eligible for multivariate analysis ($P < 0.25$). [a]A square-root transformation was applied to normalise CD4 T-cell counts. [b]After adjustment for CD4 T-cell count (or percentage) at first visit and for significant time-dependent variables among the following: age, time since HCV diagnosis, F3–F4 fibrosis stage (assessed using the FIB-4 index [25,26]), HCV treatment status (receiving vs. not receiving HCV treatment), undetectable HIV viral load, receiving a nucleoside and non-nucleoside reverse transcriptase inhibitors combined ART regimen, receiving a fusion inhibitor-containing ART regimen and time since ART initiation.

counts and percentages in HIV-HCV co-infected patients. However, a potential effect of cannabinoids on the functions of immune cells cannot be ruled out. Indeed, cannabinoids are known to influence many cellular and cytokine mechanisms [29]. The major psychoactive component of marijuana, Δ9-tetrahydrocannabinol, has been reported to suppress immune functions, including cell proliferation, antibody production, natural killer cell activity and macrophage function [8]. Impaired rosetting capacity has been observed in the peripheral blood CD4 and CD8 lymphocytes subsets of marijuana users [30,31]. Cannabinoids may also have important effects on T helper 1- and 2-specific cytokines and TGF-β secretion [32]. The inhibitory effects of cannabinoids on T-cells [33], such as decreases in number or sensitivity, have been found in some studies [34,35] but not others [36,37]. This may be partly explained by the heterogeneity of the studies' characteristics (routes of administration, type and quantity of cannabis used, tetrahydrocannabinol concentration, frequency of smoking and duration of inhalation) [33]. In the context of HIV infection, a randomised, prospective placebo-controlled study comparing the short-term effects of cannabinoids in ART-treated patients [8] showed no meaningful pattern of changes in immune phenotype or function over the 21-day study period in patients randomised to smoked marijuana or dronabinol, compared with patients receiving a placebo. However, the long-term effect of cannabinoids on the immune system of HIV-infected patients remains to be explored. This issue is of specific interest in light of the growing body of evidence on the structure and function of the endocannabinoid receptor system and its possible role in HIV disease [38]. In addition to their immunosuppressive and anti-inflammatory properties, cannabinoids are indeed hypothesised to have direct antiviral effects [39,40].

As expected, the prevalence of cannabis use was high in our study sample [2,3] in comparison with the general population [41]. Targeted actions for cannabis use prevention are thus needed, given the well-documented adverse health effects of cannabis use [42]. As some co-infected patients use cannabis to relieve disabling symptoms, research efforts on the therapeutic use of cannabinoids should be pursued.

Interestingly, in this study, tobacco use was not significantly associated with CD4 T-cell measures. This contrasts with results from several other studies showing a positive association between tobacco use and CD4 T-cell counts in HIV-infected and uninfected men [5,11,12,43]. In the present study, cannabis use remained non-significantly associated with CD4 levels after considering concomitant tobacco use. Furthermore, no significant association was observed between cannabis use and CD4 levels in the sensitivity analyses conducted in both moderate tobacco users and non-smokers. The relatively low number of non-smokers in this study may partly explain these results.

As observed elsewhere [9], use of illicit drugs other than cannabis was not associated with CD4 levels in this study.

As expected, both time since ART initiation—associated with duration of infection—and undetectable HIV viral load were identified as significant correlates of higher CD4 levels. F3–F4 fibrosis stage was associated with lower CD4 T-cell count, but was not significantly associated with CD4 T-cell percentage. Receiving HCV treatment was associated with lower CD4 T-cell count and higher CD4 T-cell percentage. These contrasting results may be related to the discordances between CD4 T-cell counts and percentages observed in HIV-infected patients with liver fibrosis [44].

