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Title: HCV screening based on dried blood samples and linkage to care in people who use drugs: A prospective study

Running head: HCV screening and linkage to care in PWUD

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Abstract

Background: The burden of hepatitis C virus (HCV) infection among people who use drugs (PWUD) is considerable. We aimed to screen for HCV infection using the fingerstick dried blood spot (DBS) test and to describe the cascade of hepatitis C care among PWUD in Madrid, Spain. We also evaluated the prevalence of hepatitis B virus (HBV) and hepatitis D virus (HDV) in this population.

Methods: We carried out a prospective study and collected samples and epidemiological data using a mobile unit. Viral infections were tested by immunoassay and RT-PCR assay. PWUD with a positive result were contacted and referred to a specialized health center to confirm and treat the HCV infection.

Results: We studied 529 PWUD samples; 49.7% were people who inject drugs (PWID). Of these, 152 (28.7%) were positive for HCV antibodies, 122 (23.1%) for HCV RNA, 23 (4.3%) for HBsAg, and two (0.4%) for HDV antibodies (8.7% of those with hepatitis B). PWID more frequently had positive HCV antibody titers (52% vs. 7.3%; $p<0.001$) and a positive HCV RNA test result (40.2% vs. 7.3%; $p<0.001$) than non-PWID. The time from sample collection to test results was 19 days. The next 104 individuals (85.2%) with active HCV infection were contacted to report their HCV test results. Of these, 63 (51.6%) had an appointment, 62 (50.8%) were evaluated in the hospital, and 56 (45.9%) started HCV therapy.

Conclusion: HCV screening using fingerstick DBS was an excellent tool for determining HCV prevalence and other chronic hepatitis viruses (HBV and HDV) in PWUD. However, linkage to care was limited, mainly with respect to the initiation of HCV therapy.

Keywords: HCV, screening, dried blood spot, people who use drugs, linkage to care, HCV treatment

Introduction

Hepatitis C virus (HCV) infection is one of the leading causes of liver-related death and disability worldwide (Stanaway, et al., 2016). Moreover, other chronic viral hepatitis viruses, such as hepatitis B (HBV) and hepatitis D (HDV), share routes of transmission similar to those of HCV; thus, their diagnoses are a priority in public health around the world (UNAIDS, 2016; World Health Organization, 2017). The World Health Organization (WHO) aims to significantly reduce the number of new viral hepatitis infections and related deaths by 2030 (World Health Organization, 2016a). However, in the absence of an effective vaccine against HCV, this strategy involves a scale-up of screening, reduction in risk behavior, and unrestricted access to treatment. The most pragmatic approach to the eradication of HCV in marginal populations is micro-elimination (Hollande, Parlati, & Pol, 2020).

The burden of HCV infection among people who use drugs (PWUD) is considerable, and transmission continues as a result of ongoing risk behaviors, such as sharing needles/syringes and paraphernalia (Morris, et al., 2017; Nelson, et al., 2011). Around 39% of people who inject drugs (PWID) are living with hepatitis C infection (9% of all global infections) (Day, et al., 2019). In Spain, the prevalence of viremic HCV infection among PWUD is estimated at 53% (Day, et al., 2019). As such, the percentage of PWID with undiagnosed HCV infection is probably higher than in the general population, given that this vulnerable group has limited access to the health system. Thus, needle and syringe sharing among PWID leads to high HCV infection rates, transmission, and reinfection (Grebely, et al., 2017; Valencia, et al., 2019).

The prevalence of HCV infection can be substantially reduced through more frequent diagnosis and access to treatment (Day, et al., 2019). HCV screening is cost-effective since early identification of HCV infection allows patients to benefit from therapy, helps limit the transmission of the infection, and prevents or reduces liver disease (Eckman, Ward, & Sherman, 2019). Direct-acting antiviral (DAA) therapy has dramatically increased cure rates for HCV to over 95%, even in PWUD (Grebely, et al., 2017; Ingiliz & Tacke, 2019; Wong, et al., 2019). Thus, therapeutic guidelines recommend that PWUD should be treated (Panel, 2018). Monitoring the HCV cascade of care in PWUD is essential if we are to reach WHO goals for elimination of hepatitis (Safreed-Harmon, et al., 2019; World Health Organization, 2016b).

