Incidence and Risk Factors for Hepatitis C Virus Infection among Illicit Drug Users in Italy

Enea Spada • Giovanni Rezza •
Anna Rosa Garbuglia • Flavia Lucia Lombardo •
Ornella Zuccaro • Francesca Menniti Ippolito •
Elisabetta Cupellaro • Stefania Capone •
Maria Rosaria Capobianchi • Alfredo Nicosia •
Riccardo Cortese • Antonella Folgori • Alfonso Mele •
The Collaborative Study Group

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Abstract So far, only three small outdated studies have investigated hepatitis C virus (HCV) incidence and risk factors among illicit drug users (DUs) in Italy. Thus,

during 2007–2010, we conducted a prospective cohort study among DUs attending 17 Italian rehabilitation centers serving urban areas. Two hundred eighty-four HCV-uninfected DUs were prospectively followed by interview and anti-HCV antibody and RNA testing every 6 months. Incidence was calculated using the person-years method. Infection predictors were assessed by time-dependent Cox analysis. Participants were mostly male (83.4%), under opioid substitution therapy (OST) (78.9%), non-injecting DUs (67.9%), and with a mean age of 30.8. Ninety-one of 224 DUs initially under OST interrupted treatment during the follow-up. Overall HCV incidence was 5.83/100 person-years at risk (PYAR) [95% confidence intervals (CI), 3.63–9.38]. The incidence did not significantly differ according the participants' sociodemographic characteristics or the degree of urbanization of the towns involved in the study. The incidence was higher for DUs under than for those not under OST (6.23 vs 4.50/100 PYAR; $p = 0.681$). Incidence was also higher for those with than for those without OST interruption (7.17 vs 5.04/100 PYAR; $p = 0.55$). However, all these differences were non-significant. At last follow-up visit, a significant decrease in frequency of sharing equipment for preparation/using drugs (by injection or not) was observed by analyzing either the whole cohort or DUs under OST only. Anti-HCV seroconversion resulted independently associated with sharing drug preparation/use equipment, backloading, having a HCV-positive sexual partner, or household and (marginally) intravenous injection. In

E. Spada (✉) • G. Rezza
Department of Infectious Diseases, Viral Hepatitis Unit, Istituto
Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy
e-mail: enea.spada@iss.it

A. R. Garbuglia • M. R. Capobianchi
Laboratory of Virology, National Institute for Infectious Diseases
“Lazzaro Spallanzani”, Rome, Italy

F. L. Lombardo • F. Menniti Ippolito
National Centre of Epidemiology, Surveillance and Health
Promotion, Istituto Superiore di Sanità, Rome, Italy

O. Zuccaro
Medical Affairs Department, MSD Italia, Rome, Italy

E. Cupellaro
Local Health Unit of Latina, Latina, Italy

S. Capone • A. Nicosia • A. Folgori
ReiThera S.r.l., Rome, Italy

A. Nicosia
Department of Molecular Medicine and Medical Biotechnology,
University of Naples Federico II, Naples, Italy

A. Nicosia • R. Cortese
Keires AG, Basel, Switzerland

A. Mele
Calabria Association of Hepatology, Reggio Calabria, Italy

this study, HCV incidence was non-negligible and OST seemed to lack effectiveness in reducing it. In Italy, implementation of combined harm reduction interventions and antiviral treatment of chronically infected DUs would be needed.

Keywords Hepatitis C virus · Illicit drug use · Incidence · Opioid substitution treatment · Risk factors · Seroconversion

Introduction

Infections are common among illicit drug users (DUs). In particular, unsafe drug injections may favor transmission of important blood-borne pathogens, including hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) [1]. HCV transmission occurs frequently among intravenous drug users (IDUs). Nearly 10 million IDUs worldwide (67%) had antibodies against HCV (anti-HCV) in 2010 [1]. Also, among non-injecting drug users (NIDUs) smoking or inhaling drugs, higher anti-HCV prevalence rates than in non-drug users have been reported [2]. Globally, reported HCV incidence rates among IDUs range from 3.8 to 66/100 person-years at risk (PYAR), with most between 20 and 40/100 PYAR [3, 4].