Study limitations

The main limitation of this study is that analyses are restricted to the effect of cannabis use on the frequency of circulating CD4 T-cells. Future studies are needed to explore a potential effect on the cells' immune function, and to analyse common immune parameters, such as cytokine and chemokines, as well as their status in lungs, which is the primary organ affected by cannabis exposure. The frequency of CD4 T-cell subpopulations, including naïve and memory cells (which are not routinely measured in the clinical follow-up of co-infected patients), as well as CD8 T-cell measures, may also be analysed. Nevertheless, this study adds interesting data to the existing body of literature about cannabis use, which is an issue of growing interest given its global increase and the debate concerning the legalisation of cannabis in certain settings. This study is also limited by the high rate of concomitant use of cannabis and tobacco (also shown elsewhere [2,3]), preventing us from disentangling the effect of each of these two behaviours on CD4 measures. Finally, the higher percentage of patients with undetectable HIV viral load in the study sample compared with the remaining cohort participants may slightly restrict the external validity of our results. However, this percentage was close to that observed in co-infected patients who participated in the nationally representative survey ANRS-VESPA2 in 2011 [3].

Conclusion

In conclusion, we found no negative effect of cannabis use on the counts and percentages of circulating CD4 T-cells in ART-treated HIV-HCV co-infected patients. Our findings, together with data on the relatively low

contribution of cannabis use to global mortality [45], may help caregivers make useful recommendations to co-infected patients. Indeed, cannabis use in co-infected patients has probably both recreational and therapeutic purposes, just as is observed in the context of HIV infection [46]. Caregivers' recommendations should therefore focus on preventing excessive use of cannabis in order to lower the risk of addiction, cognitive problems and cancer [42]. Additional studies are needed to document the potential effect of cannabis use on CD4 levels in the lungs. Indeed, some differences have been observed in the lymphocytic subpopulation profiles between bronchoalveolar lavage fluid and peripheral blood in tobacco and marijuana users [4]. Moreover, a potential deleterious effect of cannabis use on the functional properties of CD4 T-cells needs in-depth research [5]. Finally, further studies are required to disentangle the concomitant effects of cannabis use and tobacco use on the immune system of HIV-HCV co-infected patients.

Acknowledgements

We thank all members of the ANRS CO13-HEPAVIH Study Group*. We especially thank all physicians and nurses who are involved in the follow-up of the cohort and all patients who took part in this study. Finally, our thanks to Jude Sweeney for the English revision and editing of our manuscript.

*The ANRS CO13-HEPAVIH Study Group

Scientific Committee: D. Salmon (cohort manager), F. Dabis (principal investigator), L. Wittkop, L. Esterle, P. Sogni, Y. Benhamou, P. Trimoulet, J. Izopet, V. Paradis, B. Spire, P. Carrieri, C. Katlama, G. Pialoux, M.A Valantin, P. Bonnard, I. Poizot-Martin, B. Marchou, E. Rosenthal, D. Garipuy, O. Bouchaud, A. Gervais, C. Lascoux-Combe, C. Goujard, K. Lacombe, C. Duvivier, D. Vittecoq, D. Neau, P. Morlat, F. BaniSadr, L. Meyer, F. Boufassa, S. Dominguez, B. Autran, A. M. Roque, C. Solas, H. Fontaine, L. Serfaty, G. Chêne, D. Costagliola, S. Couffin-Cadiergues J. Chas, L. Piroth, A. Simon, D. Zucman, F. Boué, P. Miailhes, E. Billaud, H. Aumaître, D. Rey (ANRS).