In Spain, HCV is generally diagnosed at specialized health centers using immunoassay and a PCR test, with high rates of loss to follow-up in PWUD. Besides, given that HCV therapy can only be prescribed in hospital settings and that PWUD often do not have access to primary care services or referrals to hospitals, diagnosis of hepatitis C remains inadequate (Yehia, Schranz, Umscheid, & Lo Re, 2014). Screening via finger pricks and Whatman cards is inexpensive and straightforward and can be implemented with minimal training. Consequently, samples are easy to handle, and many patients are not lost to follow-up. The diagnostic performance of dried blood spot (DBS) sampling in screening for viremic HCV infection is excellent (Saludes, et al., 2019; Vazquez-Moron, et al., 2019; Vazquez-Moron, et al., 2018). DBS simplifies testing algorithms, increases the rate of diagnoses, and reduces the number of visits to health centers, thereby improving linkage to care for PWUD (Stéphane Chevaliez, 2019). Linkage to care is also dependent on drug use, psychiatric illness, and social and economic problems. PWUD face stigma and discrimination, even among healthcare professionals, thus posing additional barriers to health care (Ahern, Stuber, & Galea, 2007).

This study aimed to screen for HCV infection by testing fingerstick DBS samples and describing the cascade of hepatitis C care among PWUD in Madrid, Spain. We also evaluated the prevalence of HBV and HDV infections in this population.

Methods

Design and participants

We carried out a prospective study between June 1, 2017, and May 31, 2018. The study population comprised persons who visited the "Cañada Real Galiana" shantytown on the outskirts of Madrid, where 90% of illicit drugs in the region are sold and consumed. It is estimated that 4,000-6,000 people/day visit the shantytown to obtain drugs.

In phase I (virus detection), we only included people who were using drugs during participation in the study and entered the shantytown from outside. HCV screening was offered to everyone, regardless of whether they knew their serological status. Participants were incorporated consecutively by order of appearance (consecutive sampling). The inclusion criteria were as follows: 1) Age > 18 years; 2) Signature of the informed consent document; 3) Contact details. The sampling process ended when the time limit established by the research project was reached (time saturation). A trained nurse and a social educator from the mobile unit collected samples using DBS on Whatman ™ cards. Afterward, all the collected samples were sent by courier to the Instituto de Salud Carlos III (ISCIII) for analysis. In this phase, serological tests were also performed to detect HBV and HDV infection.

In phase II (linkage to care), participants with a positive HCV RNA test result were contacted and referred to specialized health centers. Patients had access to standard confirmation tests for HCV monitoring and access to antiviral treatment and were followed to assess the impact of the program on their health status. A hepatitis C patient navigator accompanied the participant during sampling. In the case of patients infected with HCV, the navigator accompanied the patient in the delivery of the HCV test result and the appointment at the hospital, where they underwent a blood test to confirm the results of screening. Then, a physician prescribed HCV therapy according to international guidelines.

The study was conducted following the Declaration of Helsinki, and participants gave their written informed consent. The study was approved by Institutional Review Board and the Research Ethics Committee of ISCIII approved the study (CEI PI 77_2015-v2) and Hospital General Universitario Gregorio Marañón (409/15).

Data sources

Epidemiological, sociodemographic, and behavioral data were collected through questionnaires. Participants provided contact data for their subsequent location. Clinical data related to the diagnosis and treatment of hepatitis C were collected from hospital medical records.

Study data were collected and managed using Research Electronic Data Capture (REDCap, Vanderbilt University, Nashville, TN, USA) (Harris, et al., 2009), which is hosted at *Asociación Ideas for Health*. Data were recorded using a mobile device with Internet capability. Researchers from the project, hospital, and laboratory had access to the database.

Laboratory assays

DBS samples were collected by finger prick using Whatman 903™cards (50-75 µl blood, approximately, depending on the characteristics of the cards used). DBS cards were dried at room temperature (~25°C) for four hours, kept in individual zipped plastic bags with a desiccant, and stored at 4°C. Samples were sent refrigerated (~4°C) to the National Microbiology Center (laboratory) within 15 days of collection and stored at –80°C until processing.

DBS samples were processed following a previously described protocol (Vazquez-Moron, et al., 2018). The eluates were tested for anti-HCV antibodies (Murex anti-HCV kit, v. 4.0), HBV surface antigen (Murex HBsAg v. 3.0), and anti-HDV antibodies (ETI-AB-DELTAK-2). All kits were from the manufacturer DiaSorin (Saluggia, Italy), and samples were processed on an ETI-Max 3000 instrument (DiaSorin, Saluggia, Italy).

HCV RNA testing was performed in a reflex manner (only if HCV antibodies were detected). We punched two 6-mm disks from the DBS cards. These disks were pretreated in 1100 µl of ATL buffer (Qiagen, Hilden, Germany) at 56°C and centrifuged at 1200 rpm for 15 minutes. HCV RNA was extracted from 300 µl of pretreated DBS sample using a commercial mini-kit DSP Virus/Pathogen (Qiagen, Hilden, Germany) (Vazquez-Moron, et al., 2018). HCV RNA was detected using the SYBR Green RT-PCR assay, which is a qualitative test (positive/negative) described elsewhere (Vazquez-Moron, et al., 2018). The limit of detection of the assay was 5000 copies/µl, which is equivalent to 960 IU/ml.

