

TITLE

Evolution of the prevalence of HCV infection and HCV genotype distribution in HIV-infected patients in Italy between 1997 and 2015

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**Running title: HCV/HIV coinfection in Italy between
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ABSTRACT (max 250 word, ora 318)

Objectives: Aim of the study was to analyze the variation of HCV prevalence and genotype distribution and their determinants in people with HIV who entered care between 1997 and 2015.

Methods: HIV-infected patients enrolled in ICONA who were tested for the HCV-Antibody (HCV-Ab) were included. We analyzed distribution of HCV-Ab positivity and HCV genotype during the calendar period of enrollment and HBsAg-/HBcAb+ status in HCV-Ab+ and investigated their determinants.

Results: Overall 3,407/12,135 (28%) of HIV-infected patients were HCV-Ab+; 9,134/12,135 (75%) were males, 1,841/12,135 (15%) had AIDS, 2,748/12,135 (23%) had a history of injecting drug use (IDU), 735/12,135 (6%) were HBsAg+ and 10,375/12,135 (84%) were Italian-born. Among patients whose HCV genotype was known, 668/1,359 (49%) had genotype 1 (435/1,359; 32% 1a, 172/1,359; 12.7% 1b), 488/1,359 (36%) genotype 3, 146/1,359 (10.8%) genotype 4 and 46/1,359 (3.4%) genotype 2. Among patients with HCV-Ab+ status and known HBcAb status, 764/1,249 (63%) were HBsAg-/HBcAb+ with the highest prevalence in IDU (68%, 559/819).

The prevalence of HCV infection decreased from 49.2% (2,565/5,217) between 1997 and 2002 to 10.2% (556/5,466) between 2009 and 2015. The relative frequency of genotype 1a increased from 29% (264/911) to 43% (129/300), whereas

genotype 3 decreased from 38.5% (351/911) to 27% (81/300). Independent predictors of HCV-Ab+ status were being female (AOR 1.23, CI 95% 1.04-1.50, p=0.01), risk category (vs IDU: MSM AOR 0.01, 0.01-0.01, p<0.001; heterosexual contacts AOR 0.01, 0.01-0.01, p<0.001; other/unknown AOR 0.02, 0.01-0.02, p<0.001), geographic area (vs being cared for HIV in Northern Italy: Center AOR 0.85, 0.73-0.98, p<0.001), being born in Italy (AOR 1.44, 1.16-1.80, p=0.001) and being enrolled in less recent calendar years (vs 1997-2002: 2009-2015 AOR 0.23, 0.19-0.27, p<0.001; 2003-2008 AOR 0.49, 0.41-0.61, p<0.001).

Conclusions: In Italy, the prevalence of HCV infection has significantly declined in more recent calendar years in HIV-infected patients. After adjusting for risk factors and calendar year of enrollment, HCV co-infection is more frequent in female and in natives.

Introduction

Globally, more than 150 million people are infected with HCV and due to their overlapping routes of acquisition, the burden of HIV and HCV co-infection is estimated to affect ~2 million people worldwide [1]. Odds of HCV infection are six times higher in HIV-infected patients: overall prevalence of HCV co-infection in HIV-positive individuals is 2.4% (IQR 0.8–5.8), 4.0% (1.2–8.4) in pregnant women or heterosexually exposed individuals, 6.4% (3.2–10.0) in men who have sex with men (MSM) and 82.4% (55.2–88.5) in current or previous injecting drug users (IDU) [2]. The HCV/HIV co-infected population is at higher risk of progression to advanced liver disease and cirrhosis and of developing liver-related complications than HCV monoinfected patients [3-5]. International expert panels recommend that treating HCV should be a priority in all HIV-infected patients because of the accelerated risk of progression to liver fibrosis and the increased risk of HCV transmission in this population [7, 17-21].

The recent introduction of oral HCV direct-acting antivirals (DAAs) to treat chronic HCV infection has increased virological efficacy enormously, has greatly simplified regimens and has improved tolerability and outcomes, with few side effects and fewer drug-drug interactions in comparison with the interferon therapy era (6-10).

Because DAAs directly target HCV non-structural proteins (NS3/4A protease, NS5B polymerase, and NS5A replication complex), oral combination drugs have reduced the negative impact of host factors (e.g. race/ethnicity, body mass index, IL28B genotype and HIV co-infection) on the likelihood of curing HCV. Data from trials and from clinical practice result in similar HCV cure rates in HIV-infected and HIV-uninfected patients. This supports the hypothesis that coinfection with HIV will not impair HCV cure rates with new regimens [16]. Eradication of chronic HCV infection in HIV-infected patients has been associated with significantly reduced morbidity and mortality related to hepatic complications, similar to what occurs in HCV monoinfected patients [11-15].

