Active HCV replication but not HCV or CMV seropositive status is associated with incident and prevalent type 2 diabetes in persons living with HIV.

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Running head: HCV replication and diabetes in HIV

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TEXT

Introduction

Antiretroviral therapy has significantly reduced HIV-associated morbidity and mortality [1]. However, the substantially increased survival has exposed the HIV-infected population to a relevant number of co-morbid conditions, such as metabolic and cardiovascular disorders [2], as well as to the long-term consequences of chronic co-infections, including chronic hepatitis C virus (HCV) infection [3]. Among the metabolic disorders, type 2 diabetes affects a relevant number of persons living with HIV. Although diabetes mellitus does not seem more frequent in HIV-infected subjects than in negative controls [4], in the HIV-infected population diabetes represents a risk factor for all specific causes of death except non-AIDS cancers [5]. Moreover, exposure to some antiretroviral agents may also favor the emergence of type 2 diabetes [6-8]. In any case, the clinical impact of diabetes, as a risk factor for cardiovascular and cerebrovascular disorders, may be more relevant in the HIV-infected population where the incidence of cardiovascular disease is already increased as compared to the age-matched general population [2].

Chronic viral infections lead to immune activation and may therefore cause a number of co-morbidities. Indeed, chronic HCV infection, apart from causing liver-related morbidity and mortality, is also associated with an increased incidence of extra-hepatic disorders, including diabetes and cardiovascular disease, in the HIV-negative population [9-10].

In HCV-infected patients, HIV co-infection has been associated with an accelerated liver disease [3]. Whether HCV co-infection favors the emergence of diabetes in HIV-infected patients remains to be fully established. Some reports indicate a similar incidence of type 2 diabetes in HCV-

antibody positive HIV-infected patients [11,12] while other studies indicate that HCV-antibody positive individuals have an increased risk [4,10]. However, it is not clear whether HCV-antibody positivity is a marker for a specific population exposed to a higher risk of diabetes due to behavioral factors or whether HCV infection by itself represents a risk factor for this disorder. In addition, latent CMV infection, as determined by a positive CMV-antibody serostatus, has been associated with an increased incidence of non-AIDS events, particularly cardiovascular events [13], due to an immune activation mechanism. Latent Cytomegalovirus (CMV) infection has also been associated with diabetes mellitus [14].

Aim of our study was to determine the impact of HCV infection, both as prior HCV exposure and as active HCV infection, and of latent CMV infection on the incidence and the prevalence of type 2 diabetes in a nationally representative Italian cohort of HIV-infected individuals.

Patients and Methods

Patients selection for the incidence and prevalence analysis and definition of type 2 diabetes.

For the incident type 2 diabetes analysis, patients enrolled in the ICONA Foundation Study cohort [15] were selected who (a) had an available CMV IgG result (time of first result=baseline) and (b) did not have diabetes at baseline. For the prevalent type 2 diabetes analysis all ICONA enrolees were evaluated at the date of diabetes diagnosis or at their last available follow-up, whichever occurred first. Type 2 diabetes was defined by one of the following criteria: (a) diagnosis by the treating clinician, (b) use of antidiabetic drugs or (c) first of 2 consecutive blood glucose levels >125 mg/dL at a verified fasting status. All the type 2 diabetes diagnoses were validated by an external monitor using the above criteria.

Statistical analysis

We used standard descriptive statistics to describe characteristics at baseline for the population analyzed for the diabetes incidence and at the date of diabetes diagnosis or last available follow up for the population analyzed for the diabetes prevalence. Time to diagnosis of diabetes was analyzed using the Kaplan-Meier method using the time of first CMV serology as baseline. Patients were followed until onset of type 2 diabetes, last available clinical follow-up, death or September 30, 2014, whichever occurred first. Predictors of incident type 2 diabetes were analysed by Poisson regression. In the multivariable model we included variables with $p<0.10$ at univariate analysis plus CMV serostatus. The type of antiretroviral treatment regimen used was analysed as time updated variable.

Factors associated with prevalent type 2 diabetes at the last available follow-up were analysed by logistic regression; multivariable models included variables with $p<0.10$ at univariate analysis.