Study groups and outcome variables

Participants were stratified into four groups according to HCV infection: i) unexposed to hepatitis C (anti-HCV antibody negative); ii) exposed to hepatitis C (anti-HCV antibody positive); iii) active HCV infection (anti-HCV antibody test and HCV RNA positive); iv) resolved HCV infection (anti-HCV antibody positive and HCV RNA negative). Moreover, participants were stratified into two groups according to drug use: i) non-PWID were drug users but never injected; ii) PWID had injected drugs.

The two events taken into account for HCV screening were as follows: i) a positive anti-HCV test; ii) a positive HCV-RNA test. We also analyzed patients exposed to HBV (HBsAg positive) and HDV (anti-HDV antibody positive).

The five events taken into account for the linkage to care of active HCV infection were as follows: i) receiving the results of the HCV test; ii) having an appointment at the hospital; iii) being seen at the hospital; iv) starting HCV treatment; v) achieving a sustained virological response (SVR), defined as undetectable HCV RNA in serum at week 12 after discontinuation of DAA therapy.

Statistical analysis

All analyses were performed using IBM SPSS v24 (IBM Corp, Armonk, NY, USA). All p-values were two-tailed and $p < 0.05$ was considered statistically significant. We used the Kruskal-Wallis test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables, as appropriate. Multivariate logistic regression was used to analyze the association between patient characteristics (age, gender, nationality, homeless, decades of drug use, benzodiazepines prescribed, injection drug use (IDU), retention in care, and lack of healthcare insurance) and groups of HCV infection (unexposed to hepatitis C, active HCV infection, and resolved HCV infection). We used the forward stepwise selection method (pin <0.05 and pout <0.10), without forcing the entry of variables into the model.

Results

Characteristics of the study population

The characteristics of the 529 participants are summarized in **Table 1**. The median age was 42 years, 79% were male, 21% were migrants, and 20% were homeless. As for drug use, 81.8% had used drugs for more than ten years. Heroin and cocaine were the most consumed drugs, with smoking as the current primary mode of administration, and around 50% had previously injected drugs. Regarding healthcare, 88% had regular medical care in a health center, mainly addiction centers (29%), hospital centers (29%), and primary care centers (26%). Around 15% of participants had never been tested for HCV infection. Furthermore, 35% of participants had not been tested for HCV infection in the previous 12 months. Overall, 28% were taking opioid substitution therapy, and 29% were taking psychiatric medications. Concerning sexual risk behaviors, 37% of participants had a stable partner, 19.1% engaged in HCV-related sexual risk behavior, and 9.6% of the women had engaged in commercial sex work in the last year.

Screening results

Of the total samples analyzed, 152 (28.7%) were positive for HCV antibodies, 122 (23.1%) for the HCV RNA test, 23 (4.3%) for HBsAg, and two (0.4%) for HDV antibodies (8.7% of those who had hepatitis B) (**Figure 1A**). HCV viral load in the hospital was 685,300 IU/ml (IQR: 215,800; 2,916,000). Moreover, compared with non-PWID, PWID more frequently had positive HCV antibodies (52% vs. 7.3%; p <0.001) and HCV RNA test results (40.2% vs. 7.3%; p < 0.001) (**Figure 1B**).

Moreover, 167 (32%) participants reported a previous diagnosis of HCV, and in 9, no HCV antibodies were detected. Among 167 individuals who reported having had a previous HCV infection, only 108 (65%) were positive for HCV antibodies, and 75 (45%) were positive for HCV RNA.

Table 1. Baseline epidemiological characteristics of the population screened.

Characteristics	Data
N (%)	529
Age, median (IQR)	42 (35-48)
<30 years	65/528 (12.3%)
30-40 years	163/528(30.8%)
40-50 years	219/528(41.4%)
≥50 years	82/528(15.5%)
Male, N (%)	420/529 (79%)
Origin, N (%)	
Spain	419/529 (79%)
Eastern Europe	55/529 (10%)
Western Europe	17/529 (3%)
North Africa	23/529 (4%)
America	7/529 (1%)
Homeless, N (%)	105/526 (20%)
Drugs use	
Decades of drug use, median (IQR)	5 (4; 7)
<1 year	6/489(1.2%)
1-9 years	83/489 (17%)
10-19 years	171/489 (35%)
20-29 years	134/489 (27.4%)
≥30 years	95/489 (19.4%)
Type of drug used, N (%)	
Heroin	412/529 (78%)
Cocaine	476/529 (90%)
Marihuana	71/529 (13.4%)
Alcohol (>50 g/day)	50/529 (9.5%)
Current route of administration	
Injected	177/529 (33.5%)
Smoked	432/529 (81.7%)
Snorted	135/529 (25.5%)
Benzodiazepines prescribed	39/529 (7.4%)
Had injected drugs (IDUs)	254/511 (49.7%)
Shared syringes in the previous year	21/246 (8.5%)
Shared paraphernalia in the previous year	90 /223(40.4%)
IDUs active in the previous year	127/478(26.6%)
Healthcare, N (%)	