Due to the specific target of antiviral drugs and to high viral variability, the virological efficacy of DAAs is strongly influenced by the viral genotype [19-20]. Data from the multicenter MASTER cohort show a steady reduction of the prevalence of HCV co-infection in HIV-infected patients in Italy between 1985–88 and 2010–13 [22].

Little is known about the longitudinal evolution of HCV prevalence and the HCV genotype distribution among HIV-infected patients entering care in Italy. Aims of this study were to analyze the variation of HCV prevalence and genotype distribution in HIV-infected patients who entered care between 1997 and 2015 and their determinants in a representative national cohort of HIV-infected patients.

Materials and Methods

We selected all patients who were tested for HCV antibodies (HCV-Ab) from the ICONA Foundation cohort study; this is a national Italian multicenter prospective cohort that enrolls adult HIV-infected individuals naïve for antiretroviral therapy who enter

care in 81 centers. The study protocol was approved by the local Ethics Committees at each study center; written informed consent was obtained from all patients prior to their participation.

We analyzed HCV-Ab and HCV genotype prevalence over a calendar period of enrollment and tested their association with epidemiological and demographic factors. We also evaluated the prevalence of HBsAg+ and of HBsAg-/HBcAb+ status in HCV-Ab+ individuals according to risk factor for HIV infection.

Descriptive statistics were used to describe patients' baseline (i.e. time of enrollment in the cohort) demographic and virological patients characteristics.

Differences between HIV-infected and HIV/HCV co-infected patients among patients with different risk factors were assessed using the Mann-Whitney or Chi-square test, as appropriate. A linear-by-linear association chi-square test was used to analyze the prevalence of HCV Ab positive status according to calendar period in the different risk categories for HIV infection (Tab.1). Correlates of HCV-Ab positive status and of the presence of specific HCV genotypes

were tested using univariable and multivariable logistic regression with forward stepwise conditional selection. The following factors were analyzed: gender, age, risk category (IDU, heterosexual contacts, other/unknown), geographic area of Italy (North, Center and South/Islands), nationality, calendar period of enrollment (1997-2002, 2003-2008, 2009-2015), HBsAg positivity.

The multivariable models included co-variables that showed a significant association ($p < 0.05$) in the univariable analysis.

All analyses were performed using the SPSS software package (version 21; IBM, Chicago, IL, USA).

Results

Overall patients characteristics at enrollment and their HCV and HBV infections status.

Of the 12,135 HIV-infected patients, 3,407 (28%) were HCV-Ab+ and 735 (6%) were HBsAg+.

The main baseline characteristics of the study population, overall and divided by HCV-Ab status, are summarized in Table 1. Most were males, who were

enrolled shortly after their HIV diagnosis, their most frequent risk factor was a history of injecting drug use (IDU) and few patients had AIDS. Most of the patients were Italian and were being treated in Northern of Italy.

Of the 1,359 with a known HCV genotype, 49.2% carried genotype 1 (32% 1a, 12.7% 1b, 4.5% not specified), 35.9% genotype 3, 10.7% genotype 4, 3.4% genotype 2 and 0.8% mixed genotypes (Fig.1).

Of the 735 HBsAg+ patients, most were heterosexual (243, 34%), 214 (29%) were IDUs , 238 (32%) were MSM, 40 (5%) had other/unknown risk factors for HIV infection.

Of the 3,228 HCV-Ab positive patients with known HBsAg status 261 (8.1%) were HBsAg+ and had a higher prevalence than among HCV-Ab negatives (5.7%, $p < 0.001$). With regard to risk factor, HBsAg+/HCV-Ab+ were 192/2,393 (8%) in IDUs, 25/285 (8.8%) in MSM, 30/455 (6.6%) in heterosexuals and 14/95 (14.7%) had other/unknown risk factors for HIV (Fig.1a Supplementary).

Of the 1,249 patients with HCV-Ab positive status

and known HBcAb status, 764 (63%) were HBsAg-/HBcAb+, with the highest prevalence among IDU (68%) (Fig.1b. Supplementary).

Evolution of HCV antibody status over calendar years and associated factors.