In Italy, HCV prevalence has declined among the general population [5–7]. The most recent seroepidemiological studies reported an overall prevalence of 2.6%, with rates increasing by age [6, 7]. According to the Italian surveillance system for acute viral hepatitis, during the period 2003–2010, the incidence of acute symptomatic hepatitis C among the general population decreased from 0.7 to 0.2 per 100,000 people, but IDUs showed still the highest infection risk [5]. In late 2000s, two studies found among DUs attending Italian public drug dependency rehabilitation centers (DDRCs) anti-HCV prevalence rates of about 71 and 64%, respectively [8, 9]. These were among the highest rates in Europe and were similar to those found among Italian DUs during the 1990s [10, 11]. So far, despite this epidemiological situation, only three small and by now quite outdated studies have investigated the incidence of and risk factors for HCV infection among DUs in Italy [11–13]. Thus, it seems that in Italy, HCV transmission among DUs has remained essentially

uncontrolled, despite the adoption of harm reduction interventions (HRI), namely injecting equipment provision (IEP) and opioid substitution therapy (OST) since mid-1990s.

Various IDUs' behaviors, collectively characterizing the "high risk drug use (HRDU) behavior", seem particularly effective in transmitting HCV. Among them, frequent drug injection, and several drug use/preparation equipment sharing practices, characteristic (typical) of group-injecting settings are those most often cited [14–18]. An increased HCV infection risk has also been found among NIDUs, being it associated with drug use duration and frequency, non-injecting equipment sharing, sexual behavior, and tattooing [2].

In this study, to bridge the gap in the knowledge of HCV epidemiology among illicit DUs in Italy, we prospectively followed up a cohort of HCV-uninfected DUs attending public DDRCs serving urban areas scattered throughout the country. The aims of our investigation were to determine HCV incidence and risk factors and to evaluate the impact of OST (the HRI with the highest coverage in Italy) [19] on them.

Methods

Settings and Study Population

The study was performed during 2007–2010 in 17 Italian DDRCs, operating in 11 public Local Health Care Districts serving urban communities scattered throughout the country (4 in the north, 4 in the center, and 3 in the south). DUs attending these DDRCs underwent 6-month interval counseling and screening for blood-borne pathogens. All consecutive DUs (without age limit) who resulted anti-HCV negative at the last screening, or were presumed to be so, based on self-reported information, were considered eligible for the study. Eligible individuals were invited to participate in the study and, if agreed, enrolled in the cohort and scheduled for baseline interview and blood sampling for HCV testing.

Baseline anti-HCV-negative DUs underwent follow-up visits planned every 6 months, for 1 year. At each follow-up visit, participants were reinterviewed, recounseled, and retested for HCV. Baseline anti-HCV-positive DUs were excluded from the follow-up but were offered referral for

further medical evaluation. Free vaccination was offered to participants testing HBV negative. Participants gave signed informed consent. The study was approved by Istituto Superiore di Sanità Ethics Committee and has been performed according to the World Medical Association Declaration of Helsinki.

Laboratory Tests

Sera were tested for anti-HCV by a third-generation chemiluminescent microparticle immunoassay (ARCHITECT Anti-HCV assay, ABBOTT, Wiesbaden, Germany). Reactive samples were confirmed using HCV recombinant immunoblot assay (RIBA) Deciscan HCV Plus (Biorad, Marnes-la-Coquette, France) and tested for HCV RNA by a commercial real-time RT-PCR assay (Real-TM HCV, Abbott Molecular Inc., Des Plaines, IL, USA) (detection limit 12 IU/mL). RIBA and/or HCV RNA-positive samples were considered true positive. All samples testing anti-HCV negative at the last follow-up visit were assessed for HCV RNA to detect window period infections.

Data Collection

A baseline interview questionnaire elicited information on sociodemographic, drug use history, as well as OST status, drug use and sexual behaviors, and other HCV risk factors within the previous 6 months. Details on collected information are displayed in Table 1.

Analysis

The final cohort included only baseline-negative anti-HCV DUs having at least two visits at least 6 months apart.

An HCV incident case was defined as the occurrence of a positive anti-HCV and/or HCV RNA test during the follow-up in a participant anti-HCV negative at baseline. The date of infection was defined as the midpoint between the last negative and first positive anti-HCV and/or HCV RNA test dates.

PYAR was computed for DUs who acquired infection as the time elapsing from the date of the first anti-HCV negative test to the date of infection. For DUs who remained anti-HCV negative, PYAR was computed as the difference between the dates of the first and last negative test. Behaviors reported at the time of the last

anti-HCV negative test and first anti-HCV and/or HCV RNA positive test (both referred to the previous 6 months) were used for nonseroconverters and seroconverters, respectively.

Incidence rates (IRs) and their 95% confidence intervals (CIs) were calculated for 100 PYAR. Poisson distribution and test-based methods were used for building CIs and comparing IRs between groups.

The time-dependent Cox proportional-hazards model was used to determine variables associated with HCV seroconversion. Time-dependent covariates were included in the model.