Regular medical care in a health center	449/510 (88%)
No health insurance	74/481 (15.4%)
Never tested for HCV	55/356(15.4%)
Last HCV test >1 year	124/356(34.8%)
Opioid substitution therapy	142/512 (27.7%)
On psychiatric treatment	148/511 (29%)
HCV-related sexual risk behavior, N (%)	
Stable partner	204/441(37%)
Sexual risk behavior in last year	57/298 (19.1%)
Men who have sex with men	9/334 (2.7%)
Commercial sex work (only women)	34/354 (9.6%)

Statistics: Values are expressed as number (percentage) and median (interquartile range).
Abbreviations: HCV, hepatitis C virus; IDU, injection drug user.

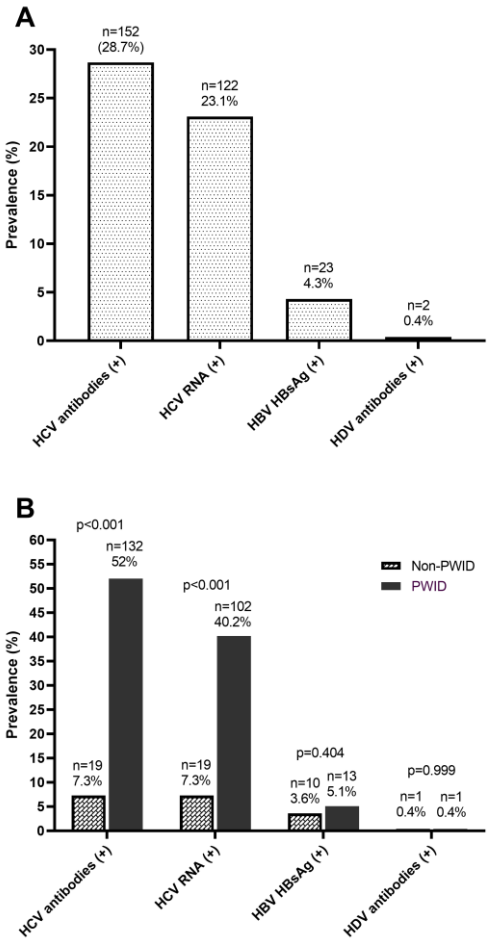

Figure 1. Prevalence of hepatitis C virus (HCV), hepatitis B virus (HBV), and hepatitis D virus (HDV) infection in people who use drugs (PWUD) in Cañada Real Galiana. A) All study population; B) population stratified by injection drug users (IDUs).

Factors related to HCV infection

The characteristics of the study population stratified by HCV infection are summarized in **Table 2**. We found significant differences ($p < 0.05$) between the study groups (unexposed to hepatitis C, active HCV infection, and resolved HCV infection) for age, nationality, homelessness, time

using drugs, type of drug used, current route of administration, benzodiazepines prescribed, and healthcare.

We also used multivariate logistic regression analysis to search for participant characteristics that were associated with belonging to the unexposed to hepatitis C, active HCV infection, resolved HCV infection, or exposed to hepatitis C group. When we compared unexposed to hepatitis C vs. active HCV infection, the characteristics associated with active HCV infection were decades of drug use (adjusted odds ratio [aOR]= 1.14 [95%CI= 1.03; 1.28]; p= 0.015), being PWID (aOR= 9.34 [95%CI= 5.23; 16.67]; p<0.001), and not having health insurance (aOR= 2.44 [95%CI= 1.24; 4.81]; p= 0.010). When we compared resolved HCV infection vs. active HCV infection, the only factor associated with active HCV infection was benzodiazepines prescribed (aOR= 0.25 [95%CI= 0.08; 0.76]; p= 0.014). When we compared unexposed to hepatitis C vs. exposed to hepatitis C (resolved HCV infection plus active HCV infection), the factors related to exposed to hepatitis C were decades of drug use (aOR= 1.14 [95%CI= 1.03; 1.26]; p= 0.011), being PWID (aOR= 9.76 [95%CI= 5.79; 16.45]; p <0.001), and not having healthcare insurance (aOR= 2.55 [95%CI= 1.36; 4.78]; p= 0.003).