Clinical Centres (*ward/participating physicians*): CHU Cochin (Médecine Interne et Maladies Infectieuses/ D. Salmon, E. Chakvetadze; Hépato-gastro-entérologie/ P. Sogni; Anatomo-pathologie/B. Terris, Z. Makhlouf, G. Dubost, F. Tessier, L. Gibault, F. Beuvon, E. Chambon, T. Lazure; Virologie/A. Krivine); CHU Pitié-Salpétrière (Maladies Infectieuses et Tropicales/C. Katlama, M.A. Valantin, H. Stitou; Hépato-gastro-entérologie/ Y. Benhamou; Anatomo-pathologie/F. Charlotte; Virologie/S. Fourati); CHU Sainte-Marguerite, Marseille (Service d'Immuno-Hématologie Clinique/CISIH/ I. Poizot-Martin, O. Zaegel, A. Ménard; Virologie/ C. Tamalet); CHU Tenon (Maladies Infectieuses et Tropicales/G. Pialoux, P. Bonnard, F. Bani-Sadr, L. Slama, T. Lyavanc; Anatomo-pathologie/P. Callard, F. Bendjaballah; Virologie/C. Le-Pendeven); CHU Purpan Toulouse (Maladies Infectieuses et Tropicales/ B. Marchou; Hépato-gastro-entérologie/L. Alric, K. Barange, S. Metivier; Anatomo-pathologie/Janick Selves; Virologie/F. Nicot); CHU Archet, Nice (Médecine Interne/E. Rosenthal; Infectiologie/J. Durant; Anatomo-pathologie/J. Haudebourg, M. C. Saint-Paul); CHU Avicenne, Paris (Médecine Interne—Unité VIH/O. Bouchaud, F. Rouges, R. Djebbar; Anatomo-pathologie/M. Ziol; Virologie/Y. Baazia); Hôpital Joseph-Ducuing, Toulouse (Médecine Interne/M. Uzan, A. Bicart-See, D. Garipuy; Anatomo-pathologie/ Janick Selves; Virologie/F. Nicot); CHU Bichat—Claude-Bernard, Paris (Maladies Infectieuses/P. Yéni, A. Gervais; Anatomo-pathologie/H. Adle-Biassette); CHU Saint-Louis (Maladies infectieuses/D. Séréni, C. Lascoux Combe; Anatomo-pathologie/P. Bertheau, J. Duclos; Virologie/P. Palmer); CHU Saint Antoine (Maladies Infectieuses et Tropicales/P.M. Girard, K. Lacombe, P. Campa; Anatomo-pathologie/D. Wendum, P. Cervera, J. Adam; Virologie/N. Harchi); CHU Bicêtre (Médecine Interne/J. F. Delfraissy, C. Goujard, Y. Quertainmont; Virologie/C. Pallier); CHU Paul-Brousse (Maladies Infectieuses/D. Vittecoq); CHU Necker (Maladies Infectieuses et Tropicales/O. Lortholary, C. Duvivier, M. Shoai-Tehrani), ANRS CO 3 Aquitaine cohort (D. Neau, P. Morlat, L. Lacaze-Buzy, S. Caldato; Anatomo-pathologie/P. Bioulac-Sage; Virologie/ P. Trimoulet, S. Reigadas)

Data collection, *management and statistical analyses*: D. Beniken, A.-S. Ritleng, A. Fooladi, M. Azar, P. Honoré, S. Breau, L. Serini, M. Mole, C. Bolliot, F. Touam, F. André, N. Ouabdesselam, S. Mellul, G. Alexandre, A. Ganon, A. Champetier, S. Gillet, J. Delaune, L. Dequae Merchadou, E. Pambrun, A. Frosch, F. Marcellin, M. Mora, C. Protopopescu, C. Lupin, V. Payssan, A. Joulie, H. Hue, L. Larmet, C. Brochier, V. Thoirain, M. Raho-Moussa, S Ogoudjobi, D. Bornarel, S. Gohier, C. Chesnel, G. Maradan, S. Hadjoudj, M. Malet, I. Kmiec, P. Fischer, A. Palacin, M.P. Pietri, V. Le Baut, P. Guet, S. Le Puil, , M. Mebarki, A. Fior, A. Adda-Lievin, V. Conte, N. Douiri, C. Gilbert, A. Jacquet, R. Kherraz, P. Lagorse

Conflicts of interest

Bruno Spire declared consultancy for Merck Sharp & Dohme-Chibret (MSD) and Gilead companies; payment for lectures from MSD, Gilead and Jensen; and travel/accommodation/meeting expenses paid by MSD.