Both univariate and multivariate Cox regression models, calculating crude and adjusted hazard ratios (HR), were used for risk factor analysis. Variables for multivariate analysis were selected by a stepwise method. Since the limited number of seroconversions, separate multivariate models for discrete blocks of variables were used including only variables with a *p* value < 0.05.

Chi-square and Fisher tests were used for comparison of frequencies between groups. A *p* value of 0.05 was considered significant.

All analyses were performed using STATA version 13 (StataCorp, LP, College Station, TX).

Results

Of 432 eligible DUs, 404 agreed participating in the study. Of them, 43 (10.6%) resulted anti-HCV positive at baseline being thus excluded. Of the remaining 361 DUs, 77 (21.3%) underwent only the baseline visit and were then lost to follow-up. The final cohort included 284 DUs (92, 85, and 107 from northern, central, and southern DDRCs, respectively); among them, 105 and 179 underwent in total 2 and 3 visits, respectively. Participants were mostly males (83.4%), NIDUs (67.9%), under OST (78.9%), with a mean age of 30.8 ± 7.6 years. They differed significantly from those lost to follow-up on some drug-related variables (abuse initiation age, drug injection frequency, heroin use, and OST) (Table 1).

Seventeen HCV seroconversions were detected during the follow-up, yielding an overall IR of 5.83/100 PYAR (95% CI, 3.63–9.38; total PYAR, 291.4). Table 2 shows IRs for all considered variables.

Table 1 Baseline features of the individuals included in the study and comparison between the final cohort and lost to follow-up

	Agreeing participants (<i>n</i> = 361)		Final cohort (<i>n</i> = 284)		Lost to follow-up (<i>n</i> = 77)		<i>p</i>
	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	
Sex (males)	305	84.5	237	83.4	68	88.3	0.296
Age ^a	30.5	± 7.4	30.8	± 7.6	29.6	± 6.7	0.218
Education							
< 9 years	190	61.1	187	67.3	46	60.5	0.278
≥ 9 years	121	38.9	91	32.7	30	39.5	
Unemployment							
No	273	80.8	217	80.7	56	81.2	0.927
Yes	65	19.2	52	19.3	13	18.8	
Homeless							
No	335	93.6	265	94.3	70	90.9	0.281
Yes	23	6.4	16	5.7	7	9.1	
Imprisonment							
No	339	94.7	266	94.7	73	94.8	0.960
Yes	19	5.3	15	5.3	4	5.2	
Sex trade							
No	348	98.6	271	98.2	77	100	0.234
Yes	5	1.4	5	1.8	0	0	
Beginning age of abuse ^a	21.0	± 5.7	21.4	± 5.6	19.6	± 5.8	0.018
Length of drug abuse ^b	8.5	5–14	8	5–14	10	6–15	0.508
Drug injection (previous 6 months)							
No	232	66.1	187	67.5	45	60.8	0.280
Yes	119	33.9	90	32.5	29	39.2	
Injection frequency (previous 6 months)							
< 3 times a week	90	55.6	39	43.3	51	67.6	0.002
≥ 3 times a week	72	44.4	51	56.7	21	33.4	
Use of heroin (previous 6 months)							
No	93	26.0	63	22.3	30	40.0	0.002
Yes	265	74.0	220	77.7	45	60.0	
Opioid substitution therapy							
No	91	25.2	60	21.1	31	40.3	0.001
Yes	270	74.8	224	78.9	46	59.7	
Syringe sharing							
No	341	95.0	267	94.7	74	96.1	0.612
Yes (ever or sometimes)	18	5.0	15	5.3	3	3.9	
Sharing drug preparation/using equipment ^c							
No	196	54.7	152	54.1	44	57.4	0.515
Yes	162	45.3	129	45.9	33	42.6	
Backloading ^d							
No	312	88.1	250	88.7	62	86.1	0.552
Yes	42	11.9	32	11.3	10	13.9	
Injecting/using drugs with > 2 DUs							
No	123	48.2	97	50.5	26	41.3	0.202
Yes	132	51.8	95	49.5	37	58.7	

Table 1 (continued)