Cascade of HCV care

The time from sample collection to availability of test results was 19 days (IQR, 13-28). Next, of all individuals with active HCV infection, 104/122 (85.2%) were located and contacted to report the results of the HCV test. Of these, 63/122 (51.6%) had an appointment at the hospital, and 62/122 (50.8%) were evaluated at the hospital (**Figure 2**). At the hospital, the mean log HCV viral was 5.7 and the main HCV genotypes found were 1a (57.5%) and 3 (22.5%). In total, 56/122 patients (45.9%) started HCV therapy, and 49/122 (40.2%) achieved SVR (**Figure 2**). SVR was achieved by 87.5% of patients who started HCV treatment. Overall, we did not find significant differences in the percentages of participants who were contacted and referred to the hospital, evaluated at an HCV clinic, started HCV therapy, and achieved SVR (p >0.05).

The main reason for not beginning HCV therapy was if patients were unreachable (34 patients); some had moved to different regions/countries (6 patients), and another was imprisoned (1 patient). No significant differences were found when the population was stratified according to IDU.

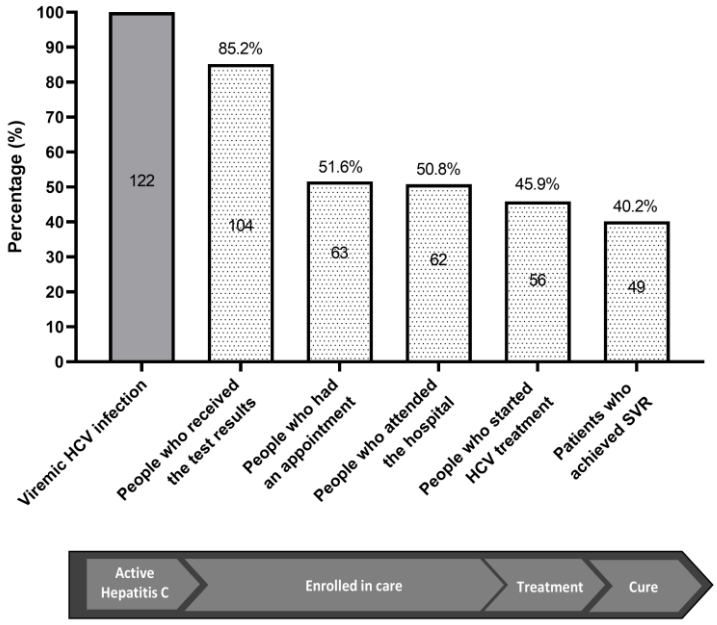

Figure 2. Cascade of hepatitis C virus (HCV) care in people who use drugs (PWUD) in Cañada Real Galiana.

Table 2. Baseline epidemiological characteristics of the screened population according to HCV infection.

Characteristics	Unexposed to hepatitis C	Active hepatitis C	Resolved hepatitis C	p-value
N (%)	359 (67.8%)	122 (23.1%)	48 (9.1%)	
Age, median (IQR)	42 (34-48)	42 (37-49)	46 (36-50)	0.273
<30 years	56/359 (15.6%)	5/122 (4.1%)	4/48 (8.3%)	**0.012**
30-40 years	100/359 (27.9%)	49/122 (40.2%)	14/48 (29.2%)	0.567
40-50 years	148/359 (41.2%)	50/122 (41%)	21/48 (43.8%)	0.721
≥50 years	55/359 (15.3%)	20/122 (14.8%)	9/48 (18.8%)	0.328
Male, N (%)	280/359 (78%)	102/122 (83.6%)	38/48 (79.2%)	0.416
Origin, N (%)				
Spain	295/359 (82.2%)	93/122 (76.2%)	31/48 (64.6%)	**0.027**
Eastern Europe	27/359 (7.5%)	18/122 (14.8%)	10/48 (20.8%)	**0.003**
Western Europe	8/359 (2.2%)	7/122 (5.7%)	2/48 (4.2%)	0.147
North Africa	20/359 (5.6%)	1/122 (0.8%)	2/48 (4.2%)	0.086
America	5/359 (1.4%)	1/122 (0.8%)	1/48 (2.1%)	0.748
Homeless, N (%)	56/356 (15.7%)	36/122 (29.5%)	13/48 (27.1%)	**0.002**
Drugs use				
Decades of drug use, median (IQR)	5 (4, 6)	6 (5, 7)	6 (5, 8)	**<0.001**
<1 year	5/328 (1.5%)	1/117 (0.9%)	0/44 (0%)	0.631
1-9 years	69/328 (21.0%)	7/117 (6%)	7/44 (15.9%)	**0.001**
10-19 years	124/328(37.8%)	39/117 (33.3%)	8/44 (18.2%)	**0.034**
20-29 years	74/328 (22.6%)	43/117 (36.8%)	17/44 (38.6%)	**0.003**
≥30 years	56/328 (17.1%)	27/117 (23.1%)	12/44 (27.3%)	0.143
Type of drug used, N (%)				
Heroin	262/359 (73%)	107/122 (87.7%)	43/48 (89.6%)	**<0.001**
Cocaine	321/359 (89.4%)	114/122 (93.4%)	41/48 (85.4%)	0.239
Marihuana	47/359 (13.1%)	15/122 (12.3%)	9/48 (18.8%)	0.512
Alcohol (>50 g/day)	32/359 (8.9%)	13/122 (10.7%)	5/48 (10.4%)	0.827
Current route of administration				
Injected	72/359 (20.1%)	70/122 (57.4%)	35/48 (72.9%)	**<0.001**
Smoked	297/359 (82.7%)	97/122 (79.5%)	38/48 (79.2%)	0.653
Snorted	109/359 (30.4%)	17/122 (13.9%)	9/48 (18.8%)	**<0.001**