Eric Rosenthal declared board membership for Gilead Sciences and Abbvie; consultancy for Bristol-Myers Squibb (BMS) and Merck companies; payment for lectures from Gilead Sciences, Amgen, and Abbvie; and travel/accommodation/meeting expenses paid by Janssen and Viiv Healthcare.

Linda Wittkop declared board membership for BMS; grants from the French National Agency for Research on HIV/AIDS and viral hepatitis (ANRS); and payment for lectures from the French National Institute of Health and Medical Research (Inserm).

Source of funding

This work was supported by the French National Agency for Research on Aids and Viral Hepatitis (ANRS), with the participation of Abbott France; Glaxo-Smith-Kline; Roche; Schering-Plough; and INSERM's 'Programme Cohortes TGIR'.

Compliance with ethical standards

All patients who agreed to participate in the study signed a letter of informed consent. The study was approved by the institutional review board of the Cochin Hospital (Paris, France). The study was designed and performed in accordance with the Declaration of Helsinki and was approved by the ethics committee of Cochin University Hospital in Paris.

References

[1] MILDECA. Interministerial mission for combating drugs and addictive behaviours website http://www.drogues.gouv.fr (downloaded 11 January 2016).

[2] Brunet L, Moodie EE, Rollet K, *et al.* Marijuana smoking does not accelerate progression of liver disease in HIV-hepatitis C coinfection: a longitudinal cohort analysis. Clin Infect Dis 2013;57:663–70.

[3] Roux P, Demoulin B, Sogni P, *et al.* La co-infection par le virus de l'hépatite C chez les personnes infectées par le VIH : données de l'enquête ANRS-Vespa2. Bull Epidémiologique Hebd Jul 2013:314–20.

[4] Wallace JM, Oishi JS, Barbers RG, Simmons MS, Tashkin DP. Lymphocytic subpopulation profiles in bronchoalveolar lavage fluid and peripheral blood from tobacco and marijuana smokers. Chest 1994;105:847–52.

[5] Chao C, Jacobson LP, Tashkin D, *et al.* Recreational drug use and T lymphocyte subpopulations in HIV-uninfected and HIV-infected men. Drug Alcohol Depend 2008;94:165–71.

[6] Lee JT, Shepherd BE, Koethe JR, *et al*. Active marijuana Use was not associated with changes in body mass index or CD4 T-cell counts in HIV-infected patients (poster), ID week 2014, October 8–12, Philadelphia, PA 2014.

[7] Abrams DI, Hilton JF, Leiser RJ, *et al.* Short-term effects of cannabinoids in patients with HIV-1 infection: a randomized, placebo-controlled clinical trial. Ann Intern Med 2003;139:258–66.

[8] Bredt BM, Higuera-Alhino D, Shade SB, Hebert SJ, McCune JM, Abrams DI. Short-term effects of cannabinoids on immune phenotype and function in HIV-1-infected patients. J Clin Pharmacol 2002;42:82S–89S.

[9] Montarroyos UR, Miranda-Filho DB, César CC, *et al.* Factors related to changes in CD4+ T-cell counts over time in patients living with HIV/AIDS: a multilevel analysis. PLoS One 2014;9:e84276.

[10] Lodwick RK, Johnson MA, Smith CJ, *et al.* Effect of patient smoking status on CD4 count change after initiation of antiretroviral therapy, Royal Free HIV Cohort Database, https://www.ucl.ac.uk/royalfreehivdb (downloaded 11 January 2016).

[11] Park LP, Margolick JB, Giorgi JV, *et al.* Influence of HIV-1 infection and cigarette smoking on leukocyte profiles in homosexual men. The Multicenter AIDS Cohort Study J Acquir Immune Defic Syndr 1992;5:1124–30.

[12] Royce RA, Winkelstein W. HIV infection, cigarette smoking and CD4+ T-lymphocyte counts: preliminary results from the San Francisco Men's Health Study. AIDS 1990;4:327–33.