Results

Incidence analysis

Six-thousand-five-hundred-five patients were suitable for the type 2 diabetes incidence analysis. Baseline patients characteristics are summarized in table 1 (left part).

CMV IgG were detected in 84.4% of 6,505, HCV-antibodies in 31.5% of 6,112 tested, of whom 83.5% of 1,033 tested had a detectable HCV RNA. During 38.062 person-years of follow up (PYFU) we observed 140 cases of incident type 2 diabetes with an incidence rate of 3.7 (95%CI 3.1-4.3) per 1.000 PYFU. Time to event analysis showed that the 5-years, 10-years and 15-years estimated probability of type 2 diabetes were 1.8% (95% CI 1.5-2.3), 3.4% (2.8-4.1) and 5.4% (4.4-6.7),

respectively (see figure 1). By multivariable Poisson regression analysis (see table 2), HCV RNA positive status HCV-antibody positive HCV RNA positive status was independently associated with a higher incidence of type 2 diabetes as compared to an HCV-antibody negative status (adjusted relative rate, ARR, 1.73 [1.08-2.78]); CMV IgG serology was not associated with incident diabetes. Other independent predictors of diabetes onset were male gender, older age, a higher baseline BMI, higher baseline glucose and triglycerides levels, presence of arterial hypertension, current use of a regimen containing NRTIs with an unboosted protease inhibitor (as compared with NRTI with NNRTI) as well as current use of stavudine+lamivudine as compared to tenofovir+emtricitabine (table 2).

Prevalence analysis

Prevalent type 2 diabetes analysis was performed on 12,001 patients at their last follow-up in ICONA. Characteristics of the patients used in the prevalence analysis are summarized in table 1 (right side). HCV antibodies were detected in 29.3% of 10,611 tested, while HCV RNA was detectable in 75.1% of 1,095 tested; CMV IgG were positive in 84.6% of 7,033 tested. Three-hundred-six patients (2.5%) had a diagnosis of type 2 diabetes at last follow-up. Factors associated with prevalent diabetes are summarized in table 3. Again, HCV RNA positive status (adjusted odds ratio, AOR 2.49 [1.08-5.74]) was independently associated with prevalent type 2 diabetes, while HCV-antibody positive, HCV RNA negative status was not. Also in this analysis CMV IgG serology was not associated with diabetes. Other factors independently associated with prevalent diabetes were older age, a more recent time from HIV diagnosis, higher BMI, as well as current use of zidovudine+lamivudine, didanosine+stavudine, didanosine+lamivudine or other (uncommon) NRTI combinations as compared to tenofovir+emtricitabine. Interestingly, transaminase levels were

associated with prevalent diabetes at univariate analysis but lost this association in the adjusted model completely.

Discussion

In this cohort study we observed how, in HIV-infected patients, active HCV replication was independently associated both with incident and prevalent type 2 diabetes. Interestingly, this association was not observed in HCV-seropositive individuals without active HCV replication. This association was independent from several confounders including ALT or AST in both incident and prevalent diabetes analysis.

Our findings contribute to resolve the contradictory observations made in the HIV positive and negative population, where the association between HCV and diabetes was mostly studied using HCV antibody status as marker of HCV infection. Indeed, an association was observed in some studies [4,10] but not confirmed in others [11,12]. In the HIV-infected population, approximately 80-85% of HCV-antibody positive patients show a detectable serum HCV RNA. The proportion of HCV-antibody positive patients without active HCV replication is deemed to increase due to the spread of highly effective HCV eradication therapies with direct acting antivirals [16]. Given the observed association with active HCV replication but not with simple HCV-antibody positive status our study reinforces the hypothesis that HCV infection and replication per se and not its associated behavioural or biological factors, is directly involved in the pathogenesis of type 2 diabetes.