	Agreeing participants (<i>n</i> = 361)		Final cohort (<i>n</i> = 284)		Lost to follow-up (<i>n</i> = 77)		<i>p</i>
	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	
Injecting/using drugs mostly in street							
No	211	60.1	165	60.2	46	59.7	0.940
Yes	140	39.9	109	39.8	31	40.3	
Pooling money to buy drugs							
No	183	52.4	143	52.6	40	51.9	0.923
Yes	166	47.6	129	47.4	37	48.1	
Nosocomial exposure (previous 6 months)							
No	296	84.8	231	84.9	65	84.4	0.912
Yes	53	15.2	41	15.1	12	15.6	
Piercing							
No	291	82.4	227	82.2	64	83.1	0.859
Yes	62	17.6	49	17.8	13	16.9	
Tattoos							
No	293	82.5	229	82.4	64	83.1	0.879
Yes	62	17.5	49	17.6	13	16.9	
Having an HCV-positive household or sexual partner							
No	342	97.7	270	98.5	72	94.7	0.050
Yes	8	2.3	4	1.5	4	5.3	
Having a drug-using sexual partner							
No	301	85.5	238	86.6	63	81.8	0.298
Yes	51	18.18	37	13.5	14	18.2	
Being homo/bisexual							
No	333	97.1	261	97.0	72	97.3	0.902
Yes	10	2.9	8	3.0	2	2.7	

DUs drug users

^a Mean $\pm$ sd^b Median and interquartile range^c It includes the sharing of all devices used for preparing/injecting/smoking/inhaling drugs^d This is a practice whereby a drug solution is squirted from a donor syringe to another by removing the plunger. In this table, frequency of backloading refers to the total number of questionnaire respondents (without distinction between intravenous and non-intravenous drug users)

Three of 17 seroconverters belonged to the group of 60 DUs not undergoing OST throughout the study. The IR for this sub-group was 4.50/100 PYAR (95% CI, 1.45–13.95; total PYAR, 66.7). These three seroconverters were NIDUs.

Fourteen of 17 seroconverters belonged to the group of 224 DUs under OST for which the IR was 6.23/100 PYAR (95% CI, 3.69–10.52; total PYAR, 224.7). Heights of these 14 seroconverters were IDUs, while 6 were NIDUs. Difference in incidence between DUs under OST and those not under OST was non-significant ($p = 0.681$).

Ninety-nine of 224 DUs under OST (44.2%) interrupted their treatment during the follow-up. Of them, 35 (35.3%), 61 (61.7%), and 3 (1.3%) interrupted OST for a period of less than 3 months, 4–6 months, and more than 6 months, respectively. Five and nine seroconversions occurred among DUs with (IR, 5.04/100 PYAR; 95% CI, 2.17–11.26; total PYAR 99.2) and without OST interruption (IR, 7.17/100 PYAR; 95% CI, 3.82–13.07; total PYAR, 125.5), respectively, without significant difference in IR between these groups ($p = 0.45$).

Table 2 Incidence rates of anti-hepatitis C virus (HCV) seroconversion according to exposure

	PYAR	Events	Incidence/100 PYAR	95% CI
Total cohort	291.4	17	5.83	(3.63–9.38)
Sex				
Male	245.2	16	6.52	(4.00–10.65)
Female	46.1	1	2.17	(0.31–15.38)
Age (years)				
< 30	144.6	12	8.30	(4.71–14.61)
≥ 30	146.8	5	3.41	(1.42–8.18)
Beginning age of abuse				
≤ 18 years	89.8	10	11.14	(5.99–20.70)
> 18 years	192.5	7	3.64	(1.73–7.63)
Length of drug abuse				
0–3 years	24.0	4	16.69	(6.26–44.46)
≥ 4 years	258.3	13	5.03	(2.92–8.67)
Imprisonment				
No	272.7	13	4.8	(2.80–8.20)
Yes	15.7	3	19.1	(6.20–59.3)
Homeless				
No	269.8	13	4.82	(2.80–8.30)
Yes	18.7	3	16.04	(5.17–49.74)
Being homo/bisexual				
No	268.8	15	5.58	(3.36–9.26)
Yes	7.27	1	13.75	(1.94–97.6)
Use of heroin				
No	116.8	3	2.57	(0.83–7.96)
Yes	171.8	14	8.15	(4.83–13.76)
Drug injection				
No	220.5	9	4.08	(2.12–7.84)
Yes	70.9	8	11.29	(5.64–22.57)
Opioid substitution therapy				
No	68.1	3	4.50	(1.45–13.95)
Yes	224.9	14	6.23	(3.69–10.52)
Injection frequency				
no injection use	220.5	9	4.08	(2.12–7.84)
< 3 times a week	34.3	3	8.74	(2.82–27.11)
≥ 3 times a week	34.5	5	14.50	(6.04–34.84)
Nosocomial exposure				
No	246.2	14	5.69	(3.37–9.60)
Yes	28.7	1	3.48	(0.49–24.70)
Backloading ^a				
No	265.6	11	4.14	(2.29–7.48)
Yes	12.4	4	32.34	(12.14–86.18)
Sharing drug preparation/using equipment ^b				
No	215.5	7	3.25	(1.55–6.81)
Yes	70.0	9	12.86	(6.69–24.71)