The prevalence of HCV-Ab positive status decreased progressively from 1997-2002 to 2009-2015 (Fig.2). An analysis of the prevalence trend over calendar years according to risk factor for HIV infection showed a clear decrease in HCV-Ab positive status in every group (Fig.3 and Tab.1 Suppl). Overall HCV-Ab positive status was detected in 2,502/2,748 (91%) patients who reported IDU: 1,927 out of 2,124 (90.7%) males and 575 out of 624 (92.1%) females reporting who reported IDU ($p=0.27$) and in 494/4,564 (10.8%) patients who reported heterosexual contacts: 228 out of 2,375 (9.6%) males and 266 of 2,189 (12.2%) females ($p=0.0056$).

Among males the proportion of HCV-Ab positive patients with decreased from 51.9% during 1997-2002 to 19.3% during 2003-2008 to 9.9% during 2009-

2015, while among females it decreased from 42.8% during 1997-2002 to 20.9% during 2003-2008 to 11.3% during 2009-2015. Notably, among HIV-infected patients with heterosexual contacts and injecting drug use as risk factors for HIV acquisition, HCV-Ab prevalence was slightly higher among females compared to males in every calendar group, except for injecting drug users in the earliest period, where identical prevalence between genders emerged. Univariable and multivariable logistic regression analyses that show factors associated with HCV-Ab positive status are summarized in Table 2. Independent predictors of HCV-Ab+ status were being female, IDU, undergoing care in Northern Italy, born in Italy and being enrolled in less recent calendar years (Tab. 2). Female sex remained independently associated even in an additional model that excluded MSM (adjusted odds ratio, AOR of females vs males 1.19, 95% CI 1.01-1.40 p=0.033) (Tab.2 Suppl).

Evolution of HCV genotypes over calendar years and associated factors

During the same calendar time, the relative frequency

of genotype 1 (including 1a, 1b and samples without an assigned subtype) increased from 45.8% to 59.3%, whereas genotype 3 decreased from 38.5% to 27% (Fig.1). In particular, the relative increase of total genotype 1 prevalence was completely attributable to the increase of genotype 1a, whereas the relative prevalence of genotype 1b remained lower and stable over time. Genotype 4 prevalence remained stable and genotype 2 showed a stable low frequency over time. Factors independently associated with being infected with HCV genotype 1a were: younger age (+10 years, AOR, 0.70, 95% CI 0.58-0.84), being MSM (vs IDU, AOR 1.89, 1.21-2.94), geographical location (Central vs Northern Italy, AOR 0.74, 0.55-1.00), and being enrolled in a more recent calendar period (2009-2015 vs 1997-2002, AOR 2.33, 1.68-3.23). Factors associated with genotype 3 infection were being IDU (MSM vs IDU, AOR 0.45, 0.28-0.72) and being enrolled in a less recent calendar period (2009-2015 vs 1997-2002, AOR 0.67, 0.50-0.90).

Discussion

As few data are available regarding the longitudinal evolution of HCV prevalence and HCV genotype distribution in HIV-infected patients entering care in Italy, in this retrospective study we investigated the declining trends in prevalence of HCV positive antibody status in HIV infected patients over two decades in a large, representative cohort in Italy and a change in the prevalent HCV genotypes. Adjusting for risk factors and calendar year of enrollment, HCV infection in HIV-infected patients is more frequent in females and in native Italians.

The prevalence of HCV-Ab positive status in patients entering in care showed a consistent steep decline that ranged from over 50% at the end of the '90s to about 10% at the beginning of the last decade. This observation is in line with reports from the Italian MASTER cohort, which showed a similar decline but with the follow-up ending in 2013 and reported HCV-Ab prevalence during the follow-up and not at cohort entry as in our study [22]. We also extended this

observation by analyzing the factors associated with HCV-Ab prevalence changes over calendar years. The observed decline was only partially explained by changes in the prevalence of the modality of HIV transmission observed in the country over this period. Indeed, IDU, the most frequent modality of transmission of HIV in Italy in the early years of the epidemic and which showed the highest prevalence of positive HCV antibodies, was much less represented in more recently HIV-infected patients. The observed HCV antibody prevalence decline in more recent calendar years, however, was independent of risk factor for HIV infection, as shown by a multivariable analysis which adjusted for this and other potential confounders. In line with this, the HCV antibody prevalence decline was observed in all transmission risk groups, including IDU, possibly because of reduced circulation of HCV in the HIV-infected population and/or of changes in behavioral risk factors. Interestingly, female HIV-infected patients showed a trend towards a higher prevalence of HCV-antibody positive status in more recently diagnosed

HIV cohorts and a significantly higher prevalence in the heterosexual transmission risk group. More importantly, female sex was a factor associated with positive HCV antibody status independently of modality of HIV transmission and other potential confounders. Although this unique observation requires confirmation in other cohort studies, it suggests that females with HIV infection may be infected with HCV more easily and thus require specific attention and intervention as a particularly vulnerable population for this co-infection. Finally, we observed a significantly higher prevalence of HBsAg-/HBcAb+ status, a condition that indicates prior HBV exposure, in HCV-Ab+ HIV-infected patients who reported IDU as compared to the other risk groups, indicating the higher exposure and acquisition risk for both hepatitis co-infections in this particularly vulnerable population.