[13] Loko M-A, Salmon D, Carrieri P, *et al.* The French national prospective cohort of patients co-infected with HIV and HCV (ANRS CO13 HEPAVIH): early findings, 2006-2010. BMC Infect Dis 2010;10:303.

[14] Marcellin F, Roux P, Winnock M, *et al.* Using patient-reported outcomes to improve the management of co-infection with HIV and HCV: the ANRS CO13 HEPAVIH cohort. Expert Rev Gastroenterol Hepatol 2014;8:351–8.

[15] Beck F, Richard J-B, Guignard R, Le Nézet O, Spilka S. Les niveaux d'usage des drogues en France en 2014 (Inpes OFDT). Tendances 2015. http://www.ofdt.fr/BDD/publications/docs/eftxfbv3.pdf (Accessed 11 January 2016).

[16] Carrieri MP, Villes V, Raffi F, *et al.* Self-reported side-effects of antiretroviral treatment among IDUs: a 7-year longitudinal study (APROCO-COPILOTE COHORT ANRS CO-8). Int J Drug Policy 2007;18:288–95.

[17] Martens KM, Gilbert DG. Marijuana and tobacco exposure predict affect-regulation expectancies in dual users. Addict Behav 2008;33:1484–90.

[18] Schane RE, Ling PM, Glantz SA. Health effects of light and intermittent smoking: a review. Circulation 2010;121:1518–22.

[19] Schane RE, Glantz SA, Ling PM. Social smoking implications for public health, clinical practice, and intervention research. Am J Prev Med 2009;37:124–31.

[20] Marcellin F, Roux P, Loko M-A, *et al.* High levels of alcohol consumption increase the risk of advanced hepatic fibrosis in HIV/hepatitis C virus-coinfected patients: a sex-based analysis using transient elastography at enrollment in the HEPAVIH ANRS CO13 cohort. Clin Infect Dis 2014;59:1190–2.

[21] Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. Arch Intern Med 1998;158:1789–95.

[22] Carrieri P, Cailleton V, Le Moing V, *et al.* The dynamic of adherence to highly active antiretroviral therapy: results from the French National APROCO cohort. J Acquir Immune Defic Syndr 2001;28:232–9.

[23] Roux P, Fugon L, Winnock M, *et al.* Positive impact of hepatitis C virus (HCV) treatment on antiretroviral treatment adherence in human immunodeficiency virus-HCV coinfected patients: one more argument for expanded access to HCV treatment for injecting drug users. Addiction 2012;107:152–9.

[24] WHO. Laboratory guidelines for enumerating CD4 T lymphocytes in the context of HIV/AIDS, World Health Organization, Regional Office for South-East Asia, 2007: http://www.who.int/hiv/amds/LaboratoryGuide-EnumeratingCD4TLymphocytes.pdf (Accessed 11 January 2016).

[25] Sterling RK, Lissen E, Clumeck N, *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317–25.

[26] Tate JP, Justice AC, Hughes MD, *et al.* An internationally generalizable risk index for mortality after one year of antiretroviral therapy. AIDS 2013;27:563–72.

[27] Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 1986;42:121–30.

[28] WHO Expert Committee. Physical status: the use and interpretation of anthropometry—WHO technical report series 854. 1995.

[29] Klein TW, Cabral GA. Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. J Neuroimmune Pharmacol 2006;1:50–64.

[30] Nong L, Newton C, Cheng Q, Friedman H, Roth MD, Klein TW. Altered cannabinoid receptor mRNA expression in peripheral blood mononuclear cells from marijuana smokers. J Neuroimmunol 2002;127:169–76.

[31] Szulakowska A, Milnerowicz H. Cannabinoids—influence on the immune system and their potential use in supplementary therapy of HIV/AIDS. HIV AIDS—InTech, Available from: http://www.intechopen.com/books/hiv-and-aids-updates-on-biology-immunology-epidemiology-andtreatment- strategies/cannabinoids-influence-on-the-immune-system-and-their-potencial-use-insupplementary- therapy-of-hiv; 2011 (Accessed 11 January 2016).