Table 2 (continued)

	PYAR	Events	Incidence/100 PYAR	95% CI
Having HCV+ household or sexual partner				
No	267.4	12	4.49	(2.55–7.90)
Yes	3.9	2	51.28	(12.83–205.04)
Having drug-using sexual partner				
No	260.7	13	4.99	(2.90–8.59)
Yes	23.7	4	16.91	(6.35–45.05)
Injecting/using drugs with > 2 DUs				
No	96.5	1	1.04	(0.15–7.36)
Yes	57.1	9	15.76	(8.20–30.29)

PYAR person-years at risk, DUs drug users

^a This is a practice whereby a drug solution is squirted from a donor syringe to another by removing the plunger

^b It includes the sharing of all devices used for preparing/injecting/smoking/inhaling drugs

At the last follow-up visit, a decrease in frequency of all reported HRDU behaviors was observed in comparison to the baseline (Table 3). A significant decrease in frequency was evident either for drug injecting equipment sharing or for drug use/preparation equipment sharing (i.e., sharing of all devices used for injecting, smoking or inhaling drugs, taken together) only when the whole cohort or the under OST sub-group were considered (Table 3). Besides, all reported HRDU behaviors were

more frequent among DUs with than among those without OST interruption during the follow-up. This difference was however non-significant (data not shown).

Results of risk factors analysis are shown in Table 4. After multivariate analysis, an independent association with HCV seroconversion was found for backloading, drug use/preparation equipment sharing (which included the sharing of all devices used for preparing/injecting/smoking/inhaling drugs), having a HCV-

Table 3 Frequency of high-risk drug use behaviors reported at baseline and end at last follow-up visit by drug users undergoing or not opioid substitution therapy

	All drug users (<i>n</i> = 284)					Drug users under OST (<i>n</i> = 224)					Drug users not under OST (<i>n</i> = 60)				
	Baseline		Last visit			Baseline		Last visit			Baseline		Last visit		
	<i>n/n</i>	<i>%</i>	<i>n/n</i>	<i>%</i>	<i>p</i>	<i>n/n</i>	<i>%</i>	<i>n/n</i>	<i>%</i>	<i>p</i>	<i>n/n</i>	<i>%</i>	<i>n/n</i>	<i>%</i>	<i>p</i>
IDU	90/277	32.4	72/263	27.4	0.229	71/219	32.4	60/208	28.8	0.486	19/58	32.7	12/55	21.8	0.274
Injection frequency ≥ 3 T/W	51/90	56.7	28/65	43.1	0.113	40/71	56.3	24/55	43.6	0.216	11/19	57.9	4/10	40.0	0.689
Sharing injecting equipment ^a	57/87	65.5	25/65	38.4	0.001	44/68	64.7	18/54	33.3	0.001	13/19	68.4	7/11	57.8	0.100
Backloading ^b	23/85	27.1	10/61	16.4	0.187	14/67	20.9	7/51	13.7	0.043	9/18	50.0	3/10	30.0	0.434
Sharing drug preparation/using equipment ^c	129/281	45.9	81/271	29.9	0.0001	101/221	45.7	59/212	27.8	0.0001	28/60	46.7	22/59	37.3	0.349

OST opioid substitution therapy, *n/n* row proportion, % percentage, *p p* value, IDU intravenous drug use, ≥ 3 T/W injecting drugs 3 or more times a week

^a Sharing of injecting equipment in this analysis includes needles/syringes sharing (an infrequent practice in this study) and also sharing of other injecting paraphernalia (water, drugs, acids, spoons, lighters, filters etc.) and backloading, all taken together

^b Backloading is a practice whereby a drug solution is squirted from a donor syringe to another by removing the plunger. In this, table is also considered alone, separated from other types of sharing procedures

^c It includes the sharing of all devices used for preparing/injecting/smoking/inhaling drugs

Table 4 Univariate and multivariate Cox regression analysis of risk factors for anti-hepatitis C virus (HCV) seroconversion