Benzodiazepines prescribed, N (%)	22/359 (6.1%)	8/122 (6.6%)	9/48 (18.8%)	**0.007**
Had injected drugs (IDUs)	113/343 (32.9%)	102/121 (84.3%)	39/47 (83%)	**<0.001**
Shared syringes in the previous year	9/110 (8.2%)	11/99 (11.1%)	1/37 (2.7%)	0.291
Shared paraphernalia in the previous year	75/197 (38.1%)	12/19 (63.2%)	3/7 (42.9%)	0.103
IDUs active in the previous year	89/317 (28.1%)	23/117 (19.7%)	15/44 (34.1%)	0.105
Healthcare, N (%)				
Regular medical care in a health center	48/344 (14%)	108/120 (90%)	45/46 (97.8%)	0.052
No health insurance	37/328 (11.3%)	26/112 (23.2%)	11/41(26.8%)	**0.001**
Never tested for HCV	46/303 (15.2%)	6/43 (14%)	3/10 (30%)	0.425
Last HCV test >1 year	107/303 (35.3%)	13/43 (30.2%)	4/10 (40%)	0.760
Opioid substitution therapy	73/345 (21.2%)	47/121 (38.8%)	22/46 (47.8%)	**<0.001**
On psychiatric treatment	101/346 (29.2%	32/120 (26.7%)	15/45 (33.3%)	0.693
HCV-related sexual risk behavior, N (%)				
Stable partner	117/298 (39.3%)	55/89 (50.9%)	12/29 (34.3%)	0.693
Sexual risk behavior in last year	36/189 (19%)	15/84 (17.8%)	6/25 (24%)	0.790
Men who have sex with men	7/214 (3.3%)	2/95 (2.1%)	0/25 (0%)	0.580
Commercial sex work (only women)	21/229 (9.2%)	9/98 (9.2%)	4/27 (14.8%)	0.633

Statistics: Values are expressed as number (percentage) and median (interquartile range). **Abbreviations**: HCV, hepatitis C virus; AHC, acute hepatitis C; RHC, resolved hepatitis C; IDU, injecting drug user.

Discussion

We conducted a prospective study in PWUD from one of the largest shantytowns in Europe in order to evaluate the utility of the DBS point-of-care (POC) fingerstick test in detecting HCV infection and, subsequently, enabling referral to specialized clinical care. Our major findings were as follows: (i) The frequency of individuals with HCV RNA was high, particularly in PWID; (ii) Linkage to care was poor because only around 50% of patients with viremic HCV infection started HCV therapy; (iii) The prevalence of HBsAg was close to 4%, and HDV antibodies among PWUD with HBsAg was close to 9%, although no differences were observed with respect to IDU.