[32] Croxford JL, Yamamura T. Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? J Neuroimmunol 2005;166:3–18.
[33] Tanasescu R, Constantinescu CS. Cannabinoids and the immune system: an overview. Immunobiology 2010;215:588–97.
[34] Nahas GG, Morishima A, Desoize B. Effects of cannabinoids on macromolecular synthesis and replication of cultured lymphocytes. Fed Proc 1977;36:1748–52.
[35] El-Gohary M, Eid MA. Effect of cannabinoid ingestion (in the form of bhang) on the immune system of high school and university students. Hum Exp Toxicol 2004;23:149–56.
[36] White SC, Brin SC, Janicki BW. Mitogen-induced blastogenic responses of lymphocytes from marihuana smokers. Science 1975;188:71–2.
[37] Lau RJ, Tubergen DG, Barr M, Domino EF, Benowitz N, Jones RT. Phytohemagglutinin-induced lymphocyte transformation in humans receiving delta9-tetrahydrocannabinol. Science 1976;192:805–7.
[38] Milloy M-J, Marshall B, Kerr T, *et al.* High-intensity cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load among recently infected people who use injection drugs. Drug Alcohol Rev 2015;34:135–40.
[39] Peterson PK, Gekker G, Hu S, Cabral G, Lokensgard JR. Cannabinoids and morphine differentially affect HIV-1 expression in CD4(+) lymphocyte and microglial cell cultures. J Neuroimmunol 2004;147:123–6.
[40] Rock RB, Gekker G, Hu S, *et al.* WIN55,212-2-mediated inhibition of HIV-1 expression in microglial cells: involvement of cannabinoid receptors. J Neuroimmune Pharmacol 2007;2:178–83.
[41] Beck F, Tovar M-L, Spilka S, Guignard R, Richard J-B. Les niveaux d'usage des drogues en France en 2010, exploitation des données du Baromètre santé 2010 [in French]. Tendances 2011; 76. http://www.ofdt.fr/publications/collections/periodiques/lettre-tendances/niveaux-usage-drogues-en-france-en-2010-tendances-76-juin-2011/ (Accessed 11 January 2016).
[42] Volkow ND, Baler RD, Compton WM, Weiss SRB. Adverse health effects of marijuana use. N Engl J Med 2014;370:2219–27.
[43] Feldman JG, Minkoff H, Schneider MF, *et al.* Association of cigarette smoking with HIV prognosis among women in the HAART era: a report from the women's interagency HIV study. Am J Public Health 2006;96:1060–5.
[44] Claassen CW, Diener-West M, Mehta SH, Thomas DL, Kirk GD. Discordance between CD4+ T-lymphocyte counts and percentages in HIV-infected persons with liver fibrosis. Clin Infect Dis 2012;54:1806–13.
[45] Degenhardt L, Ferrari AJ, Calabria B, *et al.* The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: results from the GBD 2010 study. PLoS One 2013;8:e76635.
[46] Fogarty A, Rawstorne P, Prestage G, Crawford J, Grierson J, Kippax S. Marijuana as therapy for people living with HIV/AIDS: social and health aspects. AIDS Care 2007;19:295–301.
[47] Alberti A, Clumeck N, Collins S, *et al.* Short statement of the first European Consensus Conference on the treatment of chronic hepatitis B and C in HIV co-infected patients. J Hepatol 2005;42:615–24.
[48] Dhumeaux D, Marcellin P, Lerebours E. Treatment of hepatitis C. The 2002 French consensus. Gut 2003;52:1784–7.
[49] Galmiche JP. French consensus conference on hepatitis C: screening and treatment. Gut 1998;42:892–8.

Supporting Information

Additional Supporting Information may be found in the online version of this article.