Model	Univariate		Multivariate ^a			
	Crude HR	<i>p</i>	95% CI	Adjusted HR	<i>p</i>	95% CI
Age ^b	7.66	0.037	0.85–0.99			
Block 1						
Backloading ^c	7.66	< 0.000	2.44–24.09	3.72	0.051	1.00–13.89
Nosocomial exposure	0.66	0.691	0.09–5.02			
Equipment sharing ^d	4.24	0.004	1.57–11.43	4.12	0.017	1.29–13.13
Syringe sharing	2.34	0.410	0.31–17.77			
Block 2						
Sex (F vs M)	0.31	0.254	0.04–2.32			
Unemployment	0.61	0.521	0.14–2.72			
Imprisonment	4.67	0.043	1.05–20.69			
Homeless	3.53	0.097	0.80–15.67			
Being homo/bisexual	2.32	0.415	0.31–17.57			
Having a HCV+ sexual partner or household	9.88	0.003	2.21–44.19	12.27	0.001	2.73–55.17
Having a DU ^d sexual partner	3.28	0.038	1.07–10.05			
Block 3						
Opioid substitution therapy	1.30	0.681	0.37–4.52			
Use of heroin	3.43	0.053	0.98–11.92			
Drug injection	2.87	0.030	1.11–7.43	2.58	0.053	0.99–6.72
Injection frequency						
< 3 times/week vs no injection	2.05	0.281	0.56–7.58			
≥ 3 times/week vs no injection	4.05	0.012	1.36–12.10			

HR hazard ratio, CI confidence interval

^a Multivariate models are adjusted for age

^b Age is considered in continuous form

^c Backloading is a practice whereby a drug solution is squirted from a donor syringe to another by removing the plunger

^d It includes the sharing of all devices used for preparing/injecting/ smoking/ inhaling drugs

positive household/sexual partner, and (marginally) intravenous drug injection.

Discussion

The overall HCV incidence found in this study was 5.83/100 PYAR. The incidence was higher, although not significant, for DUs under OST than for those not under OST. Anti-HCV seroconversion resulted independently associated with sharing drug preparation/use equipment, backloading, having a HCV-positive sexual partner or household, and (marginally) intravenous injection.

Previously published studies reporting HCV incidence among Italian DUs, apart from time period (all of them were performed during 1991–1993), differ from this study in several aspects, partly related to study design and size, partly to the risk exposure profile of the respective cohorts [11–13]. One study was a retrospective analysis of screening data from individuals attending a sexually transmitted disease (STD) clinic in Rome [12]. Participants having ≥ 2 anti-HCV tests included also 12 IDUs for which HCV incidence was 30.3/100 PYAR. Another study investigating prospectively a sample of 34 IDUs attending a DDRC in a northern small town found a HCV incidence of 6.2/100 PYAR [11]. The third study performed on a sample of 106 IDUs prospectively followed up in three

DDRCs in Naples reported an incidence of 28.6/100 PYAR [13].

Hence, our study is the largest and most methodologically appropriate assessment of HCV incidence and risk factors ever performed among DUs in Italy. In comparison with these Italian studies, our estimated HCV incidence was close to that by Galeazzi et al. (6.2/100 PYAR) [11]. This might be due to the low-to-moderate risk exposure profile of both study populations. Whereas, the cohort investigated by Rezza et al. [13] (all participants were IDUs, mostly daily injectors and not under OST) and the small IDUs group studied by Giuliani et al. (all participants were active IDUs and at increased risk of STDs) [12] had both a moderate-to-high risk exposure profile. Anyhow, in our study, HCV incidence was much higher than those reported in earlier and recent Italian general population studies [5, 20, 21].

Our estimated overall HCV incidence also closely paralleled those of studies performed abroad among DU cohorts with a similar risk exposure profile and/or undergoing HRI [15, 22–27]. However, in our cohort, participants with high-risk exposure characteristics showed IRs at least twofold higher than the overall cohort incidence. This is consistent with data of other prospective studies performed abroad in moderate-to-high risk exposure DUs cohorts [17, 18, 28, 29] and with the results of a recent review of 17 incidence studies among European IDUs [4]. This review reported a median HCV incidence of 13/100 PYAR (range, 5.2–42/100 PYAR), with particularly high rates among active/recent IDUs.

However, the incidence in our cohort was not negligible considering its overall risk exposure profile. The absence of significant differences in IRs between DUs under OST and those not under OST suggests a lacking effectiveness of OST in reducing HCV incidence. This view is reinforced by the observation that the IR for DUs not interrupting OST was even higher (although non-significant) than that for those interrupting OST.