Our study shows that HCV screening using fingerstick DBS can be a useful tool in PWUD. Of note, we found a seroprevalence of HCV antibodies of 28.7% (52% in PWID) and a frequency of active HCV infection of 23.1% (40.2% in PWID). In Western Europe, the estimated prevalence of PWID with HCV antibodies is 53.2% (Degenhardt, et al., 2017), and the frequency of viremic HCV infection is 39.9% (Day, et al., 2019). Given that over 300,000 individuals have a lifetime history of injecting drugs in Spain, the burden of HCV infection is considerable (Roncero, Littlewood, Vega, Martinez-Raga, & Torrens, 2017): 60-80% of PWID have HCV antibodies (Roncero, et al., 2017), and 53.3% have active HCV infection (Day, et al., 2019). Moreover, in Spain, the only study using DBS in PWID was performed in Catalonia, with a prevalence of active HCV infection of 58% (Saludes, et al., 2019). The difference in the prevalence of active HCV infection between our article and that of Saludes *et al*. (Saludes, et al., 2019) can be explained, in part, by differences in the inclusion criteria. In our study, inclusion was based on a proactive search for PWUD in a shantytown; about 50% of those screened had never injected drugs before. In contrast, the study of Saludes *et al.* (Saludes, et al., 2019) was based on PWID from harm reduction services. These variations can also be explained by geographical differences related to the prevention strategies carried out in different regions (Heffernan, Cooke, Nayagam, Thursz, & Hallett, 2019), which may have reduced the prevalence of HCV among PWUD. However, our prevalence data for active HCV infection in PWID were similar to those reported in a recent French study by Chevaliez *et al.* (S. Chevaliez, et al., 2020), who detected a prevalence of 38.6%, also using DBS sampling. Moreover, our data indicate that using fingerstick DBS to screen for HCV in PWUD can prove very useful in low-to middle-income countries with limited laboratory capacity by enabling samples to be collected in remote/rural areas and shipped to the laboratory at room temperature (Duchesne, et al., 2020).

One of the main barriers to treating and eliminating hepatitis C in PWUD is the low rate of HCV diagnosis. Our study found that belonging to the active HCV infection and exposed to hepatitis C groups was linked to a longer duration of drug use, being PWID, and not having health insurance, all of which were linked to vulnerability, thus supporting the critical nature of these features when assessing HCV infection in PWUD, as previously reported (Arasteh, Des Jarlais, Feelemyer, & McKnight, 2020; Bartholomew, et al., 2020).

Our data show that a large proportion of participants were lost to follow-up before starting HCV therapy. Thus, the HCV screening result was only delivered in 85% of those with HCV viremia, and finally, less than 50% began HCV therapy. However, previous studies in PWID have reported lower rates attended in hospitals and initiation of HCV treatment (Broad, et al., 2020; G. McAllister, et al., 2014; Georgina McAllister, Shepherd, Templeton, Aitken, & Gunson, 2015). Therefore, our data can be considered an improvement over other data on PWID, maybe because accompaniment to the hospital was more effective than in other studies. Furthermore, it should be noted that a series of factors that were not taken into account (e.g., the use of incentives, peer-based support) may have affected losses to follow-up. In fact, while 15% of the participants were lost in the first step of the cascade of care (delivery of HCV-RNA results, which is related to the DBS test), this loss was much higher in the later steps (39% of those who received their results ended up not receiving HCV therapy, which is not directly related to DBS testing). Therefore, although our patients had the support of a patient navigator, linkage to

treatment was not ideal. Our data emphasize the need for a range of strategies to facilitate better linkage to care in this population, given the many health priorities and barriers to HCV care they have.

Alternative strategies should be implemented to improve linkage to care, since decentralized HCV screening is often not sufficient in marginalized populations who have minimal contact with the health system. One strategy that has been proposed is decentralized HCV treatment, either by specialists who deliver therapy at the POC or by removal of prescriber-type restrictions for DAAs, so that drugs can be delivered in settings such as primary care and drug treatment centers (Lazarus, et al., 2019; Valerio, et al., 2020). HCV screening in the POC as part of a test-and-treat strategy and the community-based POC testing and HCV treatment initiated by general practitioners are both decentralized models of HCV treatment (Applegate, Fajardo, & Sacks, 2018) that have been already implemented in some countries to improve linkage to care and close the gaps in the HCV cascade (Draper, et al., 2020; Forns, et al., 2020). Other strategies could include incorporating peer navigators or linkage-to-care coordinators, mobile medical unit use, increased implementation of onsite laboratory visits, and medication-assisted treatment (Applegate, et al., 2018; Lazarus, et al., 2019). Moreover, the fact that our study took more than two weeks to report the HCV screening results may have been of considerable importance, as our strategy requires testing to be performed in a centralized laboratory. Shortening the response time could reduce losses to follow up and increase the effectiveness of the intervention. Using the POC test Xpert HCV VL Fingerstick (Sunnyvale, CA, USA), Saludes *et al*. (Saludes, et al., 2020) showed that 80% of PWID received their HCV RNA results on the same day. However, Chevaliez *et al.* (S. Chevaliez, et al., 2020) used the GeneXpert POC test and reported that only 47% of participants started HCV treatment, thus stressing the need for the removal of prescriber-type restrictions. It is essential to adapt the screening method to detect people with active HCV infection and rapidly refer them for treatment. Screening and access to specialized care significantly improve patient bonding, simplify the HCV care cascade, and increase the number of patients treated (Grebely, et al., 2020; Hsiang, et al., 2020; Mohamed, et al., 2020). The differences found in other studies may be due to multiple factors, as discussed in the previous section, and include differences in study design, inclusion criteria (harm reduction units, prisons, street), and HCV prevention strategies.