Several biological mechanisms have been hypothesized through which HCV infection may favor type 2 diabetes. HCV infection is thought to induce insulin resistance through multiple

mechanisms, involving both the liver as well as peripheral tissues [17-18]. Some of these mechanisms lead to an increased production of proinflammatory cytokines (such as TNF alpha and interleukin 6) [19], other result in the induction of liver steatosis, which is more prevalent in patients infected with HCV genotype 3 [20]. In this study we were unable to detect an association between a specific HCV genotype, the entity of HCV replication and type 2 diabetes. This finding may have been limited by the dispersion of the different HCV genotypes in the cohort and the proportion of missing values which may have reduced the power to detect an association. With these limitations in mind, our observations may suggest that the presence of HCV replication and not its entity or type is associated with diabetes, indicating an indirect pathogenetic role for HCV. Other factors associated with type 2 diabetes, male sex, older age, higher baseline BMI, glucose and triglycerides, hypertension, are consistent with those found in other cohorts of HIV-infected as well as uninfected individuals [21]. Interestingly, we also found that regimens containing d-drugs were associated with a higher risk of diabetes, consistent with their higher metabolic impact. Similarly, a higher incidence was observed for the association of NRTI with unboosted PI, probably representing older regimens with PI showing a deeper impact on insulin resistance.
Our findings have a very strong practical implication. Indeed HCV eradication, a goal which is now obtainable in the majority of HCV/HIV co-infected individuals, may result in an additional clinical benefit in this population. We found a 73% (95% CI 8-178%) increased incidence of diabetes in individuals with active HCV replication. Given the overall incidence in our study population, this translates in an incidence of approximately 1.5 per 100 PY of type 2 diabetes attributable to active HCV replication. Given the chronicity of type 2 diabetes and its clinical consequences and assuming that the majority of these cases could be averted by HCV eradication, HCV treatment in this population may result in a relevant benefit in terms of both patients and public health, not only due to the prevention of liver-related morbidity and mortality.

Another goal of the current study was to explore the association of latent CMV infection with type 2 diabetes. We found no association of positive CMV IgG serostatus neither with incident nor with prevalent type 2 diabetes. This is the first analysis of this kind in the HIV-infected population and its results are in contrast with some observation in the elderly HIV-uninfected population [14]. While CMV positive serostatus has been associated with increased immune activation in HIV [13], the absence of any correlation with type 2 diabetes, in contrast with the observed association with active HCV replication, suggests that distinct immune-inflammatory mechanisms are triggered by these two very different chronic viral infections. However, since we could not analyse the plasma CMV DNA status, our observation does not exclude the role of active CMV replication in determining the risk of diabetes.

The results of this study should be interpreted with caution given its retrospective nature. However, in the ICONA cohort this limitation is only partial, given the fact that patients are enrolled and followed prospectively and that the diagnosis of clinical events, including diabetes, is validated by an external monitor using standardized criteria.

In conclusion, in this large observational study, we found a significant association of active HCV replication with incident and prevalent type 2 diabetes. This result is consistent with previous observations in the HCV mono-infected population and with the hypothesized mechanism by which HCV may induce liver steatosis and insulin resistance. Future studies should aim to determine whether HCV eradication with antiviral therapy may be able to revert this increased risk.

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Table 1. Characteristics of study patients.

Characteristic	Baseline characteristics of patients used in the incidence analysis (n=6,505)		Characteristics of patients at the time of the prevalence analysis (n=12,001)	
Calendar month median (IQR)	Jun 2002	(Jan 1998-Apr 2010)	Mar 2003	J(ul 1999-Jun 2009)
Male gender, n (%)	4636	(71.3)	8980	(74.8)
Age, years median (IQR)	36	(31-42)	41	(35-49)
Mode of HIV transmission, n (%)				
Heterosexual contacts	2624	(40.3)	4581	(38.2)
IDU	1788	(27.5)	3867	(32.2)
MSM	1730	(26.6)	2747	(22.9)
Other/unknown	363	(5.6)	806	(6.7)
CDC stage C, n (%)	706	(10.8)	1681	(14.1)
BMI category (kg/m^2), n (%)				
<18	304	(4.7)	351	(2.9)
18.5-25.9	3765	(57.9)	4609	(38.4)
25-29.9	1109	(17.1)	1791	(14.9)
≥ 30	238	(3.6)	456	(3.8)
missing	1089	(16.7)	4794	(39.9)
ALT median (IQR) IU/mL	29	(19-48)	28	(19-45)
AST median (IQR) IU/mL	27	20-42)	25	(19-37)