In our cohort, at last follow-up visit, a decrease in frequency of all reported HRDU behaviors was observed in comparison to the baseline. However, this decrease was statistically significant only for drug preparation/using (by injection or not) equipment sharing and only when the whole cohort or DUs under OST were considered. This finding suggests at least a partial effectiveness of OST in reducing HRDU behavior and is consistent with the observation that at last visit all HRDU behaviors were more frequent (although non-

significant) among DUs with than among those without OST interruption. Indeed, recent studies have shown that only combination of OST and IEP, at sufficient coverage, can significantly reduce HCV incidence among IDUs [3, 24, 26, 30]. Most of these studies also reported a contemporary decrease in some HRDU behaviors (mainly injection frequency and/or injecting equipment sharing), but no significant reduction in HCV incidence when OST or IEP were administered alone [3, 24, 26]. Besides, a recent review of reviews by MacArthur et al. on the effectiveness of the full range of HRI, in relation to HIV and HCV incidence and HRDU behavior [31], found for OST a sufficient evidence of effectiveness in reducing HRDU behavior (documented either for injection frequency or for sharing of needles/syringes or other injecting equipment). Conversely, only a tentative review-level (i.e., little evidence) was found as regards OST effectiveness in reducing HCV transmission. In fact, most of reviewed studies showed no or non-significant association between OST (or remaining in OST throughout the follow-up) and HCV incidence reduction. Even one of these studies, paralleling our findings, reported a higher incidence among DUs under OST throughout the follow-up than among those who were not under OST or interrupted it during the follow-up, thus [32].

Why this different impact of OST on HCV incidence and HRDU behavior? It is possible that the decreased frequency of some HRDU behaviors induced by OST is not per se sufficient to reduce HCV incidence too, also considering that several factors may favor HCV transmission in drug using groups. This would be consistent with the reported higher efficiency in reducing HCV incidence of combined HRI respect to OST or IEP alone [3, 23, 26, 30]. Combined interventions supporting different strategies (drug use and injection frequency reduction, sterile equipment availability, etc.) have likely more chances to negatively affect HCV transmission. Another possible explanation is that OST's overall effectiveness might be influenced by underdosing. Indeed, in most studies revised by MacArthur et al., the OST dose used was unclear [31]. A recent Canadian study showed a strong dose-response protective effect of increasing methadone-maintained therapy on HCV incidence [22]. Unfortunately, we also did not record the OST dosage.

Syringes/needles sharing occurred infrequently in our cohort, while sharing of other drug use/preparation equipment, including backloading, was common. Sharing drug use/preparation equipment

other than syringes or needles is likely perceived by DUs as less risky. Instead, as evidenced by a recent meta-analysis, the risk of HCV transmission through shared drug use/preparation equipment is similar to that found for syringes/needles sharing, accounting actually for a large proportion of infections [14]. In our risk factor analysis, we considered separately syringes/needles sharing, backloading, and drug use/preparation equipment sharing, this latter including also backloading and the sharing of all devices used for preparation of injecting drug or for smoking or inhaling drugs. This choice was motivated by three reasons: not reducing the statistical power of analysis; our cohort was mostly composed by NIDUs at baseline; among IDUs (who usually smoke or inhale drugs too), sharing of filters, cups, rinsing water or backloading, or other devices rarely occur in isolation. An association between HCV infection and drug use/preparation equipment sharing (with similar IRs) among DUs has been already reported by others [16–18, 25], which have also demonstrated that most such infections could be prevented by eliminating these practices [14, 18].

The strong association we found between anti-HCV seroconversion and having a sexual/household relationship with HCV-positive individuals is consistent with the findings of a recent Italian general population study on risk factors for acute symptomatic hepatitis C [5]. However, the actual role and mechanism(s) of this exposure in acquiring HCV are still debated [33]. A higher anti-HCV prevalence in sexual and, to less extent, household HCV carrier's contacts than in general population has been reported [33, 34]. Conversely, longitudinal studies have reported no or very rare transmission among heterosexual HCV-discordant couples [35, 36]. It is now clear that apart from intercourses involving bleeding or those between individuals with STDs (particularly HIV), sexual contact is not an efficient way to transmit HCV [33]. This has been also demonstrated in HCV-discordant heterosexual DU couples among which transmission seemed associated with couples' drug-injection behavior, instead [37]. Sexual/household relationships with other DUs may result in a high rate of syringes/needles and drug use/preparation equipment sharing [17, 38]. Besides, awareness about sexual/household partner HCV status may have only limited impact on sharing practices, particularly if considering drug use/preparation equipment sharing between individuals in sexual relationship [38]. Thus, incident cases reporting sexual/household relationships with HCV-

carriers could be due to drug-related sharing practices or domestic parenteral transmission rather than to sexual transmission.