HCV genotypes are associated with liver disease prognosis as well as with response to DAA therapy [23-24]. Historically, the prevalence of genotypes in HIV/HCV co-infected populations in Italy is peculiar,

with a higher prevalence of genotypes 1a and 3 as compared to HCV mono-infected individuals [25]. Here we describe trends over calendar years of HCV genotypes in the HIV-infected population entering care and we observed a relative increase in genotype 1a and a decrease in genotype 3. Both changes over calendar time were confirmed by multivariable analysis. While HCV genotype 3 infection was largely associated with IDU, which largely confirms previous observations [26-27], genotype 1a co-infection was more prevalent in the younger population and in MSM. This suggests that these populations may have driven the more recent HCV infections in HIV-infected patients in Italy and that they should be the target for preventative interventions.

The overall picture of this observational study is positive findings, with a relevant decline in HCV antibody prevalence in HIV-infected patients who have entered care in Italy more recently and a significant relative decline in infections with HCV genotype 3, which is associated with a greater liver morbidity and with lower response rates to modern

DAA therapies [26-27]. However, the higher HCV exposure in female patients and the increase in HCV infections with genotype 1a which is associated with MSM transmission mode and younger age suggests that the HCV co-infection in HIV-infected patients in Italy has more recently shifted from an epidemics largely driven by IDU to one prevalently driven by sexual transmission, as observed in Northern European countries [28].

This study has several limitations. We analyzed the trend in HCV exposure as defined by HCV antibody status but were unable to determine the trend in active HCV co-infection status because we lacked information on plasma HCV RNA for a significant number of patients. However, the fraction of HCV RNA positivity in HCV-Ab positive HIV-infected patients is around 75-85% and there is no reason to believe that it should have changed over time. The proportion of HIV-infected patients successfully treated for HCV in the ICONA cohort before 2015 was minimal, moreover treatment was generally undertaken after entering into HIV care, which in

most cases corresponds to enrollment in the cohort. Therefore, the observed, major decline in HCV-Ab positivity over calendar years largely reflects a decline in active HCV co-infection status. Moreover, HCV genotyping was only available for a subset (about 40%) of HCV-Ab positive individuals. However the fraction of genotyped HCV-positive individuals remained stable over time (not shown).

The strengths of this study are the size of the population analyzed, the national representativeness of this prospective cohort, the inclusion of patients entering into HIV care and the large time span, almost two decades analyzed.

The efficacy of current hepatitis C therapy with DAA in HIV/HCV-coinfected patients largely depends on HCV genotype. In the era of highly effective DAA therapies, continuous surveillance of HCV co-infection in newly diagnosed HIV-infected individuals, of incident HCV infections in persons already in care, of HCV genotype distribution and correlated risk factors is necessary to eradicate HCV in this vulnerable population.

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Transparency declaration

Dr. Rossetti has nothing to disclose.

BR designed the study, wrote the first draft of the manuscript, also performing the appropriate literature search.

FB, AT, ACL performed the statistical analyses.

FC, MG, AA, GP, SB, ADM, MP contributed to data interpretation.

ADL conceived and designed the study, contributed to data interpretation and to write the manuscript.

Preliminary or partial data have been presented during the following scientific meetings

VIII Congresso Nazionale ICAR, 6-8 June 2016,
Milano, Italy (ABSTRACT: OC 9)

EASL Conference 2016, 13-17 April 2016, Barcelona,
Spain (ABSTRACT: P_FRI 157)

-IWHOD'S 20th International workshop on HIV
observational databases 7-9 April 2016, Budapest,
Hungary (OC 65)

14th European Meeting on HIV & Hepatitis
Treatment Strategies & Antiviral Drug Resistance, 25-
27 May 2016, Rome, Italy (ABSTRACT: P60)

V Riunione annuale SIMIT Toscana 2016.
L'appropriatezza nell'impiego degli antinfettivi. 13-14
October 2016, Pistoia, Italy (OC3)

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