Creatinine median (IQR) mg/dl	0.9	(0.7-1.0)	0.9	(0.7-1.0)
Tryglicerides median (IQR) mg/dl	109	(77-157)	115	(81-172)
Total cholesterol median (IQR) mg/dl	165	(138-193)	179	(150-210)
Hypertension, n (%)	1116	(17.2)	2540	(21.2)
CD4 nadir, median (IQR) cells/µL	380	(192-569)	238	(147-420)
CD4 nadir, n (%) in category of cells/µL				
0-199 n (%)	1548	(23.8)	3763	(31.4)
200-349 n (%)	1187	(18.2)	3458	(28.8)
350+ n (%)	3271	(50.3)	4131	(34.4)
missing	499	(7.7)	645	(5.4)
HCV status				
HCV Ab-	4185	(64.3)	7505	(62.5)
HCV Ab+ and HCV-RNA-	170	(2.6)	273	(2.3)
HCV Ab+ and HCV-RNA+	863	(13.3)	822	(6.8)
HCV Ab+ and HCVRNA nd	894	(13.7)	2011	(16.8)
HCV Ab nd	393	(6.1)	1390	(11.6)
HCV genotype* n (%)				
1	362	(41.9)	197	(24.0)
2	25	(2.9)	11	(1.3)
3	263	(30.5)	115	(14.0)
4	90	(10.4)	52	(6.3)
Mixed/unknown	129	(14.9)	447	(54.4)
HCV RNA load, median (IQR) $\log_{10}$ IU/mL	5.9	(5.4-6.3)	5.9	(5.5-6.4)

HBsAg				
positive	330	(5.1)	554	(4.6)
negative	6009	(92.4)	9827	(81.9)
Not known	166	(2.5)	1620	(13.5)
CMV IgG				
positive	5488	(84.4)	1085	(9.0)
negative	1017	(15.6)	5948	(49.6)
Not known	0	(0)	4968	(41.4)
Patients initiating ART (Incidence analysis) or on ART (prevalence analysis)	5442	(83.7)	7451	(62.1)

IDU, injecting drug users; MSM, men having sex with men; CDC, Centers for Disease Control; BMI, body mass index; NRTI, nucleoside reverse transcriptase inhibitors, NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; CMV, Cytomegalovirus; ART, antiretroviral therapy

* among the HCV RNA positives.

Table 2. Predictors of incident type 2 diabetes using a Poisson regression analysis. Variables assayed in the multivariable model include only those associated with p<0.1 at univariable analysis plus Cytomegalovirus IgG status.

Variable	**Univariable analysis**		**Multivariable analysis**	
	RR (95% CI)	**p**	**ARR (95% CI)**	**p**
Male gender vs female	1.72 (1.15-2.57)	0.009	1.73 (1.41- 2.12)	<0.001
Age (per 10 years increase)	2.05 (1.77-2.38)	<0.001	1.06 (1.04-1.08)	<0.001
CDC stage C vs A/B	2.30 (1.53-3.46)	<0.001	1.67(0.97-2.88)	0.065
BMI at baseline (kg/m^2)				
<18.5	2.35 (0.58-9.60)	0.234	0.26 (0.04-1.93)	0.189
18.5-24.9	1		1.00	
25-29.9	4.98 (1.20-20.66)	0.027	2.10 (1.31-3.38)	0.002
≥ 30	13.20 (3.08-56.68)	0.001	6.47 (3.49-12.01)	<0.001
Blood glucose at baseline mg/dL				
< 125	1.00		1.00	
>125	28.64 (15.72-52.17)	<0.001	5.17 (1.95-13.71)	0.001
AST at baseline (per 50 U/L higher)	1.00 (1.00-1.00)	0.071	1.08 (1.00-1.17)	0.057
Current triglycerides (per 100 mg/dL higher)	1.00 (1.00-1.00)	<0.001	1.05 (1.12-4.50)	<0.001
Current hypertension status	2.35 (1.68-3.27)	<0.001	1.85 (1.23-2.78)	0.003