Our study has several limitations; firstly, the study population was a convenient sample of DUs attending Italian DDRCs, but might not represent all DUs. Besides, sample size and limited number of seroconversions could have hindered identification of infection predictors. The sample size was anyway comparable or larger than that in previous studies [13, 15–17, 22, 25, 29, 30]. The differential loss to follow-up might have also influenced study findings, even if loss to follow-up was not associated with exposures predicting seroconversion. In addition, OST dosage was not recorded and exposures were based on self-reported data; thus, both recall and social desirability bias might have diluted associations. Finally, we did not collect individual accurate information on the participants' residence. Indeed, since DUs participating in our study attended DDRCs of densely populated towns and cities, the overwhelming majority of them were very likely real urban residents. Given that the degree of urbanization has been reported to be associated with illicit drug use and its consequences [39], we conducted a post hoc analysis using population density of participating towns and cities (taken as a marker of urbanization) to verify the existence of this association also in our study. In particular, we chose a cut-off value that of the town/city having the median population density in the ordered list of participating centers. We found no significant difference in HCV incidence between the groups of towns/cities with higher and lower population density compared to cut-off value (5.97 /100 PYAR (CI, 3.4–10.1) vs 5.10/100 PYAR (CI, 32.5–11.3; $p = 0.81$).

DUs' low risk perception of acquiring HCV through equipment sharing is a major obstacle to infection prevention, particularly considering that sharing practices are embedded in drug using group and interpersonal relationships. Educational programs should consider this aspect.

Combined HRIs, given at high coverage, are the best mean for limiting both high risk behavior and HCV transmission among DUs [3, 23, 26, 28, 30]. Nevertheless, global provision of key HRIs remains low [1, 28]. In Italy, HRI programs exist but they are far from being fully implemented, particularly as regards IEP [19]. The importance of IEP in blood-borne infections prevention among IDUs was already emphasized by one of us in a study analyzing the impact of a morphine prescription program on HIV spread among IDUs [40].

In conclusion, our study showed a not negligible HCV incidence in a cohort of Italian drug users, mostly composed by NIDUs and DUs under OST. More efforts in reducing high-risk behaviors and further implementation and effective combination of HRIs would be needed. The reduction of reservoir of chronic carriers should be a priority. DUs should be systematically screened for HCV, and chronic carriers should be treated. HCV treatment with new direct acting antiviral agents could become the best prevention intervention, even if it is still too expensive [41].

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Spada and Rezza are with the Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy; Garbuglia and Capobianchi are with the Laboratory of Virology, National Institute for Infectious Diseases "Lazzaro Spallanzani," IRCCS, Rome, Italy; Lombardo and Menniti Ippolito are with the National Centre of Epidemiology, Surveillance and Health Promotion, Istituto Superiore di Sanità, Rome, Italy; Zuccaro is with the Medical Affairs Department, MSD Italia, Rome, Italy; Cupellaro is with the Local Health Unit of Latina, Latina, Italy; Capone, Nicosia, and Folgori are with the ReiThera S.r.l, Rome Italy; Nicosia and Cortese are with Keires AG, Basel, Switzerland; Nicosia is with CEINGE, Naples, Italy; Nicosia is with the Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy; Mele is with the Calabria Association of Hepatology, Reggio Calabria, Italy.

Other members of the collaborative study group are the following persons and institutions:

Carlo De Mei (Drug Dependency Service of Latina); Angela Coia (Drug Dependency Service of Priverno, Latina); Vittorio Santi (Drug Dependency Service of Formia, Latina); Marco Masci (Drug Dependency Service of Terracina, Latina); Anna Maria Cioce (Drug Dependency Service of Aprilia, Latina); Vincenzo Mellace, Egidio Battaglia, Franco Montesano (Drug Dependency Service of Soverato, Catanzaro); Vincenzo Biancolilli (Drug Dependency Service of Benevento); Pasquale Farina (Drug Dependency Service of Anzio, Roma); Marco Cucuini (Drug Dependency Service of Terni); Giuliano Dozzini (Drug Dependency Service of Foligno, Perugia); Antonio De Luna (Drug Dependency Service of Battipaglia, Salerno); Raffaele Lovaste, Silvana Chiasera (Drug Dependency Service of Trento); Antonia Cinquegrana, Enrico Elba, Elisabetta Secchi (Drug Dependency Service of Brescia); Umberto Nizzoli, Angela Zannini (Drug Dependency Service of Reggio Emilia); Henri Margaron, Lucia Mancino (Drug Dependency Service of Livorno); Salvatore De Masi (Public Health Care Districts of Livorno); Salvatore Lobello, Andrea Vendramin (Drug Dependency Service of Padova).

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