Reducing the prevalence of HBV and HDV infection is also one of the challenges for the WHO (World Health Organization, 2016a). The prevalence of HBV infection is around 1% in western Europe (World Health Organization, 2017). PWUD are a population at risk, with a higher prevalence of hepatitis B than the general population (Schillie, et al., 2018); they also have a higher risk of developing chronic HBV infection due to impaired immune function and coinfections with HIV and HCV (Tran, Grimes, Lai, Troisi, & Hwang, 2012). In our study, the prevalence of HBsAg was 4.3% for PWUD, which is similar to 3.2% (0.9–5.6%) in Western Europe, as reported in a previous study (Degenhardt, et al., 2017). Moreover, HBV/HDV coinfection is the most severe form of viral hepatitis and has an underrecognized role in chronic liver disease. In Europe, the prevalence of anti-HDV antibodies among people with HBsAg is 3.0% (2.1–4.2%), although this is higher in PWID (Stockdale, et al., 2020). In Spain, the prevalence of anti-HDV antibodies was 8.2% among PWID with hepatitis B (Ordieres, et al., 2017), that is, very similar to the percentage we report (8.7%); both HBV and HDV infections are still endemic in PWUD in Spain. Another remarkable finding of our research was that around 45% of PWUD with a positive HBsAg test were immigrants, mainly from Eastern Europe; the two individuals with a positive anti-HDV test result were also from this area. These data support the role of immigration as a risk factor for HBV and HDV infection, as highlighted in other studies (Coppola, et al., 2019; Coppola, et al., 2015).

The use of fingerstick DBS sample can be an effective strategy, and it can easily be incorporated into public health programs at addiction or harm reduction facilities. EASL guidelines

recommend using DBS sample as an alternative to serum or plasma obtained by venipuncture for anti-HCV antibody testing and HCV RNA testing after a central laboratory shipment. The simplicity and relative ease of specimen collection, preparation, transport, and storage make DBS specimens a potential option in vulnerable populations with poor access to healthcare and provide invaluable tools for large-scale HCV hepatitis screening, diagnosis, and treatment decisions. The DBS sample also enables the diagnosis of other prevalent infections in PWUD, such as HBV, HDV, and HIV. Therefore, fingerstick DBS sample may be essential to organize and implement local or national HCV elimination plans because it may reduce costs associated with sample collection, storage, transportation, and allow for batch testing in a centralized laboratory.

Limitations of the study

Our study is subject to a series of limitations. First, the sample was small, and the data are for a single city, Madrid, thus reducing the generalizability of our findings. Second, some patients may have changed their residence to another region, where they were treated; consequently, they may not be registered as having received treatment. Third, by not offering incentives, some PWUD users might not have been sampled, although higher-risk patients, such as PWID, may also have been more interested in being sampled. Fourth, the risk behavior data may not be completely reliable; however, this bias may be minimized by the fact that data were collected by trained personnel. Finally, the diagnostic performance of the DBS samples was not compared with the diagnostic performance of plasma samples because plasma was not collected. However, we already demonstrated excellent performance of DBS samples compared with plasma in previous articles (Vazquez-Moron, et al., 2019; Vazquez-Moron, et al., 2018).

Conclusion

In conclusion, HCV screening using fingerstick DBS proved to be an excellent tool for determining the prevalence of HCV in PWUD, as well as other chronic hepatitis viruses (HBV and HDV). However, linkage to care was limited, mainly for initiation of HCV therapy. New strategies to improve rapid diagnosis and access to HCV treatment in PWUD are needed to strengthen the link with medical care and to help achieve the elimination goals of the WHO.

Declarations and Ethics

Ethics approval and consent to participate

The study was conducted following the Declaration of Helsinki, and participants gave their written informed consent. The Institutional Review Board and the Research Ethics Committee of Instituto de Salud Carlos III (ISCIII) approved the study (CEI PI 77_2015-v2), as did that of Hospital General Universitario Gregorio Marañón (409/15).

Consent for publication

Not applicable.

Originality

The authors confirm that the material contained herein is entirely original.

Availability of data and materials

Datasets used and analyzed during the current study may be available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

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Study concept and design: PR, JV, SR.

Patient selection and clinical data acquisition: AR, CR, JTM, JV, PR, and JT.

Laboratory assays: SV, IC, and MJMG.

Statistical analysis and interpretation of data: PR, SR, and SV.

Drafting the manuscript: PR, JV, SR, and SV.

Critical revision of the manuscript for relevant intellectual content: JV and JT.

Supervision and visualization: PR and SR.

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