CD4 nadir cells/µL				
0-199	1.00		1.00	
200-349	0.51 (0.30-0.86)	0.012	0.66 (0.35-1.22)	0.185
350+	0.53 (0.36-0.77)	0.001	0.67 (0.39-1.14)	0.138
HCV infection status at baseline				
HCV Ab-	1.00		1.00	
HCV Ab+ / HCV RNA-	0.17 (0.02-1.25)	0.082	0.28 (0.04-2.02)	0.206
HCV Ab+ / HCV RNA+	1.25 (0.85-1.85)	0.250	1.73 (1.08-2.78)	0.023
HCV Ab+ / HCV RNA nd	0.70 80.36-1.35)	0.285	0.71 (0.30-1.69)	0.439
HCV Ab nd	0.82 (0.38-1.78)	0.622	0.86 (0.34-2.17)	0.752
HCV genotype 2 vs 1	1.53 (0.20-11.88)	0.683		
3 vs 1	1.64 (0.73-3.66)	0.227		
4 vs 1	1.81 (0.63-5.21)	0.271		
HCV RNA (per 1 log IU/mL	1.10 (0.91-1.32)	0.322		
higher)				
HBsAg (positive vs negative)	0.55 (0.20-1.49)	0.242		
Baseline CMV IgG neg. vs pos.	0.97 (0.62-1.52)	0.911	1.11 (0.64-1.91)	0.721
Drug classes in the current				
regimen				
NRTI + NNRTI	1.00		1.00	
NRTI + PI/r	1.37 (0.82-2.28)	0.231	1.15 (0.65-2.02)	0.635
NRTI + unboosted PI	2.11 (1.27-3.50)	0.004	2.07 (1.17-3.68)	0.013
Only NRTI	1.47 (0.81-2.66)	0.205	1.38 (0.65-2.90)	0.398
Other	1.72 (0.75-3.91)	0.198	1.54 (0.61-3.87)	0.361

Current NRTI combination				
tenofovir+emtricitabine	1.00		1.00	
tenofovir+lamivudine	0.26 (0.03-1.88)	0.181	0.27 (0.04-2.04)	0.205
abacavir+lamivudine	0.97 (0.40-2.37)	0.950	1.16 (0.47-2.90)	0.749
zidovudine+lamivudine	1.24 (0.73-2.12)	0.423	1.07 (0.57-2.02)	0.835
stavudine+lamivudine	2.53 (1.39-4.59)	0.002	2.52 (1.22-5.20)	0.013
stavudine+didanosine	1.18 (0.45-3.07)	0.741	1.09 (0.39-3.06)	0.872

CDC, Centers for Disease Control; BMI, body mass index; NRTI, nucleoside reverse transcriptase inhibitors, NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; RR, relative risk; ARR, adjusted relative risk

Table 3. Factors associated with prevalent type 2 diabetes using logistic regression. Variables assayed in the multivariable model included only those associated with p<0.1 at univariable analysis.

Variable	**Univariable analysis**		**Multivariable analysis**	
	OR (95% CI)	**p**	**AOR (95% CI)**	**p**
Male vs female	1.55(1.15-2.08)	0.004	0.70 (0.40- 1.21)	0.202
Age (per 10 years increase)	1.32 (1.19-1.46)	<0.001	1.04 (1.02-1.06)	<0.001
Mode of HIV transmission				
Heterosexual	1.00		1.00	
IDU	0.57 (0.43-0.76)	<0.001	0.90 (0.48-1.69)	0.753
MSM	0.78 (0.58-1.04)	0.090	0.84 (0.37-1.92)	0.684
Other/unknown	0.86 (0.54-1.35)	0.511	0.63 (0.24-1.64)	0.340
Years from HIV diagnosis (per 1 yr more)	0,89 (0.87-0.91)	<0.001	0.95 (0.91-0.99)	0.014
CDC stage C vs A/B	1.29 (0.95-1.74)	0.099	1.20(0.64-2.23)	0.568
BMI (kg/m^2)				
<18.5	0.58 (0.18-1.87)	0.365	NE	
18.5-24.9	1.00		1.00	
25-29.9	2.15 (1.50-3.08)	<0.001	1.12 (0.61-2.06)	0.715
≥ 30	6.52 (4.35-9.77)	<0.001	3.55 (1.82-6.92)	<0.001
ALT per U/L increase	1.00 (1.00-1.00)	0.006	1.00 (1.00-1.00)	0.920

AST per U/L increase	1.00 (1.00-1.00)	0.007	1.00 (1.00-1.01)	0.372
Hypertension (present vs absent)	1.81 (1.42-2.31)	<0.001	1.09 (0.66-1.79)	0.734
CD4 nadir cells/µL				
0-199	1.00		1.00	
200-349	0.57 (0.40-0.81)	0.002	1.09 (0.61-1.95)	0.770
350+	0.55 (0.39-0.77)	<0.001	0.84 (0.42-1.67)	0.613
HCV infection status at baseline				
HCVAb-	1.00		1.00	
HCVAb+ and HCV-RNA-	0.66 (0.21-2.09)	0.482	1.33 (0.77-6.48)	0.720
HCVAb+ and HCV-RNA+	2.49 (1.68-3.68)	<0.001	2.49 (1.08-5.74)	0.032
HCVAb+ and HCVRNA nd	0.99 (0.67-1.46)	0.972	0.66 (0.27-1.59)	0.354
HCVAb nd	5.27 (4.05-6.84)	<0.001	0.63 (0.15-2.70)	0.529
HCV genotype 2 vs 1	NE			
3 vs 1	0.93 (0.33-2.59)	0.891		
4 vs 1	1.04 (0.28-3.86)	0.959		
HCV RNA (per 1 log IU/mL higher)	0.91 (0.66-1.24)	0.542		
HBsAg (positive vs negative)	0.66 (0.31-1.41)	0.283		
CMV IgG at baseline				
positive	1.00		1.00	
negative	1.08 (0.71-1.66)	0.717	1.02 (0.51-2.05)	0.946
not known	1.35 (1.07-1.72)	0.013	0.50 (0.27-0.91)	0.024
Current CD4 count (per 100 cell/ µL higher)	0.95 (0.90-0.99)	0.022	1.04 (0.99-1.09)	0.141

Drug classes in the current regimen				
NRTI + NNRTI	1.00		1.00	
NRTI + PI/r	1.26 (0.79-2.03)	0.332	1.29 (0.66-2.51)	0.455
NRTI + PI	5.44 (3.40-8.72)	<0.001	1.67 (0.73-3.81)	0.222
Only NRTI	4.07 (2.32-7.16)	<0.001	1.52 (0.52-4.48)	0.446
Other	2.24 (0.86-5.80)	0.097	2.15 (0.52-8.82)	0.287
Current NRTI combination				
tenofovir+emtricitabine	1.00		1.00	
tenofovir+lamivudine	1.14 (0.27-4.78)	0.858	1.49 (0.19-11.65)	0.704
abacavir+lamivudine	0.83 (0.35-1.98)	0.676	0.99 (0.34-2.82)	0.978
zidovudine+lamivudine	4.26 (2.63-6.92)	<0.001	3.34 (1.55-7.20)	0.002
stavudine+lamivudine	7.10 (4.00-12.60)	<0.001	3.02 (0.92-9.87)	0.068
stavudine+didanosine	6.91 (3.14-15.22)	<0.001	5.58 (1.51-20.71)	0.010
didanosine+lamivudine	6.81 (2.35-19.72)	<0.001	8.33 (1.86-37.22)	0.006
other	3.67 (2.27-5.96)	<0.001	2.78 (1.09-7.12)	0.033

CDC, Centers for Disease Control; BMI, body mass index; NRTI, nucleoside reverse transcriptase inhibitors, NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; OR, odds ratio; AOR, adjusted odds ratio; NE, not estimable.

Figure legends

Figure 1. Estimated probability of type 2 diabetes. Kaplan Meier method. Baseline was the time of the first available CMV serology. Patients with pre-baseline diabetes were excluded.